ANTIMICROBIAL EFFECTS OF *GOMPHRENA* AND *VERNONIA* EXTRACTS AGAINST *STAPHYLOCOCCUS* SPECIES AND *CANDIDA* SPECIES FROM WOMEN WITH VULVOVAGINITIS IN ZARIA, NIGERIA.

BY

Esther Iyadunni, OGUNDANA, B.Sc BOTANY (KSU, 2007)
M.Sc/SCIE/8486/2010-2011

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN BOTANY

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE, NIGERIA

MARCH, 2015

DEDICATION

This thesis is dedicated to the Almighty God, the Maker and the Finisher, the beginning and the end and to all women, both singles and pregnant.

DECLARATION

I declare that the work in this thesis titled '' Antimicrobial Effects of *Gomphrena* And *Vernonia* Extracts Against *Staphylococcus* Species and *Candida* species from Women with Vulvovaginitis in Zaria, Nigeria'' has been performed by me in the Department of Biological Science, Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria under the supervision of Dr. M. A. Adelanwa, Prof. A.K Adamu and Dr. E. E. Ella. The information derived form the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Esther Iyadunni OGUNDANA _____ _____

Signature Date

CERTIFICATION

This thesis entitled '' ANTIMICROBIAL EFFECTS OF *GOMPHRENA* AND *VERNONIA* EXTRACTS AGAINST *STAPHYLOCOCCUS* SPECIES AND *CANDIDA* SPECIES FROM WOMEN WITH VULVOVAGINITIS IN ZARIA, NIGERIA'' by Esther Iyadunni OGUNDANA (M.Sc/SCIE/8486,2010-2011) meets the regulations governing the award of degree of Master of Science in Botany of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Dr.M.A Adelanwa Chairman, Supervisory Committee Department of Biological Sciences Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Prof. A. K. Adamu Member, Supervisory Committee Department of Biological Sciences Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Dr.E.E. Ella Member, Supervisory Committee Department of Microbiology, Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Prof. A.K. Adamu Head of Department, Department of Biological Sciences Faculty of Science, A.B.U, Zaria	_____ Signature	_____ Date
Prof A.Z. Hassan Dean, School of PostgraduateStudy Ahmadu Bello University, Zaria	_____ Signature	_____ Date

ACKNOWLEDGEMENTS

I am grateful to God Almighty for His mercies throughout this research. You made it possible, Lord.

My gratitude goes to my supervisors: Dr. M.A. Adelanwa, Prof. A.K. Adamu and Dr. E.E. Ella, for their time, support, efforts and corrections throughtout the period of this research. May God crown your efforts worthy and your children will excel by His grace. Remain blessed.

My sincere appreciation to my parents, Mr. and Mrs. A.A. Ogundana for their parental love, words of encouragement, support and prayers. My sister and her husband, Solicitor and Barrister and Mrs Babajide Aminu, for their financial support and words of encouragement. My brother, flying Sergent Olarenwaju Ogundana, for his financial contribution and for bieng there for me. Also to my brother, Emmanuel Durojaiye Ogundana, my niece Kolawole Folakemi Faith, Akinyele Tunrayo and my nephew, Emmanuel Tope Ogundana, for their lovely support.

My gratitude goes to the school, Ahmadu Bello University and the Departments, of Biological Sciences and Microbiology for giving me the opportunity to pursue my dreams. To the Heads of the Departments, Biological Sciences and Microbiology, lecturers and Staff that have helped in one way or the other, God bless you.

My thanks to all laboratory technicians for their guidance and time during the course of this work. God will always be there for you all.

Special thanks to Dr. Uchendu Chidiebene for his contributions and the knowledge shared towards this research. The wisdom God has given you, the devil will not steal it from you. Thanks for being there.

How can I forget my fiancé for his love, support and word of encouragement. May God take our love beyond this level, amen.

I can not afford to forget my friends and colleaques. My stay on campus would have been borring without you guys. God bless you all.

ABSTRACT

A total of 300 samples of High Vaginal swabs (HVS) were collected from women of reproductive age (15-50 years) for suspected vaginitis at Ahmadu Bello University, Health Care Center and Samaru Clinic, Zaria, Kaduna State, Nigeria. The specimens were investigated for the presence of *Candida* species and *Staphylococcus* species and the effect of *Gomphrena celosioides* and *Vernonia perrottetii* extracts on the isolates. The antimicrobial activity of the extracts was performed against *Candida* spp and *Staphylococcus* spp isolated using agar well diffusion method. Total number of 153(51%) patients were found to be infected, with 79(26.3%) having Candidiasis and 74(24.7%) having Staphylococcal vaginal infection. Organisms were found to be more prevalent among the age group of 21-30 years followed by 31-40 years old. There was a significant association in the use of birth control pills, tight underwear, tight clothing, pregnancy and vaginitis (χ^2= 82.78, p<0.001 OR =20.07 CI = 9.192 to 43.79, χ^2= 23.06 p<0.001 OR = 4.836 CI = 2.440 to 9.587, χ^2= 8.292 p<0.004 OR = 2.385 CI = 1.306 to 4.354, χ^2 = 30.95 p<=0.0001 OR = 4.338 CI = 2.541 to 7.405, respectively). There was no significant (p>0.05) association between age and vaginitis, marital status, use of vagina cream by the respondents and the use of antibiotics and vaginitis infections (χ^2 = 4.560, p = 0.2070, df = 3; χ^2 = 0.06609, p = 0.7971, df = 1; χ^2 = 2.796, p = 0.0945, OR = 1.679, CI = 0.9108 to 3.096, and χ^2 = 0.05774, p = 0.4473, OR = 0.8367, CI=0.5281 to 1.326 respectively). Biochemical characterization of the 50 randomly selected *Candida* spp using API 20 AUXC showed that *Candida tropicalis* were isolated from 13(4.33/26%) followed by *C. glabrata* and *C. albicans* 3(1%/6%) and (1%/6%), *C. parasiplosis* and *C. lambica* 1(2%/0.33%) and 1(2%/0.33%), respectively. The results of the ten randomly selected *Staphylococcus* spp using Microgen kit also showed that *S.*

xylosus, *S. aureus* and *Staphylococcus* spp were present in 3 samples 3(1%) and 3(1%) respectively and, *S. warneri* in one sample 1(0.3%). Antibiotic sensitivity testing was performed for both *Candida* spp and *Staphylococcus* spp using agar well diffusion method. The results showed that the organisms exhibited multiple resistances against ketoconazole among the 5 antifungal drugs used. The plant extracts sensitivity showed that almost all the *Candida* spp isolated were resistant to the plant extracts both singly and in combination, except for only 7 isolates that were susceptible at 2000mg/ml to *V. perrottetii* extracts. The results of the aqueous plant extracts sensitivity against *Staphylococcus* species at 500mg/ml and 1000mg/ml showed that *V. perrottetii* produced a zone of inhibition greater than the one produced by the antibiotics used for *S. xylosus* (a). The methanol extracts of *Vernonia perrottetii* (100mg/ml) showed that *S. xylosus* (a) had the same zone of inhibition as the antibiotic used (amoxycillin). Both aqueous and methanolic extracts of the two plant also did better for *S. aureus* where the antibiotics did not produce any zone of inhibition. The results of the minimum inhibitory concentration of *Vernonia perrottetii* (aq) $MIC_{500}/MBC>_{1000}$ showed bacteriostatic activity against only one organism. *V. perrottetii* (Me) tested against all the organisms exhibited bacteriostatic activity $MIC_{500}/MBC>_{1000}$ respectively. Aqueous combination of the extracts $MIC_{500}/MBC>_{1000}$ exhibited bacteriostatic activity to *S. aureus* and methanol combination of the extracts was bactericidal to *S. xylosus*. The present study showed that there was a high prevalence of *Candida* spp and *Staphylococcus* spp as well as strong association with some of the risk factors for vaginitis, and this suggest that, public enlightening should be carried out among women. It can be deduced from this study that the combination of the plant extracts did not perform better against both *Candida* spp and *Staphylococcus* spp isolated than when applied singly or in combination, implying that the two selected plant extracts might not

be combine together. It is therefore suggested that the individual plants be combined with other plants to see if a better result could be obtained.

TABLE OF CONTENTS

Title page ..... ….i

DEDICATION.....ii

DECLARATION..... iii

CERTIFICATION iv

ACKNOWLEDGEMENTS v

ABSTRACT.....vi

TABLE OF CONTENTS ix

LIST OF TABLES xv

LIST OF APPENDICES xvii

LIST OF PLATES.....xix

1.0 INTRODUCTION 1

1.1 Background to the study.....1

1.2 Statement of the Research Problem.....7

1.3 Justification for the Research Problem 9

1.3 Aim of the study.....11

1.5 Objectives of the study 12

1.6 Hypotheses of the research.....12

CHAPTER TWO.....14

2.0 LITRATURE REVIEW 14

2.1 Prevalence of Vulvovaginitis 14

2.2 Factors Associated with Vulvovaginitis 18

2.2.1 Immunological factors assosciated with vulvovaginitis.....18

2.2.2 Risk factors associated with vulvovaginitis.....20

2.3 Causes of Vaginitis **21**

2.4 Pathophysiology of Vaginitis **23**

2.5 Diagnosis of Vaginitis **24**

2.5.1 Vaginal pH 25

2.5.2 Amine test 26

2.5.3 Microscopy 26

2.6 Treatment of Vaginitis **27**

2.7 Yeast Vaginitis **28**

2.8 Morphology **35**

2.9 Epidemiology of Candidiasis **35**

2.10 Pathogenesis of *Candida* vulvovaginitis **38**

2.10.1 Host 39

2.10.2 Adaptation and propagation of *Candida albicans* 40

2.11 Clinical Presentation of *Candida* vulvovagintis **42**

2.12 Distribution of *Candida* spp in Vulvovaginal Candidiasis **43**

2.13 Virulence Factors Involved in Pathogenicity of *Candida albicans* **44**

2.13.1 Adhesion 45

2.13.2 Morphogenesis 46

2.13.3 Phenotypic switching 47

2.13.4 Phospholipases 49

2.13.5 Proteinases 49

2.13.6 Biofilm formation 51

2.14 Diagnosis of *candida* vulvovaginitis **52**

2.15 Treatment of vulvovaginal candidiasis **55**

2.15.1 Treatment of uncomplicated VVC. 55

2.15.2 Treatment of complicated vulvolvaginal candidiasis and recurrent vulvolvaginal candidiasis. 55

2.16 *Candida* Infection 63

2.17 Antifungal Resistance in *Candida* Isolates 64

2.18 Bacterial Vaginosis 65

2.19 Epidemiology of Bacterial Vaginosis 67

2.20 Pathogenesis of Bacterial Vaginosis 69

2.21 Clinical Presentation of Bacterial Vaginosis 70

2.22 Diagnosis of Bacterial Vaginosis 70

2.23 Treatment of Bacterial vaginosis 72

2.24 *Gomphrena celosioides* 76

2.24.1 Morphology of *Gomphrena celosioides* 76

2.24.2 Description of *Gomphrena celosioides* 76

2.24.3 Taxonomy and classification *Gomphrena celosioides* 77

2.24.4 Classification of *Gomphrena celosioides* 77

Plate I: *Gomphrena celosioides* 78

2.24.5 Distribution of *Gomphrena celosioides* 78

2.24.6 Biology and ecology *Gomphrena celosioides* 78

2.24.7 Uses of *Gomphrena celosioides* 79

2.24.8 Phytochemical properties of *Gomphrena celosioides* 80

2.24.9 Antibacterial activity of *Gomphrena celosioides* 81

2.25 *Vernonia* 81

2.25.1 Ethnopharmacognostic relevance of *Vernonia* species 83

2.25.2 Description of *Vernonia perrottetii* 83

Plate II: Picture of *Vernonia perrottetii* 84

2.25.3 Uses of *Vernonia perrottetii* 84

3.0 MATERIALS AND METHODS 85

3.1 Background Information on Study Area 85

3.2 Study Design 85

3.3 Questionnaire Administration 85

3.4 Study Population 86

3.5 Determination of Sample Size 86

3.6 Plant Collection and Authentication 86

3.7 Extraction of Plant Materials 87

3.8 Phytochemical Screening of the aqueous and methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottetii* 87

3.8.1 Test for carbohydrates 87

3.8.2 Test for reducing sugars 88

3.8.3 Test for Tannins 88

3.8.4 Test for saponins 88

3.8.5 Test for flavonoids 88

3.8.6 Test for alkaloids 88

3.8.7 Test for phenols 89

3.8.8 Test for anthraquinones 89

3.8.9 Test for steroids 89

3.9 Preparation of Culture Media 89

3.9.1 Preparation of Sabouraud Dextrose Agar 89

3.9.2 Preparation of Blood Agar 90

3.9.3 Sample collection and isolation 90

3.9.4 Gram's staining 90

3.9.5 Identification and characterization of *Candida* and *Staphylococcus* isolates 91

3.9.6 Confirmatory test (germ tube formation) 91

3.9.7 Haemolysis of blood using blood agar 92

3.9.8 Coagulase test 92

3.9.9 Catalase test 92

3.9.10 DNase test 93

3.10 Preparation of MacFarland Standard 93

3.10.1 Antimicrobial susceptibility testing of the isolates 93

3.10.2 Determination of Minimum inhibitory concentration and minimum bactericidal concentration of the plant extracts. 94

3.10.3 Determination of Minimum bactericidal concentration of the extracts 95

3.11 Statistical Analysis 95

CHAPTER FOUR 96

4.0 RESULTS 96

4.1 Phytochemical characteristics of the plant extracts 96

4.2 Distribution and relationship between age and vulvovaginitis caused by *Candida* spp and *Staphylococcus* spp 96

4.3 Distribution and Relationship between Age and Vaginitis Caused by *Candida* spp 99

4.4 The distribution and relationship between age and vaginitis caused by *Staphylococcus* spp 99

4.5 Relationship between risk factors and vaginitis 99

4.6 Gram's reaction behaviours, Germ Tube, and Arthrospore Formation of *Candida* species on Corn Meal Agar 100

4.7 Distribution of *Candida* species based on Biochemical Characterization Using API 20c Aux 100

4.9 Biochemical Distribution of Ten Randomly- Selected *Staphylococcus* Species (Using Microgen Kit) 107

4.10 Antifungal Pattern of *Candida* isolated from women attending A.B.U (sick bay) and Samaru clinic, Zaria, Nigeria 107

4.11 Sensitivity Pattern of *Candida* species to aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii* 111

4.12 Pattern sensitivity of *Candida glabrata, C. albicans, C. parasiplosis* 112

4.13 Antibacterial Sensitivity Pattern of *Staphylococcus* species Isolates 112

4.14 Sensitivity Pattern of *Staphylococcus* species to Aqueous Extract of *G. celosioides* and *V. perrottetii* at Different Concentration 112

4.15 Sensitivity pattern of methanolic extracts of *G. celosioides* and *V. perrottetii* against *Staphylococcus* spp at Different Concentration 121

4.16 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Plant Extracts 125

4.17 Minimum Inhibitory Concentrations of *Vernonia perrottetii* Methanol extracts 128

CHAPTER FIVE 130

5.0 DISCUSSION 130

CHAPTER SIX 143

6.0 SUMMARY AND CONCLUSION 143

6.1 Summary and Conclusion 143

6.2 Recommendations 143

REFERENCES 145

APPENDICES 184

LIST OF TABLES

Table Page

4.1 Phytochemical characteristics of aqueous extracts of *Gomphrena Celosioides* and *Vernonia perrottetii* extracts……....97

4.2 Distribution and Relationship between Age and Vaginitis Caused by *Candida* spp and *Staphylococcus* sp in women attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria……...98

4.3 Distribution and Relationship between Age and Vaginitis Caused by *Candida* spp in women attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria……..101

4.4 Distribution and Relationship between Age and Vaginitis Caused by *Staphylococcus* spp in women attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria…….....102

4.5 Relationship between Risk factors and vaginitis in women attending ABU (Sickbay) and Samaru Clinic…….....104

4.6 Gram's reaction behaviours in *Candida* species isolated from women attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria……..106

4.7 Biochemical Distribution of *Candida* Species isolated from women Attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria (Using API 20C Aux)……...107

4.8 Biochemical Distribution of *Staphylococcus* species isolated from women attending ABU (Sickbay) and Samaru Clinic, Zaria Nigeria 109

4.9 Biochemical Distribution of Ten Randomly Selected *Staphylococcus* Species (Using Microgen Kit) from women attending ABU (Sickbay) and Samaru Clinic…….....110

4.10 Antibiotic Sensitivity Pattern of *Candida* species isolated from women

attending ABU(Sickbay) and Samaru Clinic, Zaria Nigeria……111

4.11 Susceptibility Pattern of *Candida tropicalis* against various concentration of the aqueous and Methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottetii*…….....114

4.12 Plant extracts sensitivity of *Candida glabrata, C. albicans, C. parasiplosis* and *C. lambica* at various concentration……..116

4.13 Antibacterial Pattern Sensitivity of *Staphylococcus* species Isolates……117

4.14 Aqueous Plant Extracts Sensitivity Pattern against *Staphylococcus* spp at 50mg/Ml…….....118

4.15 Aqueous Plant Extracts Pattern Sensitivity of *Staphylococcus* spp at 1000mg/ml…….119

4.16 Sensitivity Pattern of *Staphylococcus* spp against methanolic Extracts of *Gomphrena celosioides* and *Vernonia perrottetii* at 500mg/ml …….....123

4.17 Sensitivity Pattern of *Staphylococcus* spp against methanolic Extracts of *Gomphrena celosioides* and *Vernonia perrottetii* at1000mg/ml……...124

4.18 Minimum Inhibitory Concentration of *Vernonia perrottetii* aqueous Extracts against *Staphylococcus* species……..128

4.19 Minimum Inhibitory Concentration of *Vernonia perrottetii* Methanolic Extracts against *Staphylococcus* species……...…130

LIST OF APPENDICES

Appendix		Page
I	Ethical clearance……	184
II	Structured questionnaire……	185
III	Biochemical characteristic of *Staphylococcus* species isolates (conventional method)……	...187
IV	Microgen *Staphylococcus* spp identification report……	..190
V	Antibiotic sensitivity Pattern (mm) of *Staphylococcus* species…….	191
VI	Plant extracts sensitivity Pattern of *Staphylococcus* species…….	192
VII	Morphological and Biochemical characteristic of *Candida* species using conventional method…….	195
VIII	Biochemical characteristics of *Candida* species using API 20 AUXC……	198
IX	Antibiotic sensitivity Pattern (mm) of *Candida* spp isolated using antifungal agents…….	200
X	Plant extracts sensitivity Pattern (mm) of *Candida* species…….	….201
XI	Arthropores formation in *Candida* species growing on cornmeal agar medium ……	...203
XII	Biochemical characterization of *Candida* spp using API 20 AUXC……	204
XIII	Antibiotic sensitivity Pattern of *Candida* spp ……	..205
XIV	Sensitivity Pattern of *Candida* spp against aqueous and methanol extracts of *G. celosioides* and *V. perrottetii* …….	206
XV	Culture of *Staphylococcus* species on mannitol salt agar medium…….	207
XVI	Haemolysis of *Staphylococcus* species on blood agar medium……	208
XVII	Microgen kit identification of *Staphylococcus* species…….	209

XVIII Sensitivity Pattern of *Staphylococcus* species against aqueous and methanolic Extracts of *Gomphrena celosioides* and *Vernonia perrottetii*.....210

XIV MIC for *Staphylococcus* species against the aqueous and methanolic Extracts of *G. celosioides* and *V. perrottetii*……...…….211

XX MBC for *Staphylococcus* species against the aqueous and methanolic Extracts of *G. celosioides* and *V. perrottetii*……...212

LIST OF PLATES

Plates Page

I *Gomphrena celosioides*……...78

II *Vernonia perrottetii*……...84

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Sexually transmitted diseases (STDs) are a group of infectious or communicable diseases in which the primary mode of transmission is through sexual contact (Gilson and Mindel, 2001). They are among the major causes of illnesses in the world especially in the developing countries (WHO, 2001a). More than 25 infectious organisms are transmitted primarily through sexual activity and studies reveal that STDs are among the many related factors that affect the broad continuum of reproductive health (Schafer, 2006).

The staphylococci are one of the most common bacterial organisms found apparently in clean environment. Staphylococci are the causative agents of many opportunistic human and animal infections, and are considered among the most important pathogens isolated in clinical microbiology laboratory (Fidalgo *et al*., 1990). Bacterial components and exoproducts that promote staphylococcal virulence are numerous (Projan and Novick 2000), and it has been difficult to identify the role that individual virulence determinants play in the pathogenic process. Coagulase-negative staphylococci (CNS) represent the majority of the species and are considered to be saprophytic or potentially pathogenic. Several species of the CNS have been involved in nosocomial infections related to implanted medical devices such as intravenous catheters, prosthetic heart valves and orthopedic implants. The species that most frequently cause diseases are *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*. Other significant opportunistic pathogens include *Staphylococcus hominis, Staphylococcus*

warneri, *Staphylococcus capitis*, *Staphylococcus simulans*, *Staphylococcus cohnii*, *Staphylococcus xylosus*, *Staphylococcus saccharolyticus* and *Staphylococcus iugdunensis* (Shuttleworth *et al*., 1997, Calvo *et al*., 2000).

Staphylococcus aureus is an opportunistic bacterial pathogen that can infect, replicate and persist in diverse hosts, including humans and domestic animals of economic importance (Waldvogel, 2000). Though present on the skin of only 5-20% of healthy individuals, as many as 40% of individuals carry it elsewhere on their body such as throat, vaginal or rectum for varying periods of time, from hours to years without developing symptoms (Amir, 2002). It is sometimes found in breast tissue, the mouth, the genital, urinary, and upper respiratory tracts (Bradley, 2002). *Staphylococcus aureus* exists on the skin or inside the nostrils of 20 to 30 percent of healthy people (Bradley, 2002). They are usually harmless; however, when an injury or a break in the skin enables the organisms to invade the body and overcome the body's natural defenses, consequences can range from minor discomfort to death (Cabell *et al*., 2002).

Similarly, vaginal candidiasis is one of the most common types of vaginal infection (Nwankwo *et al*., 2010). It was first identified in the 1950's, as candidiasis or moniliasis and was estimated that over 85% of the American population have an overgrowth of *Candida* that is raging out of control (Jacqueline and Bettina, 2010). Vaginal candidiasis is an inflammatory condition caused by yeast, predominantly *Candida albicans* (Sobel, 2007; Akah *et al*., 2010). Nevertheless, over the last decades, there have been reports demonstrating an increase in the frequency of cases caused by non-*albicans* species with *Candida glabrata* consistently being the lead species after *C. albicans* (Nwonkwo *et al*., 2010). *Candida albicans* is said to be responsible for 90% of cases of infectious vaginitis (Adad *et al*., 2008). Vaginal candidiasis is a common

gynaecological finding among women worldwide, and up to 75% of the sexually-active women have at least, at a time experience symptomatic vaginal candidiasis (Jombo *et al*., 2010). It is the fourth most common cause of nosocomial blood stream infection in the United States (Pappas *et al*., 2009). New data indicate that the relative proportions of organisms causing nosocomial bloodstream infections have changed over the last decade, with *Candida* species now firmly established as one of the most frequent agents (Iikit and Guzed, 2011). It is a part of the lower genital flora in 20- 50% of healthy asymptomatic women (McClelland *et al*., 2009; Akah *et al*., 2010).The symptoms manifest in many ways, and it is now postulated that vaginal candidiasis in humans has increased at an alarming rate in the last 20 years, mainly among immuno-compromised individuals (Duerr *et al*., 2003; Reed *et al*., 2003; Akah *et al*., 2010). These conditions result in severe genital itching, vaginal odour and severe irritation with discharge. Occasionally, there may be no discharge or there may be discharge without inflammation. The problem of vaginal discharge is probably the most frequently narrated complaint of women of reproductive age (Nwankwo *et al*., 2010). All women experience vaginitis at least once in their lifetime, commonest organisms implicated being *Candida albicans*, and the predisposing factors include; prolonged or repeated use of antibiotics (Singh, 2003), steroid hormones medication, hormone replacement therapy (HRT), contraceptives with high estrogen content (Akah *et al*., 2010), pregnancy, diabetes mellitus (De leon *et al*., 2002; Donders, 2002), and changes in mucus lining of the vagina encourages *Candida* to flourish (Akah *et al*., 2010 and Jombo *et a*l., 2010). Less reported risk factors include use of sponge, intrauterine devices (IUDS), diaghragms, condoms, orogenital sex, douching, intercourse (Reed *et al*., 2003; Mardh, 2004) and diet with high glucose content (De leon *et al*., 2002; Akah *et al*., 2010). *Candida albicans* is believed to be present in the intestines, mouth and

vagina and its activities are normally kept under control by friendly bacteria in the body. However, when there is imbalance in the suppressive factor of *C. albicans*, due to low pH status of the vaginal fluid and antibiotic usage among other factors, the *Candida* yeast grows causing discomfort and an intense vaginal itch (Akah *et al*., 2010).

Herbal medicine is the oldest form of health care known to mankind and over 50% of all modern clinical drugs are of natural products origin. Natural products play important roles in drug development in the pharmaceutical industry (Preethi *et al*., 2010). Historically, plants have provided a good source of anti-infective agents (Erdemeier *et al*., 1996; Abo *et al*., 2000).

The global demand of herbal medicines is increasing rapidly because of their higher safety margin and low cost (Musyimi *et al*., 2008). In developing countries, medicinal plants have been the most accessible source of medicaments and in rural areas, traditional medicine is part of the first line of treatment for common pathologies (Schuster, 2007). The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Shariff, 2001). There is therefore the need to search for plants of medicinal value.

Africa has a long and impressive list of medicinal plants (Dickson *et al.*, 2006) and many African plants are used in traditional medicine as antimicrobial agents but only a few have been documented (Gorinstein and Arruda, 1991; Bellomaria and Kacou, 1995; Elvin- Lewis and Lewis, 1995). The discovery of the connection between plant and

health is responsible for launching a new generation of multi- component botanical drugs, dietary supplements and plant-produced recombinant proteins (Raskin *et al.*, 2002; Akinjogunla *et al*., 2010). Plant-derived medicines are widely used because they are relatively safer than synthetic alternatives and are easily available and cheaper (Iwu *et al*., 1999). Natural products of higher plants may give new source of antimicrobial agents with novel mechanisms of action (Sofowora, 1993; Bhat and Jacobs, 2009). Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity, and are also known to play an important role in the modern medicine (Bhat and Jacobs, 2009). It is well known that even the most potent synthetic drugs have their origin from plant products (Sofowora, 1993). Recently, scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant-derived drugs and raising concern about the side effects of modern medicine.

In recent years, drug resistance to human pathogenic bacteria has been reported from all over the world (Piddock and Wise, 1989; Singh *et al*., 1992; Mulligen *et al*., 1993; Davis, *et al*., 1997; Robin *et al.*, 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and other parasites. Over 60% of people in Nigerian rural areas depend on traditional medicine for treatment of their ailments (Ghani *et al*., 1989). Medicinal plants are believed to be a potential source for the discovery of new drug candidates (Mohajer *et al.*, 2006; Dey *et al*., 2010; Roy and Banerjee, 2010; Kayode and Kayode, 2011). A number of active compound classes like alkaloids, terpens, flavonoids, glycosides, lignins, phenolics, saponins, etc, have been used in the modern system of medicines for

their wide therapeutic activities (Saadabi *et al*., 2006; Mukherjee *et al*., 2009; Agrawal *et al*., 2011; Gantait *et al*., 2011; Sohail *et al*., 2011).

The development of synthetic drugs from petroleum products caused a decline in the pre-emergence of drugs from live plant sources (Akinjobi *et al*., 2004). But with the emerged trend of high percentage resistance of microorganisms to present day antibiotics (Arias and Murray, 2009), efforts have been intensified by researchers towards a search for more potent antimicrobial agents. The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha *et al*., 2006).

The Amaranthaceae family comprises many species with biological activities, which are used in nutrition and alternative medicine (Ahmad *et al*., 1998). This family includes approximately 65 genera and 1000 species and many species of Gomphreneae tribe have shown antimicrobial activity, such as *Blutaparon portulacoides* (Salvador *et al*., 2002); *Gomphrena martiana*, *G. boliviana* (Pomilio *et al*.,1992; Pomilio *et al*.,1994). *Gomphrena celosioides* belongs to the Amaranthacea family and over 120 species of the family are found in America, Australia and Indo-Malaysia, while 46 species occur in Brazil. Few species occur in the East and West of Africa (Vieira *et al*., 1994). *G. celosioides* is an annual documbent ascending herb up to 30cm tall, with branches cloths with shaggy white hairs, and used as antimalarial against *Plasmodium falciparum* in traditional medicine system of Ghana (Kohler *et al*., 2002). Alcohol extracts of *G. celosioides* is reported to be diuretic (Dhawan *et al*., 1997) and antimicrobial (De Moura *et al*., 2004). In South America, some species of *G. celosioides* are employed in the treatment of bronchial infections, diarrhea and malaria fever, while others had found

application as analgesic, tonic/carminative, diuretics (Gessler *et al.*, 1994; Vieira *et al.*, 1994) and abortifacient (Burkill, 1985). A decoction of the whole plant and a related species *Gomphrena globosa* is applied to gangrenous wound. *G. martiana* and *G. boliviana* are employed as antimicrobial agents by the natives of Nupe (Dosumu *et al*., 2010). *G. celosioides* is used in ethno-medical practice in Nigeria for treatment of various skin diseases, worm infections and infectious diseases (Vieira *et al.*, 1994)

Vernonia perrottetii, called 'Burzu' in Hausa, 'Doko' chintara in Nupe, is a herb, annual with branched stem attaining a height of 60cm, it belongs to the family Asteraceae (Compositeae). Its leaves are linear, long and slender about 3cm long. A decoction of the whole plants with *S. madagascariensis* and *P. thonningii* is used as a fever remedy (Abdullahi *et al*., 2003).

1.2 Statement of the Research Problem

About 75% of adult women have at least one episode of vulvovaginal candidiasis (VVC) during their life time, with prevalence of *C. albicans* in 70- 90% (Chong *et al*., 2003). *Candida* spp causes 20-25% of VVC cases whereas 40-50% cases are caused by bacteria (Sobel, 1997). *Candida albicans* is notorious for causing candidiasis. It causes a variety of infections that range from non-life threatening to life threatening diseases. *Candida* infections face a number of problems including ineffective antifungal agents, toxicity of the available antifungal agent, and, resistance of *Candida* to commonly used antifungals, relapse of *Candida* infections and non-cost effective antifungal agents (Casidharan *et al*., 2008). It accounts for about 17%-39% of vaginitis (American College of Obstericians and Gynaecology, 2006). Higher proportions of non- *C. albicans* have been isolated from women with recurrent and complicated VVC

(Anchkar and Fries, 2010). Recent epidemiological data revealed a mycological shift from *C. albicans* to non- *C. albicans. Candida* spp such as *C. glabrata, C. parasiplosis*, *C. tropicalis* and *C. krusei* (Beck-saque and Javis, 1993; Wingard *et al*., 1993). *C. glabrat*a infections are difficult to treat and are often resistant to many azole antifungal agents, especially fluconazole (Hitchcock *et al*., 1993).

Vaginal candidiasis is a gynaecological disorder that affects millions of women and can cause great discomfort affecting sexual and job performance and is considered an important problem of world public health (Corsello *et al*., 2003). It is treatable and mild but when left untreated, is a possible risk for acquisition of HIV/AIDS as well as other complications (Kenneth, 2003). Other complications include pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, menstrual disorders, spontaneous abortion and premature birth (Abebe *et al*., 2001; FMOH, 2005). Management of treatment with vaginal infections is often difficult because of the few available therapeutic options, and furthermore, cross- resistance of vaginal *C. albicans* has been detected to Itraconazole and Fluconazole, which are the antifungal choice of drugs used for this pathology (Corsello *et al*., 2003; Sojakowa *et al*., 2004). *Candida albicans* and other *Candida* species had been isolated from several clinical specimens from different parts of Nigeria (Donbraye-Emmanuel *et al*., 2010) and different parts of the world (Adad *et al*., 2008; Choudhry *et al*., 2011; Hedayati and Shafiei, 2010). *Candida* species has also been reported among immunocompromised patients with vaginitis and secondary to haematogenous spread.

A marked decline in bacterial sexually-transmitted infections (STIs), resulting in an apparent increase in other microbial STIs has been observed in previous studies by several authors. This has been reported from different regions of India (Kumar and

Chandrashedkar, 2011; Ray *et al*., 2006). Bacterial vaginosis is currently the most prevalent cause of infectious vaginitis among women seeking for medical service for genitourinary disease (Akah *et al*., 2010). It accounts for 10-30% cases of infectious vaginitis in women of child bearing age. *Staphylococcus aureus* has been reported by many workers to have developed resistance to most antibiotic. The ease of transmission and wide spread of *S. aureus*, a normal flora of the anterior nares and skin of individual leads to colonization and infect both healthy, immunologically competent people in the community. The high propensity to colonize skin causes wound infection, furuncles, carbuncles and bullous impetigo, through locally invasive diseases such as cellutis, osteomylitis, sinusitis and pneumonia to major life threatening septicemia and meningitis. Sexually transmitted disease in the body weakens immunity of the victim which has rendered many marriages childless and consequently led to separation and divorce (Marieb and Elaine, 1997). Bacterial vaginosis, when left untreated is a possible cause of increase in the risk of pelvic inflammatory disease (infection of the fallopian tube that leads from the ovaries to the uterus). Many works have been done with so many medicinal plants, herbs and especially with plant species of the present study such as *Gomphrena matiana*, *Vernonia amydalina* and *V. globosa*. However, little its known about *V. perrottetii* as well as it combination to the best of my knowledge.

1.3 Justification for the Research Problem

Despite the increase in fungal and bacterial infections, therapeutic options are very limited and are often unsatisfactory because of elevated toxicity and inability to eradicate infections, and increase in resistance to antifungal agents including Amphotericin B (AmB), fluconazole and itraconazole by many fungi and resistance to antibacterial agents such as methicillin, penicillin and vancomycin (Sheehan *et al.* 1999,

Masoko and Eloff, 2005). This therefore, highlights the need for search of new antimicrobial agents with potent and broad-spectrum fungicidal and bactericidal activities for effective management of these infections (Franklin and Snow, 1989; Graybill, 1989; Prescott *et al.,* 2002). Due to increasing development of drug resistance in human pathogens as well as appearance of undesirable effects of certain antimicrobial agents, there is need to search for new antimicrobials without toxicity and side effects. The discovery of antibiotics was thought to bring an end to microbe originated diseases (Anand *et al*., 2006). This was proved wrong as a result of continuous resistance of pathogens to these chemotherapeutic agents, therefore, it is necessary to search for more effective and less toxic novel antimicrobial agents that will overcome these disadvantages. Various factors have contributed to continuous incidence of microbial resistance to antibiotics (Uzair *et al*., 2008). Among these are indiscriminate use of antibiotics and horizontal transfer of resistance gene between bacteria and fungi. Worldwide increase in resistance to antibiotics has prompted scientists and other researchers to seek for other possible potential antimicrobials (Mahesh and Satish, 2008). Due to this search, plants have been seen as a good source of antimicrobials (Del Campo *et al*., 2000). In order to alleviate the problem of reduced availability of drugs needed to treat vaginitis, traditional medicines derived from plants are still being used in different parts of the world. Hence, the combination of *Gomphrena celosioides* and *Vernonia perrottetii*.

Medicinal plants are renewable in nature unlike the synthetic drugs that are obtained from non-renewable sources of basic raw materials such as fossil sources and petrochemicals (Samanta *et al*., 2010). Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases; this

practice being an alternative ways to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1993). Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. But now with the upsurge in the use of herbal medicine, a thorough scientific investigation of the plants will go a long way in validating their folkloric usage (Sofowora, 1993). In folklore, herbal preparation is made from cocktail of plant extract for the use in the treatment of several ailments. However, there is limited scientific evidence regarding the use of the combination of medicinal plants and the additive or synergistic effects of their use. This research would therefore help to elucidate the additive/synergistic effects of these two selected medicinal plants as to their antimicrobial effects.

In spite of these infections, there is inadequate statistical data on the prevalence of STDs in women in Zaria, Kaduna State, Nigeria. The research would provide a source of relative revenue to the economy and also the potential source of drugs. The results obtained would also provide more information about the treatment of vaginal infections caused by bacterial and fungal colonization using combination of plant extracts, which would be useful for drug formulation using organic plant source.

1.3 Aim of the study

The aim of this study was to determine vulvovaginitis associated with *Staphylococcus* spp and *Candida* spp in women attending some Hospitals in Zaria and the antimicrobial effect of two selected plant extracts.

1.5 Objectives of the study

1. To determine the prevalence of vaginitis caused by *Staphylococcus* species and *Candida* species among women attending Ahmadu Bello University Health Care Services and Samaru Clinic, Zaria, Nigeria
2. To determine the phytochemical constituents of aqueous and methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottetii*
3. To isolate, identify and characterize *Candida* species and *Staphylococcus* species among women attending Ahmadu Bello University Health Service and Samaru Clinic, Zaria, Nigeria
4. To determine the susceptibility pattern of isolated *Candida* species and *Staphylococcus* species to aqueous and methanolic extracts of *V. perrottetii* and *Gomphrena celosioides*
5. To determine the antimicrobial effect of the combination of aqueous and methanolic extracts of *Gomphrena Celosioides* and *Vernonia perrottetii* against the *Candida* species and *Staphylococcus* species.

1.6 Hypotheses of the research

1. Prevalence of *Staphylococcus* species and *Candida* species are not significant.
2. There are no *Candida* spp and *Staphylococcus* spp among women attending University Health Service and Samaru Clinic in Zaria, Nigeria
3. There are no secondary metabolites present in the plant extracts
4. The isolated *Candida* species and *Staphylococcus* species are not susceptible to the aqueous and methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottetii*.

5. There is no difference in the susceptibility of the micro-organisms when exposed to the extracts either singly or in combination.

CHAPTER TWO

2.0 LITRATURE REVIEW

2.1 Prevalence of Vulvovaginitis

The reported prevalence of bacterial vaginosis varies widely from 5 to 51% between different populations. In the United States, Bump and Buesching (1988) reported a prevalence of approximately 13% among adolescent girls. The incidence is higher in women undergoing termination of pregnancy (28%) (Blackwell *et al*., 1993) and in a group of women having *in vitro* fertilization treatment (24.6%) (Ralph *et al*., 1999). In the United States, a high incidence was reported for some populations, e.g., inner-city pregnant women (32.5%) (McGregor *et al*., 1995). The highest incidence, however, was reported from Rakai in rural Uganda, where 50.9% of women had bacterial vaginosis, along with prevalence for *Trichomonas vaginalis* of 23.8% (Paxton *et al*., 1998) and 80% of these women were asymptomatic. Vulvovaginal candidiasis (VVC) is a common problem in women and may affect their physical and emotional health as well as with their partners (Chapel *et al*., 2000). *Candida* species cause 20 to 25% of VV cases, whereas 40 to 50% of VV cases are by bacteria (Sobel, 1997).

VVC is characterized by pruritus, soreness and changes in discharges, dyspareunia, vulvar erythema, oedema, and fissures (Fischer and Addison, 1985). The condition is rare before puberty but by the age of 25 years, nearly 50% of all women will have had at least one clinically diagnosed episode of VVC (Geiger *et al*., 1995; Geiger and Foxman, 1996). The condition is less common in postmenopausal women. It is estimated that 75% of all women experience an episode of VVC in their lifetime of which about half have at least one recurrence (Sobel *et al*., 2001). *Candida albicans* is

responsible for 78.75% of all cases of VVC in Shiraz, South-West of Iran, (Pakshir *et al*., 2007) and 80 to 92% of all cases of VVC worldwide (Sobel, 1997).

This is very difficult to gauge as many women self-treat using over-the-counter (OTC) medication. A Swedish survey of OTC and prescribed antifungal preparations for vaginal candidiasis in the mid-1990s showed about 85-90% cases per 1,000 women in the age group of 15-45 year Post-marketing surveillance of women prescribed quinolone and related antibiotics revealed an incidence of around 600 cases per 100,000 women (Mardh, 2004). The control population had about 150 cases per 100,000 and this is undoubtedly common and estimated to affect about 75% of women in their reproductive years (Mitchell, 2007). About 10-20% of women have asymptomatic vaginal colonization with *Candida* spp however, peak incidence age is 20-40 years (Wilton *et al*., 2003).

In the incidence of vulvovaginal candidiasis, mostly *C. albicans*, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non-pregnant women at any single point in time and in up to 70% if followed longitudinally over a year period (Bauters *et al*., 2002; Beigi *et al*., 2004). If the balance between colonization and the host is temporarily disturbed, *Candida* can cause disease such as VVC, which is associated with clinical signs of inflammation. Such episodes can happen sporadically or often can be attributed to the presence of a known risk factor, e.g., the disturbance of local microbial flora by antibiotic use.

VVC is often diagnosed without confirmatory tests and treated with over-the-counter (OTC) medications, and thus the exact incidence is unknown. It is reported that 55% female of Midwestern university students had at least one episode of VVC by age of 25

years (Geiger *et al*., 1995). Chong and co-workers, 2003 estimated that 56% of women throughout the United States will experience at least one episode of VVC during their lifetime and that 8% of women experience recurrent vulvaginal candidiasis (Foxman, 1990).

Many attributes contribute to *C. albicans* virulence; among them are adhesion, hyphal formation, phenotypic switching (PS), extracellular hydrolytic enzyme production, and biofilm formation. There has also been a steady increase in the incidence of non-*C. albicans* strains such as *Candida*-related urinary isolates by *C. glabrata*, and *C. tropicalis*, which is the third most common species (De Francesco *et al*., 2007; Xess *et al*., 2007; Horn *et al*., 2009). In neonates, *C. parapsilosis* has become a dominant fungal species that is associated with candidiasis, including candiduria. In a large multicenter study from Spain, *C. albicans* was recovered in 68%, followed by *C. glabrata* (8%) and *C. tropicalis* (4%) (Alvarez-Lerma *et al*., 2003). *C. albicans* was also recovered in 54% of the cases, followed by *C. glabrata* (36%) and *C. tropicalis* (10%) (Jain *et al*., 2007). Other studies reported that 11 to 30% of nosocomial urinary tract infections (UTIs) are caused by *Candida* (Richards *et al*., 2000; Lundstrom and Sobel, 2001).

In the National Nosocomial Infections surveillance done between 1992 and 1997 in 112 intensive care units (ICUs) across the United States, *C. albicans* was the most commonly reported pathogen (including bacteria) from urine (21%), constituting more than half of the fungal isolates (Alvarez-lerma *et al*., 2003). *C. albicans* was also more commonly reported in catheter-associated UTIs than in non-catheter-associated infections (21% versus 13%, $P = 0.009$). Fungal urinary infections occurred more frequently in patients with urinary catheters than in those without urinary catheters (40% versus 22%, $P < 0.001$) (Richards *et al*., 2000).

In the United States, Europe and Australia, *C. albicans* is the most common species identified in women with VVC (76 to 89%), followed by *C. glabrata* (7 to 16%) (Corsello *et al*., 2003; Holland *et al*., 2003; Richter *et al*., 2005; Vermitsky *et al*., 2008).

The overall percentage of non-*C. albicans* species associated with VVC in these countries/continents ranges from 24% to 11%. Some studies have reported an increasing trend in the occurrence of non-*C. albicans* species over the time (Spinillo *et al*., 1997), while a recent U S study of over 90,000 samples found a consistent yearly distribution from 2003 to 2007 (Vermitsky *et al*., 2008). In contrast to the case in the United States, Europe and Australia, non-*C.albicans* species, in particular *C. glabrata*, appear to be more commonly associated with VVC in some Asian and African countries (Okulicz *et al*., 2011; Helbig *et al*., 2009). In Turkey, India and Nigeria, cases due to *C. glabrata* range between 30 to 37% while the *Candida* spp distribution in China resembles closely to the one in the United States (Jain *et al*., 2007).

Higher percentages of non-*C .albicans* species have been isolated in women with recurrent vulvovagina candidiasis (RVVC) than in women with VVC (40 to 32% versus 20 to 11%, respectively) (Spinillo *et al*., 1997; Holland *et al*., 2003; Richter *et al*., 2005). Other populations with higher rates of non- *C. albicans* species include HIV-infected women (Fidel *et al*., 1999). Interestingly, higher percentages of non- *C. albicans* species are associated with increasing age in women with VVC (Holland *et al*., 2003; Vermitsky *et al*., 2008). In all of these populations, *C. glabrata* is the most common among the non-*C. albicans* species. The data obtained highlight the importance of determining *Candida* spp and susceptibilities in women at high risk for non-*C. albicans* VVC in order to provide effective therapy. Again, in most studies *C. albicans* dominates and accounts for 50% to 70% of all *Candida*-related urinary

isolates, followed by *C. glabrata* and *C. tropicalis*, which is the third most common species. There has also been a steady increase in the incidence of non-*C. albicans* strains producing nosocomial infections (DeFrancesco *et al*., 2007; Horn *et al*., 2009; Xess *et al*., 2007). In neonates, *C. parapsilosis* has become a dominant fungal species that is associated with candidiasis, including candiduria (Linder, 2008**;** Trofa *et al*., 2007). An increased incidence of VVC was found in African-American compared to white American women in two different population-based studies (Foxman *et al*., 2000). Furthermore, increased incidence of blood group ABO-Lewis nonsecretor phenotype was found in women with RVVC compared to the controls (Chaim *et al*., 1997), and more recently, polymorphism in the mannose-binding lectin gene was found to be associated with RVVC (Babula *et al*., 2003; Giraldo *et al*., 2007). HIV positive women have higher rates of vaginal colonization with *Candida*, often non-*C. albicans* species, than HIV-negative women (Fidel, 1999; Duerr *et al*., 2003). Similar to other genital diseases that cause damage of the mucosa, VVC has been associated with increased vaginal HIV shedding (Wang *et al*., 2009) and in a recent meta-analysis was found to be associated with a 2-fold increase in the risk of HIV acquisition (Rottingen *et al*., 2001).

2.2 Factors Associated with Vulvovaginitis

2.2.1 Immunological factors assosciated with vulvovaginitis

The state of commensalisms is temporarily disturbed when *Candida* causes diseases such as Vulvovaginal Candidiasis. While in Recurrent Vulvovaginal Candidiasis, this balance may be more permanently disturbed. With respect to genitourinary candidiasis, there is an impressive body of literature on host immune responses to VVC (Cassone *et al*., 2007; Fidel, 2007). The infective organism is a fungus that reproduces by budding.

About 90% of vaginal infections are due to *Candida albicans* and 5% are due to *Candida glabrata* (Sobel, 2007).

Other fungal infections of the vagina are caused by *Saccharomyces cerevisiae* (brewer's yeast) and, rarely *Trichosporon* spp. *Candida* is a normal commensal organism in the vagina. Pathological infection usually follows a change in the local environment or a decrease in the host's susceptibility to infection. However, recent research suggests that symptomatic candidiasis is due to an exaggerated immunological response to the presence of *Candida*, rather than a failure of immune mechanisms (Fidel, 2007)

In primary, the host response to the presence of *Candida* in the vagina determines whether or not a woman is symptomatic (Sobel *et al*., 1998 and Fidel, 2004). Fidel. (2004) hypothesised that women with recurrent vulvovaginal candidiasis have an impaired host organism interaction in the vagina such as; an organ specific and antigen specific abnormality. A recent intravaginal *Candida* challenge study has cast doubt on this theory. Fidel. (2004) now proposes that symptoms relating to the presence of *Candida* in the vagina are due to an infiltration of polymorphonuclear neutrophils. That is, symptoms of Candidal infection are not the result of a failing by the woman's immune system, but rather 'an aggressive innate response (Fidel, 2004). Two theories have been proposed to explain the recurrence of vulvovaginal candidiasis post-treatment – relapse and reinfection. Relapse is the favoured hypotheses by those that have shown sequential episodes of recurrent vulvovaginal candidiasis and are caused by an identical strain type of *C. albicans*. Vazquez *et al*. (1994) in a retrospective review, found eight out of 10 women with recurrent vulvovaginal candidiasis over a mean of 3.1 years consistently demonstrated the same *C. albicans* strain.

In secondary vulvovaginal candidiasis, either host or microbial factors are considered the causative factor for the condition. Host factors include the use of antibacterials or systemic corticosteroids and conditions affecting a patient's immunologic status, e g., uncontrolled diabetes, lupus, thyroid disease and human immunodeficiency virus (HIV). Microbial factors chiefly consist of non- *C. albicans*, most commonly *C. glabrata*. Behavioural factors hypothesised to trigger episodes of vulvovaginal candidiasis include sexual practices, clothing habits and diet (Cathy and Marie, 2011). Conflicting results have been reported and it is difficult to design a study that can demonstrate behavioural factors contributing to vulvovaginal candidiasis. In a recent study where several behavioural factors were associated with recurrence of symptoms in women with recurrent vulvovaginal candidiasis, the authors advised caution in extrapolating the results due to study design limitations (Patel *et al*., 2004). Behavioural risk factors that have been significantly associated with a higher incidence of VVC include frequent sexual intercourse and receptive oral sex as well as the use of high-estrogen (not low-dose) oral contraceptives, condoms and spermicides (Eckert *et al*., 1998; Cetin *et al*., 2007). Among university students, tight clothing and type of underwear were not associated with VVC (Foxman, 1990), while among women with RVVC, the use of panty liners or pantyhose was positively associated with symptomatic recurrence (Patel *et al*., 2004).

2.2.2 Risk factors associated with vulvovaginitis

Host-related risk factors that have been significantly associated with VVC and RVVC include antibiotic use, uncontrolled diabetes, conditions with high reproductive hormone levels and genetic predispositions (Goswami *et al*., 2000; Sobel, 2007). Antibiotics alter the bacterial microflora of the vaginal and gastrointestinal tracts and

thus allow for overgrowth of *Candida* spp. After antibiotic use, the increase in vaginal colonization with *Candida* spp, mostly *C. albicans*, was estimated to range from 10 to 30%, and VVC occurs in 28 to 33% of cases (Sobel, 2007). It is commonly hypothesized that the reduction of lactobacilli in the vaginal tract predisposes women to VVC. Lactobacilli play a key role in the vaginal flora through the production of hydrogen peroxide, bacteriocins and lactic acid, which protect against invasion or overgrowth of pathogenic species (Ronnqvist *et al*., 2006). However, studies have failed to provide evidence that an altered or abnormal vaginal bacterial flora predisposes women to recurrent episodes of VVC in the absence of antibiotics intake (Vitali *et al*., 2007; Zhou *et al*., 2009). Prospective study demonstrated that vaginal *Lactobacillus* colonization was associated with a nearly 4-fold increase in the likelihood of symptomatic VVC (McClelland *et al*., 2009). Episodes of VVC occur mostly during childbearing age and are rare in premenarchal and postmenopausal women. An increased frequency of VVC has been reported during the premenstrual week (Eckert, 1998) and during pregnancy (Cotch *et al*., 1998).

2.3 Causes of Vaginitis

The exact incidence of vaginitis is unknown, but it is estimated that most women experience at least one episode in their lifetime. The associated distress and discomfort often lead women to self-diagnose vaginitis and use over-the-counter preparations improperly. The three most common causes of vaginitis include bacterial vaginosis, Candidal vulvovaginitis and *Trichomonas* vaginitis.

Natural protective mechanisms and factors associated with susceptibility to infection have remained elusive. Until recently when through a live challenge model in humans,

it was revealed that protection against vaginitis coincides with a non inflammatory innate presence, whereas symptomatic infection correlates with a neutrophil infilteration in the vaginal lumen and elevated fungal burden. Thus, instead of VVC being caused by a putative deficient adaptive immune response, it is now being considered that symptomatic vaginitis is caused by an aggressive innate response. Bacteria, yeast, viruses, chemicals in creams or sprays, or even clothing can cause vaginitis (Sobel *et al*., 1998). Sometimes, vaginitis occurs from organisms that are passed between sexual partners. In addition, the vaginal environment is influenced by a number of different factors including a woman's health, personal hygiene, medications, hormones (particularly estrogen) and the health of her sexual partner; a disturbance in any of these factors can trigger vaginitis. Less than 5% of healthy women are affected by this condition. Pathogenetic factors contributing to Recurrent Vulvovaginal Candidiasis include infection with *C. glabrata* and other non-*albicans Candida* species, persistence of *C. albicans* due to inadequate treatment, diabetes mellitus and impaired glucose tolerance, recent antibiotic use, estrogen use, immunosuppressive therapy and behavioural factors (Fidel, 2004). Behavioural factors associated with RVVC include vaginal douching as well as contraceptive practices associated with a higher risk of candidiasis such as diaphragm use, spermicidal use and intrauterine devices (Fidel, 2004). Sexual intercourse has not been associated with an increased incidence of *Candida* colonization. Hypotheses regarding *Lactobacillus* deficiency in women with RVVC have not been substantiated. Most women have a vaginal yeast infection at some time. *Candida albicans* is a common type of fungus often found in small amounts in the vagina, mouth, digestive tract and on the skin. Usually it does not cause disease or symptoms (Biggs and Williams, 2009). *Candida* and many other germs that normally live in the vagina keep each other in balance. However, sometimes the number of

Candida albicans increases, leading to a yeast infection (Eckert *et al*., 2007). Apart from the nuisance and discomfort of vaginal irritation, anal pruritus and in some patients, dyspareunia and dysuria (Noble, 1982), vaginal candidiasis in recent times has taken on new dimensions in importance. This is because it can lead to placental candidiasis during pregnancy, either as an isolated infection or in association with congenital cutaneous and/or systemic candidiasis in the newborn (Noble, 1982). The source of infection in vaginal candidiasis is a problem of some importance with the gastrointestinal tract being the most important focus of dissemination (Warrick *et al* 1986; Opaneye 1999).

2.4 Pathophysiology of Vaginitis

Candida is commonly present on normal human skin and in areas of moisture such as the mouth and vagina. It is estimated that about 20% to 50% of healthy women normally carry yeast in the vaginal area. Vaginitis can be caused by a number of infectious as well as noninfectious agents such as trauma or chemical irritation. Infectious vaginitis has numerous causes including bacteria such as *Gardnerella* and *Neisseria gonorrhae*, Protozoans such as *Trichomonas* and yeast (*Candida*). Vaginal yeast infection, which is the most common form of vaginitis, is often referred to as Vaginal Candidiasis. *Candida* infections of the latter category are also referred to as Candidemia and are usually confined to severely immunocompromised person, such as cancer, transplant, and AIDS patients as well as non trauma emergency surgery patients (Kourkoumpetis *et al*., 2010). Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are common in many human populations (Fidel, 2004; Pappas, 2006). While clearly attributable to the presence of

the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes (Fidel, 2004).

At menarche, under the influence of estrogen, stratified squamous epithelium develops in the vagina. Lactobacilli become the dominant organisms. The source of the vaginal lactobacilli in an individual woman has not been determined. Lactic acid is produced by both bacterial metabolism and that of the epithelium, and the vaginal pH falls to a level usually between 4.0 and 4.5. Physiological discharge consists of mucus, desquamated epithelial cells and lactobacilli. The pH may rise above 4.5 at the time of menstruation, when the concentration of lactobacilli is reduced. Cervical mucus and semen have pH between 7 and 8. In mice, exogenous treatment with progesterone alters the flora to one that resembles bacterial vaginosis (Taylor-Robinson and Hay, 1997).

If bacterial vaginosis develops, the pH rises to a level between 4.5 and 7.0. The anaerobic or facultative anaerobic organisms, which are usually present in low numbers increase between 100 and 1000 fold, to considerably outnumber the lactobacilli, which may eventually disappear. Trimethylamine and the polyamines putrescine and cadaverine are produced by anaerobic metabolism, and they are thought to be responsible for the fishy smell. Microscopy of vaginal fluid shows multiple small bacterial and epithelial cells with large numbers of adherent bacteria, which are called clue cells as they give a clue to the diagnosis of nonspecific vaginitis (Gardner and Dukes, 1995).

2.5 Diagnosis of Vaginitis

Nonspecific vaginitis has been defined as a clinical entity recognizable from the symptoms of a fishy-smelling vaginal discharge confirmed by detecting thin

homogenous vaginal fluid adherent to the walls of the vagina and confirmed by finding clue cells on microscopy. Initially, it was thought to be a straightforward infection by one organism, now called *Gardnerella vaginalis.* Subsequently, other bacteria were identified as part of the bacterial vaginosis flora. In 1983, the term bacterial vaginosis (BV) was coined, with the recognition that there are many bacterial species contributing to the condition and that inflammation is usually absent. The KOH or "Whiff" test was added as a fourth criterion for the diagnosis of vaginosis (Sobel *et al*., 1998).

Scoring systems for interpreting Gram-stained vaginal smears have been used to diagnose BV (Nugent *et al*., 1991; Hay *et al*., 1994). Self-administered vaginal swabs can be used, making noninvasive screening possible. They are smeared on a glass slide, which is air dried and subsequently read in a central laboratory. The composite criteria define a dichotomy of BV or normal flora. Scoring systems for interpreting Gram-stained smears allow a gradation to accommodate intermediate patterns. Due to the nonspecific nature of the symptoms and physical examination findings, the diagnosis of vaginitis is mainly made by using a combination of vaginal pH, amine test, microscopy findings and sometimes vaginal cultures (Sobel *et al*., 1998).

2.5.1 Vaginal pH

Vaginal pH is obtained by placing a drop of the vaginal discharge on a pH strip. It must be remembered that the pH can be altered by vaginal lubricants as well as other factors such as blood or urine. Vulvovaginal candidiasis is associated with a normal vaginal pH between 4.0 and 4.5. A vaginal pH greater than 4.5 suggests infections such as bacterial vaginosis and trichomonal vaginitis (Sobel *et al*., 1998)

2.5.2 Amine test

The amine or Whiff test is done by placing a drop of the vaginal discharge on a slide and adding a drop of 10% KOH. A fishy amine odour indicates bacterial vaginosis. The odour is caused by volatilization of amines, a by-product of anaerobic metabolism. The amine test is occasionally positive with trichomoniasis (Sobel *et al*., 1998)

2.5.3 Microscopy

2.5.3.1 Saline Mount

Cells that can be seen in the saline mount include vaginal epithelial cells, rods and cocci, polymorphonuclear cells (PMNs), clue cells, motile trichomonads and candidal hyphae. Normal vaginal epithelial cells are squamous. Parabasal cells appear in postmenopausal women and are associated with atrophic vaginitis. Rods are the predominant microorganisms in normal vaginal discharge and with candidiasis. Loss of rods and increased coccobacilli occur with bacterial vaginosis and atrophic vaginitis. Abundant PMNs are seen with the purulent discharge associated with trichomoniasis. Motile trichomonads, which are slightly larger than PMNs, are only seen in 60% to 70% of culture-confirmed cases of trichomoniasis (Sobel *et al*., 1998)

Clue cells are the single most reliable predictor of bacterial vaginosis. These are epithelial cells studded with adherent coccobacilli and represent between 5% and 50% of the epithelial cells seen in bacterial vaginosis.

2.5.3.2 Potassium Hydroxide Mount

The KOH mount is particularly useful for diagnosing candidal vaginitis. Branching hyphae of *Candida albicans* can be seen.

2.5.3.3 Vaginal Cultures

Due to the low sensitivity of microscopic tests, vaginal cultures can be obtained in microscopy-negative cases. Vaginal candidiasis often manifests with a compatible history and normal pH and negative microscopy. In these cases, hypersensitivity, contact dermatitis and allergic or chemical vaginitis can be excluded by obtaining a vaginal culture. Similarly, culture techniques have a high sensitivity in *Trichomonas* vaginitis (95%) and should be considered in patients with an elevated vaginal pH, increased numbers of PMNs and absence of motile trichomonads and clue cells or when microscopy is unavailable or yields unreliable results (Sobel *et al*., 1998)

2.6 Treatment of Vaginitis

In 1990, more than 13 million American women received prescriptions for treating vaginitis, the most common reason for visiting the gynaecologist (Weisberg, 1988). Vaginitis caused by yeast species accounts for approximately 40% of these cases, second only to bacterial vaginosis 45% (CDC, 2006).

Topical azoles remain the first line of Vulvovaginal candidiasis treatment, in achieving mycologic and clinical cures with minimal systemic effects in 85% to 90% of episodic infections. Oral agents may be more convenient, but confer some risks of side effects and drug interactions. In addition, the proportion of non albican *Candida* infections caused by resistant strains may be on the rise (Workowski and Berman, 2006)

2.7 Yeast Vaginitis

Yeast infection, also called candidiasis, this condition is caused by an over abundance of *Candida*, a microscopic fungus that normally inhabits the vagina. A variety called *Candida albicans* causes the vast majority of vaginal yeast infections. This fungus, which also grows normally in the mouth and digestive tract, can infect other moist regions of the body as well, including the skin folds and nail beds. When the mouth is infected, the condition is called thrush (Workowski and Berman, 2006)

Almost 75 percent of all adult women will have a yeast infection at some point in their lives, according to the CDC, 2002 and approximately 5 percent of these women will develop a condition called recurrent vulvovaginal candidiasis (RVVC). RVVC is classified as more than three symptomatic vaginal yeast infections over the course of a year. Women who experience RVVC should notify their gynaecologist, who will attempt to identify the underlying cause of the condition. Although yeast infection is not generally considered a sexually transmitted disease, in rare instances it may be transmitted to male partners through sexual intercourse (James and Berger, 2006)

Out of more than 200 species, the most commonly encountered in medical practices are *C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis.* About 8%–15% of nosocomial blood-stream infections are reported to be caused by *Candida* spp (Pfaller *et al*., 2005). Candidal infections are a serious problem in individuals with weakened immune defense. Interestingly, *C. albicans* differs from other medically important fungi such as *Histoplama*. *capsulatum*, *Aspergillus*. *fumigatus*, and *Chryptococcus*. *Neoformans.* It is being isolated from soil. Therefore, infections caused by *candida* are categorized as endogenous and not exogenous as with

others. *C. albicans* and related species have been isolated from several body locations as a carrier in the oral cavities, gastrointestinal tract, anus, groin, vaginal canal and vulva of healthy people, and may attain sufficiently high density without symptoms of the disease (Mari and Martin, 2000). Among these, *C. albicans* was predominant at all body locations (70%), *C. glabrata* and *C. tropicalis* (7%) (Ilkit and Guzel, 2011). In normal conditions, it exists with other normal microbial flora of host organs; about 50% of a healthy population is supposed to be a benign carrier of *Candida* spp, but in immunocompromised patients who have undergone chemotherapy, bone-marrow transplantations or diabetic treatment, it behaves like an opportunistic pathogen and produces superficial to systemic infection. Broad-spectrum antibiotic therapy may also alter the population of normal bacterial flora, resulting in *Candida* spp taking over the niche and assisting in flourishing and establishing secondary infections. Oral and vaginal thrushes are very common even in individuals with slightly weakened immunity (Soll, 2002a; Fluckiger *et al*. 2006; Odds *et al*., 2006). The ability of *C. albicans* and other *Candida* spp to colonize and survive at different anatomic sites of the host has made them more harmful than other commensals of the human body. As a commensal, *Candida* resides in yeast form and multiplies by budding into blastospores, but during weakened immunity of the host, it transforms into hyphal form at the start of pathogenesis. *In-vivo* study has revealed that change in pH, oxygen, carbon dioxide or glucose concentration in host tissue triggers this transition (Cladera one and Fonzi, 2002; Haynes 2002). Filamentous forms are more adhesive due to increased expression of adhesins on the surface, and also secretion of a higher amount of hydrolytic enzyme enhances the invasiveness. Moreover, the pathogenic stage has to resist recognition by the immune system or damaging macrophages and neutrophils. This interaction with host tissue in favour of *Candida* results in deep tissue penetration and the establishment

of infections. It is speculated that host (tissue environment and immune system) alone determines the balance between commensalism and pathogenicity (Soll, 2002a; Hube and Naglik, 2004). The work of several researchers has shown that certain genes such as pH regulated PHR1 and PHR2 genes encoding secreted aspartyl proteinases (SAPs1–9) and genes encoding phospholipases (PHL A–D) are expressed differentially in specific tissues at different stages of infections (Yang 2003; Naglik *et al*. 2004). Expression or modulation of these genes on the same mucosal surfaces only during transition from the commensal to the parasitic stage reflects a weakness in the immune system responsible for this shift (Casadevall and Pirofski, 2001). *Candida* survives and proliferates as commensal in competition with other microbial flora and is affected by epithelial cell proliferation and the immune system. Proliferation of epithelial cells constitutively forces *Candida* to attempt deeper invasion into tissues. Prolonged antibiotic therapy provides more available nutrients and space for *Candida* to multiply as other commensal microbial flora are diminished (Senet, 1998). Immune suppression in HIV patients and inhibition of epithelial cell proliferation such as in cancer therapy changes the tissue environment in terms of pH, osmolarity and oxidative stress. This changed condition is perceived by the candidal cell and subsequently down- or up-regulation of certain genes provokes *Candida* to switch over from commensal to opportunistic pathogens (Claderon and Fonzi 2002; Hube and Naglik, 2004). Advanced medical equipment and surgery have also led to the increased spread of commensal *Candida* to tissues as pathogens. Medical devices such as catheter dental implants, artificial joints, pacemakers, central nervous system shunts and others have provided the opportunity to form biofilms; a stage more resistant to drugs and capable of greater invasion to tissues. These devices are easily colonized `by candidal cells from mucosal surfaces and blood stream, and frequently get spread from one tissue site to another. Furthermore, candidal

cells can also migrate via blood flow to all inner organs, leading to septicemia and life-threatening diseases (Douglas 2003; Hall-Stoodley *et al*. 2004). Biofilm-forming cells have been reported to be more virulent than planktonic cells (Ramage *et al*. 2005; Seneviratne *et al*. 2007). Recently, several workers have reported increased production of proteinases, phospholipases and adhesins in biofilms compared to planktonic cells (Chandra *et al*. 2001; Al-Fattani and Douglas 2006; Seneviratne *et al*. 2007). All these collectively aid in establishing infections by heightening the adherence and invasion of tissues, leading to increased virulence. Genetic changes in biofilms result in elevated drug resistance, pronounced quorum sensing and regulated carbohydrate synthesis, thereby influencing the pathogenicity of *Candida*. Therefore, biofilms-forming capacity has greatly increased the potency of *Candida* to convert from the commensal stage into a virulent pathogen.

2.7.1 Taxonomy and classification of vulvovaginal *Candida*

Over a decade ago, Vulvovaginal candidiasis (VVC) was classified into uncomplicated and complicated cases, a classification that has been internationally accepted and adapted (Workowski and Berman, 2006; Pappas *et al*., 2009**)**. Candidiasis or thrush is a fungal infection (mycosis) of any species from the genus *Candida* (one genus of yeasts). *Candida albicans* is the most common agent of Candidiasis in humans (Walsh and Dixon, 1996). Also commonly referred to as a yeast infection, candidiasis is also technically known as candidosis, moniliasis and oidiomycosis (James and Berger, 2006). Candidiasis may be divided into the following types;

2.7.1.1 Uncomplicated vulvovaginal candidiasis (VVC)

Uncomplicated VVC is characterized by sporadic or infrequent occurrence of mild to moderate disease caused by *C. albicans* in immunocompetent women (James and Berger, 2006).

2.7.1.2 Complicated vulvovaginal candidiasis (VVC)

Complicated VVC includes cases of severe VVC, VVC caused by non-*C. albicans* species, VVC associated with pregnancy or other concurrent conditions such as uncontrolled diabetes or immunosuppression and recurrent VVC (RVVC) in immunocompetent women (Mari and Martin, 2000)

The genus *Candida* and species *C. albicans* was described by a botanist – Christine MarieBerkhout - in her doctoral thesis at the University of Utrecht in 1923 as reported by Bodey, 1993. Over the years, the classification of the genera and species has evolved. Obsolete names for this genus include *Mycotorula* and *Torulopsis*. The species have also been known in the past as *Monilia albicans* and *Odium albicans*. The current classification is Nomen conservandum, which means the name is authorized for use by the International Botanical Congress (IBC) (Jones *et al*., 2004). The genus *Candida* includes about 150 different species. However, only a few are known to cause human infections. *C. albicans* is the most significant pathogenic species (Jones *et al*., 2004; Bishe *et al*., 2012). Yeast infections of the vulva and vagina have been referred to as vulvovaginal candidiasis (VVC).

The history of the discovery and naming of *Candida* extends from the ancient Greeks to modern day researchers. The perception of *Candida* has evolved from the presence of an exudate in the human host to a known infectious agent. Two years of medical history was recorded before the etiological agent of oral thrush, the first form of candidiasis

described, was correctly identified as a fungal pathogen. “Thrush” appears as whitish plagues within the oropharynx or the buccal mucosa or tongue. One of the main points of contention when defining thrush was whether it originated from the host or was an infectious agent, or a combination of the two. This is yeast that forms ascospores and so belongs to the Ascomycotina family. This genus is of medical importance, *C. albicans* especially. The earliest reports of thrush predated the concept of a microbial pathogen. In “Of the Epidemics,” Hippocrates described oral candidiasis (around 400 B.C.) as “mouths affected with aphthous ulcerations” (Barnett, 2004). In 1665, Pepys Diary reported “a patient hath a fever, a thrush and a hiccup” (Martin and Jones, 1940), perpetuating the idea that oral thrush originates from the host. Mycologists accepted this perception as late as the early 1900s where Castellani quoted previous accounts of thrush as “morbid secretions of the oral mucosa” (Calderone and Fonzi, 2002). However, a few clinicians and mycologists swayed popular belief towards the idea of an infectious agent causing thrush. Langenbeck, 1839 was credited with first recognizing a fungus in a patient with typhoid fever. Oropharyngeal and esophageal thrush with pseudomembranes were found at autopsy. “Under the microscope magnified, the pseudomembranes consisted of an immense number of fungi” (translated from German) (Bernharddt and Knoke, 2008). He describes in detail what is now referred to as septate hyphae, branched pseudohyphae and blastoconidia. However, he ascribed the entity to the typhoid bacterium rather than the fungus (Bernharddt and Knoke, 2008). In 1844, J.H. Bennett observed a similar fungus in the sputum and the lungs of a patient with a pneumothorax and criticized the conclusion by Lagenbach (Calderone and Fonzi, 2002). The morphologic description of Bennett was essentially that as described by Langenbeck. Bennett concluded that the disease was “indicative of great depression of the vital powers and impairment of the nutritive functions of the economy (host)”

(Calderone and Fonzi, 2002). Two years later, Berg *et al*. (1984) explicitly concluded that thrush was caused by a fungus and found that spread could occur from communal feeding bottles. Most importantly, he also stated "descriptions of the disease unsupported by demonstration of the fungus could not substantiate the diagnosis". He was able to reproduce the infection in healthy children and thereby confirmed his hypothesis that the fungus caused the infection (Calderone and Fonzi, 2002). After this discovery, other infections would be ascribed to this dimorphic fungus including vaginitis and gastrointestinal candidiasis. Once the etiology was conclusively demonstrated by mycologists, the next point of contention was the identity of the pathogen. While Langenbeck, (1839) first documented the fungus associated with thrush, he failed to make the direct connection. Charles Philippe Robin, (1847) the distinguished French mycologist, classified the fungus as *Oidium albicans* using *albicans* ("to whiten") to name the fungus causing thrush. Hill, (1993) as well as Martin and Jones (Martins and Jones, 1940) misclassified *Candida* into the genus *Monilia*, a genus containing fungi that commonly grow on plants. Subsequently, clinicians erroneously referred to the etiology of thrush as "Monilias" despite the fact mycologists had already elucidated the morphological differences between the fungus associated with thrush and the fungus in the genus *Monilia*. Christine Berkhout and others noted these differences, particularly the ability of this fungus to infect humans. Berkhout reclassified it under the current genus *Candida* in 1923 (Barnett, 2004). *Candida* is derived from Latin where toga *Candida* was a white robe worn by Roman Senators. Berkhout's taxonomy was later heralded by the prominent French mycologists, Maurice Langeron and Paul Guerra, as "…the beginning of the rational systematics of the non-ascosporogenous yeasts" (Barnett, 2004). However, it was not until 1954 that the Eighth Botanical Congress officially endorsed the binomial *Candida albicans* as the *nomen*

conservandum formally ending the 200 years long uncertainty over the etiology and taxonomy of *Candida*.

Currently, there are some 200 species within the genus *Candida*. These yeast-like cells are anamorphic (sexual imperfect) fungi belonging to the class Blastomycetes. They are characterized by their polymorphic nature and ability to produce budding yeast cells (blastoconodia), mycelia, pseudomycelia and blastospores (Georgiev *et al*., 2003). Of the nearly 200 species, six species, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, Candida krusei* and *C. lusitaniae* are the most commonly associated with human infection.

2.8 Morphology

An interesting feature of *C. albicans* is its ability to grow in two different ways; reproduction by budding forming an ellipsoid bud and, in hyphal form, which can periodically fragment and give rise to new mycelia, or yeast-like forms (Bao and Bacon, 2004).

2.9 Epidemiology of Candidiasis

Prevalence of self-reported history of yeast vaginitis varies highly. In a population of university students, 54.7% reported a diagnosis of yeast vaginitis by age of 25 years (Geiger *et al*., 1995). In another student population, the prevalence of yeast was reported to be 20% (Foxman, 1990) and in a family practice clinic the prevalence was approximately 72% (Berg *et al*., 1984). Overall estimates are that approximately 75% of women will have at least one episode of yeast vaginitis in their lifetime. Recurrent episodes are also common. In a study of 2000 women over 18 years of age, 8% reported four or more episodes in a 1-year period (Foxman *et al*., 2000). The incidence may be

higher because many women never seek medical attention because of self-diagnosis and subsequent treatment with over-the-counter (OTC) preparations. In 1995, OTC antifungal vaginal medication sales were reportedly approximately $269 million (DP Hamacher and Associates, 1996). Although yeast usually causes characteristic signs and symptoms, asymptomatic colonization is present in 25% to 50% of immunocompetent females (Holland *et al*., 2003). Approximately 80% to 92% of yeast infections are caused by *Candida albicans*. *Saccharomyces cerevisiae* and other candidal species such as *C. glabrata* can be found in immunocompetent women with recurrent disease (Nyirjesy *et al*., 1995). Candidiasis is one of the three most common vaginal infections along with bacterial vaginosis and trichomonas (Iikit and Guzel, 2011). Approximately 20% of women get an infection yearly (Iikit and Guzel, 2011). Approximately 75% of women will experience at least one episode of vulvovaginal candidiasis (Mardh, 2004) and up to 5% of these women will have recurrent infections meeting the definition of recurrent vulvovaginal candidiasis (Mardh, 2004). The incidence of vulvovaginal candidiasis is highest for women aged 20–40 years (Sobel *et al*., 1998; Mardh, 2004). It is however, rare in prepubertal (Sobel *et al*., 1998) and postmenopausal women (Mardh, 2004). Higher oestrogen levels are thought to make women more susceptible to vulvovaginal candidiasis. This is seen with an increased incidence in pregnant women and postmenopausal women on hormone therapy (Sobel *et al*., 1998; Bauters *et al*., 2002). A study showed that while on the combined oral contraceptive were no more likely than controls to have vulvovaginal candidiasis, women on high dose oestrogen oral contraceptives had higher rates of *Candida* colonization (Bauters *et al*., 2002). Oestrogen increases the glycogen content of the vagina and has a direct effect on *Candida* growth and increases its adherence to the vaginal epithelium (Bauters *et al*., 2002; Mardh, 2004). Vulvovaginal candidiasis is most often caused by *Candida*

albicans. *Candida glabrata* is the most commonly reported 'nonalbicans' species (Sobel *et al*., 1998). In Europe, fungal infections account for 17% cases associated with intensive care units (Rupp, 2007) while in the USA it has become the seventh most common cause of deaths among hospitalized patients (Martins and Jones, 1940). About 15% of allogenic haemopoietic stem cell transplant recipients and 20% of lung transplant recipients suffered fungal infections (Ribaud *et al*., 1999). Approximately 60% and 20% of AIDS patients present with pneumonia and esophageal candidiasis respectively (Moore and Chaisson, 1996). Data from the late 1950s and early 1960s indicate that invasive fungal infections were extremely rare, even in immunocompromised cancer patients (Chakrabarti, 2005). Now, fungal infections have dramatically increased in the past two decades as a result of improved diagnostics, high frequency of catheterization, instrumentation and an increasing number of immunosuppressed patients. Particularly, invasive fungal infections are showing extremely high mortality rate. The use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, prosthetic devices and grafts, and more aggressive surgery have led to the development of complicated infections, including invasive fungal infections. Furthermore, patients with burns, neutropenia and HIV infections are now seriously exposed to fungal infections (Kuleta *et al*., 2009). Fungal infections have now also become more common in the healthy population. The National Nosocomial Infections Surveillance System (NNIS) has reported *Candida* spp as the fourth most common bloodstream isolates in nosocomial infections in USA. Over 95% of all fungal infections have been associated with *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* (Richardson, 2005). A number of *Candida* spp are encountered in candidiasis such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. dubliniensis* and *C. parapsilosis* (Hayens and Westerneng, 1996). *C. albicans* is a

member of the commensal microflora of the intestine. It is pleomorphic and undergoes reversible morphogenic transitions between budding yeast, pseuodohyphal and hyphal growth forms. Healthy persons generally encounter superficial infections but in immunocompromised patients invasive infections could also occur. Among other *Candida* spp, *C. glabrata* has emerged as a frequent pathogen due to increased use of immune suppressive agents. *C. krusei* is a pathogen of importance in patients with haematological malignancies and transplants. *C. parapsilosis* is frequently isolated from blood cultures due to insertive medical devices. *C. tropicalis* is one of the causative agents of candidemia and isolated from patients with leukemia and those who have undergone bone-marrow transplantation. *C. dubliniensis* is found associated with systemic infections in AIDS patients (Ilkit and Guzel, 2011)

2.10 Pathogenesis of *Candida* vulvovaginitis

Multiple factors contribute to a patient developing yeast vaginitis. Not only are vaginal epithelial cells more prone to infection with *C. albicans* (King *et al*., 1980), but the vaginal environment seems to be inherently susceptible. Estrogen, for example, has been shown to enhance the adherence of *Candida* to vaginal epithelium, which may explain why increased infection is seen during pregnancy (Foxman, 1990; Syed *et al*., 2004). A woman also is predisposed to yeast vaginitis when he has taken broad-spectrum antibiotics, which eradicate the normal vaginal flora, allowing for overgrowth of yeast. The association of antibiotics was shown in a recent study to have an odds ratio of 1.75 in relation to yeast vaginitis (Spinillo *et al*., 1999). Other associations with yeast growth include diabetes mellitus and other immunodeficiency states, such as HIV infection. In women with recurrent yeast vaginitis, presence of an immunocompromised state, particularly HIV, should be considered. Recurrent yeast infections also occur in

women with an acquired, reduced T-cell reactivity to *Candida* antigen (Sobel, 1993). Although yeast vaginitis is not considered an STD, asymptomatic penile carriage as well as yeast balinitis can be responsible for yeast transmission to women. Treatment of male sexual partners has not been shown to prevent infections in women (Sobel, 1993). Links also have been made to orogenital sexual transmission (Geirger and Foxman, 1996). Other risk factors include tightly fitting clothing, enuresis and stress. Links related to recurrent yeast infections include the use of feminine hygiene products and sexual activity (Spinillo *et al*., 1999). Yeast infections can occur in any age group, but occur most commonly in reproductive-aged women, whether sexually active or not.

The pathogenesis and prognosis of *Candida* infections are affected by the host immune status and also differ greatly according to disease presentations. Therefore, diagnosis, management and treatment choices vary and need to be considered in the overall setting of the affected human host. The precise contribution to pathogenesis of VVC and recurrent VVC is still not clear (Soll, 1988).

2.10.1 Host

Other studies reported that 11 to 30% of nosocomial urinary tract infections (UTIs) are caused by *Candida* (Febre *et al*., 1999; Richards *et al*., 2000; Lundstrom and Sobel, 2001). In the National Nosocomial Infection Surveillance done between 1992 to 1997 in 112 ICUs across the United States, *C. albicans* was the most commonly reported pathogen (including bacteria) from urine (21%), constituting more than half of the fungal isolates. *C. albicans* was also more commonly reported in catheter-associated UTIs than in non-catheter-associated infections (21% versus 13%, $p = 0.009$). Fungal urinary infections occurred more frequently in patients with urinary catheters than in those without urinary catheters (40% versus 22%, $p < 0.001$) (Richards *et al*., 2000).

VVC and RVVC are associated with considerable morbidity. Symptoms of vaginitis can cause substantial distress, resulting in time lost from work and altered self-esteem (Eckert, 2006). Thus, it is not surprising that vaginal complaints are the most common reason for gynaecological consultation. Among the many causes of vaginitis, VVC is the second most common after bacterial vaginosis, and it is diagnosed in up to 40% of women with vaginal complaints in the primary care setting (Anderson *et al*., 2004). In the United States, prior to the availability of OTC treatment, approximately 13 million cases of VVC annually accounted for 10 million visits to the gynaecologist (Kent, 1991). In 1990, the first topical treatment for VCC was approved by the Food and Drug Administration for OTC use, and since then the combined antifungal prescription and OTC sales have almost doubled (Lipsky and Waters, 1999). In 1995 alone, the annual cost of VVC was estimated to be $1.8 billion, with approximately half of this amount consisting of charges for Doctors visit (Foxman *et al*., 2000). Furthermore, industry sources reported that in 1995 OTC sales of vaginal antifungals were the largest component of the feminine health care sales in drug stores, generating nearly 60% of the category's sales and resulting in approximately $290 million (Drew *et al*., 2005). In contrast, Sobel *et al*. (2000) did not observe complications of fungal urinary tract infection in over 330 hospitalized patients (not only ICU patients), including pyelonephritis, candidemia, systemic candidiasis and fungus-related death.

2.10.2 Adaptation and propagation of *Candida albicans*

For a fungus to survive in its niche it has to adapt to constantly changing parameters. Therefore, fungi respond to change in a specific environmental component by inducing transcriptional and translational changes that promote survival under the newest environmental conditions. When fungi enter the mammalian host, their life style

changes from saprophytic to parasitic. As saprophytes, fungi survive in an environment with a moderate ambient temperature and pH, essential nutrients such as carbon and metal ions, and atmospheric concentrations of carbon dioxide and oxygen. Once having invaded a human host, these environmental factors are suddenly replaced by drastic changes. In the different niches of a host, completely different nutrient compositions may exist and specialized features of fungal pathogens may be involved in the establishment, dissemination and manifestation of an infection (Brock, 2009). For example, ambient temperature is replaced by the high temperature of the human body. Fungal survival at the elevated temperature of a human host is essential for virulence. The fungal pathogens, *C. neoformans* and *A. fumigatus* are simply better able to survive at 37^0C than their nonpathogenic counterparts (Hogan, 2006). Fungi often develop morphogenetic virulence mechanisms, e.g., formation of yeasts, hyphae, and spherules that facilitate their multiplication within the host at higher temperature. Yeast cells of many *Candida* species form filamentous pseudohyphae and hyphae in tissues, whereas *C. neoformans* yeasts become coated with a capsule, and *Coccidioides immitis* develops swollen, septated spherules in the host. Other fungi such as *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Penicillium marneffei* form filamentous mycelia in the environment, but convert to yeast morphology upon contact with the human host (Rappleye and Goldman, 2006). Hyphae that grow in the skin or nail as dermatophytes can fragment into arthroconidia or other conidial types. On the other hand, ambient pH is replaced with acidic conditions of mucosal surfaces or neutral to slightly alkaline pH of blood and tissues. One pathway used by fungi in response to changing pH involves activation of the transcription factors such as PacC in *A. nidulans* and Rim101 in *C. albicans* (De Bernardis *et al.*, 1999). Carbon and metal ions are lacking in host tissues; iron is sequestered from microbes by iron carrier proteins in tissues, creating an iron-

limited environment. In order to survive, fungi encode certain mechanisms by employing siderophores, high affinity iron chelators, to efficiently bind host iron into fungal cytoplasm (Haas *et al.*, 2008). Fungi aslo have to face hypoxia and high levels of carbon dioxide in tissues. In *C. albicans*, the response to hypoxia is dependent on coordination of specific transcriptional regulators; for example, transcription factor Ace2 represses oxidative metabolic processes and promotes filamentation (Mulhern *et al*., 2006). All these specialized adaptations help fungi in sustaining infection at the host site. Most of the free living pathogenic fungi possess an extremely versatile metabolism, which allows them to adapt immediately to changes in the environmental conditions during life in the soil. Therefore, success of infection depends on rapid adaptation to changing micro-environments (Sobel, 2007)

2.11 Clinical Presentation of *Candida* vulvovagintis

Women with *Candida* vaginitis typically present with a complaint of vulva itching, burning, redness, irritation or discharge, dysuria, particularly external dysuria and, urinary frequency are symptoms that often mislead clinicians to a diagnosis of cystitis but may indicate presence of yeast vaginitis instead. The patient usually describes the discharge as white, thick and odourless. Typical ‘‘yeast symptoms’’ do not predict disease consistently (Anderson *et al*., 2004). In a study of 545 women with symptoms characteristic for yeast vaginitis, only 28% had positive cultures for *C. albicans* (Eckert *et al*., 1998). Thus, a thorough history and a consideration of STD testing are needed.

The clinical symptoms of VVC are nonspecific and can be associated with a variety of other vaginal diseases and infections such as bacterial vaginosis, trichomoniasis, *Chlamydia* infection and gonorrhea.Vulva pruritus and burning are the hallmark

symptoms in most women with VVC, frequently accompanied by soreness and irritation leading to dyspareunia and dysuria (Anderson *et al*., 2004). On physical examination, vulva and vaginal erythema, edema, fissures and a thick curdy vaginal discharge are commonly found (Eckert *et al*., 1998). The clinical features of candidiasis are dependent on the sites of infection. Oropharyngeal candidiasis, angular chelitis, balanoposthitis, oral thrush and vulvovaginal candidiasis are features of mucous membrane (mucosal candidiasis) infection; inter digital candidiasis, paronychia and nappy rash are features of cutaneous candidiasis; and pulmonary candidasis, disseminated candidiasis, gastrointestinal candidiasis and candidaemia are features of systemic candidiasis, involving internal body fluids and organs (Jacob and Nall, 1990).

2.12 Distribution of *Candida* spp in Vulvovaginal Candidiasis

The distribution of *Candida* spp identified in women with VVC varies widely depending on the locations as well as the populations studied. Typically, a single species is identified, but two or more species have been found in the same vaginal culture in a minority of women (2 to 5%) with complicated as well as uncomplicated VVC (Fan *et al*., 2008; Richter *et al*., 2005). In the United States, Europe and Australia, *C. albicans* is the most common species identified in women with VVC (76 to 89%), followed by *C. glabrata* (7 to 16%) (Holland, 2003; Richter *et al*., 2005; Vermitsky *et al*., 2008). The overall percentage of non-*C. albicans* species associated with VVC in these countries/continents ranges from 24% to 11%. Some studies have reported an increasing trend in the occurrence of non-*C. albicans* species over time (Spinillo *et al*., 1997), while a recent U S study of over 90,000 samples found a consistent yearly distribution from 2003 to 2007 (Vermitsky *et al*., 2008). In contrast to the case in the United States, Europe and Australia, non-*C. albicans* species, in particular *C. glabrata*,

appear to be more commonly associated with VVC in some Asian and African countries. In Turkey, India and Nigeria, cases due to *C. glabrata* range between 30 to 37%, while the *Candida* spp distribution in China resembles closely the one in the United States. With higher resistance levels of most non-*C. albicans* species to the commonly used azole-based treatments and limited possibilities for yeast identification and susceptibility testing in some of these settings, the consequences for women affected by these strains might be incapacitating. Molecular typing of serial *Candida* isolates from patients with candiduria demonstrates that patients continue to have infection or reoccurrence with the same strains (Jain *et al*., 2007). Similar to the case for VVC, around 5% to 8% of patients with candiduria will have two or more species simultaneously (Kauffman *et al*., 2000). Strains may differ in phenotypic characteristics such as biofilm (BF) formation; however, it was demonstrated in serial isolates that these characteristics remain stable, analogous to serial *Candida* isolates derived from blood (Jain *et al*., 2007; Hasan *et al*., 2009). Molecular typing of *Candida* strains derived from the vagina of pregnant women indicated that the women, who were followed longitudinally through pregnancy, became symptomatic (average time of 14 weeks) with their colonizing *Candida* strains (Daniels *et al*., 2001). The study found that five of the eight patients with positive vaginal secretions later showed the presence of the same yeast species in their urine. Therefore, it has been suggested that vaginal *Candida* ascending from the genital to the urinary tract could explain the greater incidence of candiduria in women (Bauters *et al*., 2002).

2.13 Virulence Factors Involved in Pathogenicity of *Candida albicans*

Like other fungal pathogens, *C. albicans* also regulates expression of certain genes and their products as virulence factors to produce disease. This is the most common

opportunistic pathogen, utilizing several kinds of virulence factors. Some of the commonly studied virulence factors in *C. albicans* are briefly described below:

2.13.1 Adhesion

Adherence of candidal cells to host tissues is a complex multifactorial phenomenon utilizing several types of adhesins expressed on morphogenetically changing cell surfaces. But the striking feature of *Candida* cells is the formation of biofilms in host tissue, resulting in enhanced adherence. Ramage *et al*. (2006) reported that in the last few decades, *Candida*-related infections have been found associated with biofilm-forming capacity. Well-known adhesins are agglutinin-like sequences (ALS) that are members of a family of seven glycosylated proteins. Als1p, Als3p and Als5p (Ala1p) on the cell surface of hyphae adhere to human buccal epithelial cells (HBEC) and fibronectin, collagen, laminin and endothelial cells (Hawser and Douglas 1994; Hoyer, 2001). Als6p and Als9p bind to collagen and laminin respectively. Als4p binds to endothelial cells and Als5p is additionally needed for cell aggregation. However, the role of Als7p is unclear (Filler *et al*. 2006; Kuleta *et al*. 2009). Another 34 kDa adhesin molecule, Hwp1 (hyphal wall protein), encodes an outer surface mannoprotein on the hyphal wall; the amino terminal sequences of this adhesin are recognized as mammalian transglutaminase substrate (TGase) and form covalent binding with HBEC. Studies with hwp1 knockout mutant and HWP1 deficient mutant of *C. albicans* have shown reduced adherences and mortality in murine models (Chaffin *et al*. 1998; Staab *et al*. 2002). An integrin-like protein (Int1p) which is a plasma membrane receptor and antigenic functionally similar to human complement receptors 3 and 4, has been isolated from *C. albicans* and found to bind with extracellular matrix (ECM) ligands such as fibronectin,

laminin and collagen I and IV, and induce morphological changes in response to extracellular signals (Cladderone and Fonzi, 2002; Ruiz-Herrera and Hard, 2006).

2.13.2 Morphogenesis

Morphogenesis in *C. albicans* is defined as transition from unicellular yeast form to filamentous form (pseudohyphae or hyphae). Of all the species, only *C. albicans* and *C. dubliniensis* are able to undergo morphogenesis. Transition from yeast form to hyphal form is facilitated by nutrients, near-neutral pH, temperature of 37^0C–40^0C, $C0_2$ concentration about 5.5%, and presence of N acetyl-D-glucosamine, serum, some amino acids and biotin (Eckert *et al.* 2007). Reverse transition from hyphal to yeast form is provoked by lower temperatures, acidic pH, absence of serum and higher concentration of glucose (Corner and Magee 1997; Eckert *et al*. 2007). This transition is strongly required for pathogenesis. Yeast forms are more suited for dissemination in tissues and to other hosts, whereas hyphal forms are required for tissue damage and invasion. For example, the yeast cell, when phagocytosed by macrophages, produces hyphae and secretes hyphae-associated proteinases that kill macrophages; these factors also prevent hyphal cells from being killed by neutrophils enabling *Candida* cells to escape from the bloodstream (Molero *et al*., 1998; Gow *et al*., 2002; Hube and Naglik, 2004). Furthermore, hyphal cells have stronger adherence capacity due to expression of ALS adhesins and also exhibit greater invasiveness to tissues. Increased expression of superoxide dismutase (SOD) antagonizes oxidative burst of phagocytic cells. Several genes have been identified which regulate phase transition, namely PHR1, ECE1, HYR1, RBF1, CHS2, CHS3, which are differentially expressed during morphogenesis (Haynes, 2002; Cladderone and Fonzi, 2002).

Of these, ECE1 correlates with hyphal elongation although ECE1 null mutants displayed no morphological alterations. Similarly, null mutants for expression of CHS2, CHS3 and HYR1 did not show any obvious morphological type. But disruption of RBF1 demonstrated alteration in cell morphology and strongly involved in yeast–hypha transition (Yang, 2003). Studies with homozygous null mutants for Hst7p, Cph1p and Cst20p have shown defective hyphal formation (Leberer *et al*. 1996); in addition, three genes (TUP1, EFG1, CLA4) were found to be regulating candidal morphogenesis (Liu and Chu, 2001). Transcription factors such as Tup1 and Rbp1 are negative regulators of filamentation (Braun and Johnson, 2000). A tup1 mutant strain resulted in constitutive filamentous growth under all conditions, indicating a role in filament formation. Deletion of homozygous allele of Ste20 encoded by CLA4 showed impaired hyphal formation in a wide range of medium, and decrease in virulence in a murine model (Braun and Johnson 2000; Claderone and Fonzi, 2002). A protein of bHLH class encoded by EFG1 acts as transcriptional activator as well as repressor, and is required for pseudohyphal and hyphal morphogenesis (Liu and Chu, 2001). A study with homozygous mutants cfg1 and cph1 showed failure of germ tube and hyphae production in a murine model (Liu and Chu, 2001).

2.13.3 Phenotypic switching

Unlike other pathogens, phenotypic switching in *Candida* is pleitropic by affecting several phenotypic and metabolic parameters, with subsequently a number of virulence traits such as SAP gene regulation. This allows *Candida* to adapt to a different host environment during infection (Soll, 2002b). Colonies of *C. albicans* show morphological variation, including smooth, rough, star, stippled, hat, wrinkle, and fuzzy at high frequency. This switching is reversible, occurs spontaneously in stress, and

results in changes in cell surface behaviour, colony appearance, and metabolic, biochemical and molecular attributes to become more virulent and effective during infection (Soll, 2002b; Odds *et al*. 2006). Strains isolated from vaginitis or systemically infected patients showed higher frequencies of switching, indicating a strong role for the switching phenomenon in establishing diseases (Kvaal *et al*. 1999). In the case of yeast hypha transition, all cells of a population express the same phenotype under the same environmental conditions, whereas in the case of switching, some cells of a population express different phenotypes under the same set of environmental conditions. Earlier research had reported that laboratory isolate 3153A, grown on amino acid rich agar, which was limiting for zinc and incubated at 25^0C, showed a smooth phenotype as dominant, while variant colonies of star, ring, irregular and wrinkle occurred spontaneously. Such types of variation were also observed with cells of strain 3153A treated with low doses of UV irradiation (Soll, 2002b). At present, of all the phenotypes described, the white opaque system in strain WO-1 is the most studied. This system is characterized by transition from smooth, white colonies to flat, gray opaque colonies. White cells are round ovoid while opaque cells are elongated or bean-shaped (Soll, 2002b). Study of gene expression with the WO-1 system revealed an association of OPA1 (SAP1) and SAP3 in opaque cells, in contrast to SAP2, WH11and EFG1 in white cells (Soll, 2002a; Miller and Johnson, 2002). Study with efg1 null mutants exhibited no involvement of EFG1 in switching, but rather control of phenotypic characteristics. It has been reported that white cells in WO-1 hardly form hyphal stages, but this was achieved by opaque cells (Staab *et al*., 2002). There is good evidence that opaque cells are more virulent than white cells in several murine models (Yang, 2003).

2.13.4 Phospholipases

Phospholipases are enzymes that hydrolyze ester linkages of glycophospholipids and hence impart tissue invasiveness to *Candida* cells. In *C. albicans*, four types of phospholipases are classified by researchers on the basis of the ester bond they cleaved viz., phospholipase A, B, C and D. All types possess hydrolase activity, but PLB in addition also possesses lysophospholipase transacylase activities; therefore, it is able to release fatty acids from phospholipids and the remaining fatty acid from lysophospholipids, and then transfer a free fatty acid to lysophospholipids, producing phospholipids. Of these, only PLB1, a 84 kDa glycoprotein isolated from hyphal tip in the course of tissue invasion, has been shown to be required for virulence in a murine model of candidiasis (Ghannoum, 2000; Yang, 2003; Theiss *et al*. 2006). A study conducted by Ibrahim *et al*. (1995) revealed an increased level of phospholipase production in blood isolates compared to commensal isolates.

2.13.5 Proteinases

Secretion of proteinases by pathogen is mandatory in order to degrade the tissue barriers and obtain nutrition at the infection site. Secreted aspartyl proteinases (SAPs) from *Candida* have been reported that hydrolyze many proteins such as albumin, hemoglobin, keratin, collagen, laminin, fibronectin, mucin, salivary lactoferin, interleukin1b, cystatin A and Immunoglobulin A (Hube and Naglik, 2004). To date, ten proteins have been recognized as SAP family (SAPs 1–10) and found to be responsible for tissue invasion. Several researchers have reported that production of SAPs is also correlated with hyphal formation, adherence and phenotypic switching (Monod and Zepelin, 2002; Naglik *et al*. 2003). Such researches have highlighted the complex role played by SAPs in the pathogenicity of *C. albicans*. Several models using SAP

inhibitors such as pepstatin A and SAP-disrupted or over-expressing mutants demonstrated the need for these factors in candidal pathogenesis. *In-vitro* studies have reported that SAPs 1, 2 and 3 are expressed by the yeast phase only, while SAPs 4, 5 and 6 are expressed in the hyphal phase (Hube and Naglik 2004; Schaller *et al*. 1999; Naglik *et al*. 2004) whereas SAPs 9 and 10 are expressed by both forms (Albrecht *et al*. 2006). Structural analysis revealed that SAPs 1–8 are secreted extracellularly, but that SAPs 9 and 10 are anchored to the cell wall by glycophosphotidyinositol (GPI) protein (Naglik *et al*. 2003; Albrecht *et al*. 2006). Models of epidermal and vaginitis candidiasis revealed involvement of SAPs 4–6 in invasive systemic disease whereas SAP 7 was never detected *in-vitro*. The role of SAPs 1–3 is associated with early adherence, invasion and cutaneous infections as studied in the WO1 strain, whereas SAP8 is associated with extensive penetration. SAPs 6 and 9 were found expressed in later stages of hyphal growth (Hube and Naglik 2004; Schaller *et al*. 1999; Kvaal *et al*. 1999). Different properties of SAPs are exploited in the pathogenicity of *Candida*. For example, SAPs are active across a broad range of pH 2.0–7.0, as SAPs 1–3 are active at pH 3.5, SAPs 4–6 at pH 5.0–7.0 and therefore make *Candida* capable of colonizing and invading different tissue sites of varying pH. In addition, SAPs show varied levels of protein specificity as SAPs 1, 2, 3 and 6 cleave peptide bonds in larger hydrophobic amino acids; SAPs 1, 2 and 6 act onphenylalinine whereas SAP 3 attacks leucine and SAPs 9 and 10 hydrolyze yapsin and kexins (Naglik *et al*., 2004). This attribute enables *Candida* to obtain nitrogen at different tissue make up and aids pathogenicity by revealing potential binding sites from tissue for adhesion of candidal cells, and also dissemination via circulatory systems. *In-vivo* studies have confirmed the role of SAPs in colonization, increased adhesion and tissue penetration (Hube and Naglik, 2004; Naglik *et al*., 2004). Disruption of SAPs 1, 2 and 3 has resulted in decreased virulence

in mouse models (Hube and Naglik, 2004). Several reports have supported functional role of SAP2 in invasion and dissemination of systemic infections (De Bernardis *et al*., 1999; Kvaal *et al*. 1999; Naglik *et al*., 2004). Further research data have indicated increased expression of SAP genes, especially SAPs 5, 6 and 9 mRNA transcripts, in biofilm rather than planktonic cells (Green *et al*. 2004; Naglik *et al*., 2008). Recently, in addition to SAPs, a 60 kDa metallopeptidase and 50 kDa serine peptidases have also been isolated and reported to hydrolyze extracellular matrix proteins and serums (Biswas *et al*., 2007). Expression of SAPs has been found to be correlated with other virulence determinants to enhance the pathogenicity of *C. albicans*.

2.13.6 Biofilm formation

Biofilms are the organized structures involving microbial communities that are attached to some inanimate surfaces or tissues and circumvented in a matrix of exopolymeric materials (Thewes *et al*., 2007). Biofilm formation is initiated by irreversible adherence of microbial cells to tissues or devices and followed by growth and maturation to form a mesh of cells with altered phenotype, growth rate and gene expression compared to planktonic cells. Studies with scanning electron microscopy of biofilms revealed the presence of both adherent yeast cells and invasive hyphal forms constructing basal and upper layers respectively, enclosed in an extracellular polymer matrix consisting of polysaccharides and proteins and forming a three-dimensional structure with water channels (Dominic *et al*., 2007). These forms differ in ultrastructure, physiological behaviour and composition of cell walls, and are required for candidal pathogenicity, as mutants lacking genes for any one became less virulent both *in-vitro* and *in-vivo* (Chandra *et al*., 2001). Heterogeneity of these biofilms depends on the substrate composition, environmental conditions and type of strains involved. Although yeast–

hypha transition is necessary for full maturation of the biofilm, strains that are unable to grow as yeasts or to form hyphae can still form biofilms but are easily detachable (Baille and Douglas, 1999). The ability of *Candida* to form biofilms on catheters, endotracheal tubes, pacemakers and other prosthetic devices has contributed to its predominant prevalence in nosocomial infections (Douglas, 2003; Ramage *et al*., 2005).

2.14 Diagnosis of *candida* vulvovaginitis

A diagnosis of yeast vaginitis is made by the combination of clinical presentation, physical examination findings and observation of yeast on the wet preparation. *C. albicans* is the organism responsible for most uncomplicated cases (Ilkit and Guzel, 2011). Upon physical examination, the labia and vaginal walls may appear erythematous and edematous. Because of the itching, involved perineal skin may be excoriated. The intertriginous groin area also may be erythematous with ‘‘satellite’’ lesions present (Mari and Martin, 2000). A thick, white discharge adhering to the vaginal walls may be observed. The diagnosis is supported by the observation on a wet preparation (saline and 10% potassium hydroxide) or Grams stain of vaginal discharge, which reveals the buds or pseudohyphae most commonly seen with *C. albicans* (Georgiev *et al*., 2003). Other less common species such as *C. glabrata* and *S. cerevisiae*, produce only blastospores, which may be more difficult to detect with saline microscopy (Nyirjesy *et al*., 1995). The use of 10% potassium hydroxide in wet preparations improves the visualization of yeast by disrupting cellular material that might obscure the yeast and also associated with a normal vaginal pH of less than 4.5 and a lack of amine odour when potassium hydroxide is added (Metzger, 1998). Because the sensitivity of saline or potassium hydroxide microscopy is about 50%, often the diagnosis must be made based on history and physical examination. A culture

is usually unnecessary to confirm a diagnosis, and a trial of antifungal treatment can be initiated. A culture may be indicated in cases of recurrent yeast vaginitis or for concerns of less common varieties of yeast that may be more resistant to standard therapy (Nyirjesy *et al*., 1995).

In the majority of women, a diagnosis of VVC is made at least once during their childbearing years (Sobel *et al*., 1998). A pelvic examination will be done. It may show swelling and redness of the skin of the vulva, in the vagina, and on the cervix. The health care provider may find dry, white spots on the vaginal wall. There may be cracks in the skin of the vulva (Merit, 2011). A small amount of the vaginal discharge is examined using a microscope. This is called a wet mount and KOH test. Sometimes, a culture is taken when the infection does not improve with treatment or comes back many times. There is an automated continuous-monitoring blood culture system available for critically ill patients. A diagnosis of vaginal thrush is often made based on symptoms. However, there are many other conditions of the vagina and vulva that have symptoms in common with thrush, so if there is the slightest doubt about the diagnosis, it is essential that doctor takes a vaginal swab and sends it for analysis before treatment is started (Spence, 2007). Diagnosis of VVC based solely on patients history and genital examination is not possible because of the low specificity of symptoms and signs since other causes like leucorrhoea and pruritus vulvae mimic VVC (Geiger *et al.*, 1995). Therefore, to have a positive specific diagnosis of VVC, a number of steps are recommended namely; determination of vaginal pH (normal 4-4.5), which means that a higher pH more than 5 is suggestive of bacterial vaginitis or trichomoniasis (CDC, 2002); preparation of a wet mount of the vaginal discharge for identification of the yeast cells and mycelia and to rule out other diagnoses, e.g., bacterial vaginosis and

trichomoniasis (Marrazzo *et al*., 2002); a 10% potassium hydroxide (KOH) preparation of vaginal discharge (Geiger *et al*., 1995). Gram's stain preparation may also be used since yeast is Gram-positive. If microscopic studies are negative and the index of suspicion of VVC continues to be high, vaginal swab for fungal culture is done (Sherrard-Stratfrod and James 2003; Sobel *et al.*, 1998). In all cases of pruritus vulvae, the urine should be tested for glucose (urinalyisis). The commonest cause of vulva pruritus in pregnancy is VVC, which may be associated with the lowered renal threshold for sugar, which occurs in pregnant women (Cladérone and Fonzi, 2002). The signs and symptoms of VVC are relatively non-specific; the most *Candida* specific symptom is pruritus without discharge, which correctly predicts VVC in only 38% of patients. Some patients have the typical "cottage-cheese" vaginal discharge, which may vary from watery to thick and usually does not have an odour. Occasionally, patients complain of vaginal soreness and dyspareunia or external dysuria. Physical examination may reveal vulva erythema and swelling, often with discrete pustulopapular peripheral lesions. The cervix appears normal and the vagina erythematous. Vaginal cultures are usually not necessary and should be reserved for monitoring recurrences or determining whether recalcitrant infections are due to resistant organisms. About 50% of patients have positive microscopy of a wet mount or saline preparation, where yeast cells and hyphal filament can be seen. A 10% potassium hydroxide (KOH) preparation is more sensitive than a saline preparation in identifying yeast cells or hyphae (Sobel, 2007). Processed clinical samples are cultured on Sabouraud dextrose agar, which allows growth of any fungi and their isolation.

2.15 Treatment of vulvovaginal candidiasis

2.15.1 Treatment of uncomplicated VVC.

Successful treatment of uncomplicated VVC is achieved with single-dose or short-course therapy in over 90% of cases. Several topical and oral drugs are available, without evidence for superiority of any agent or route of administration (Pappas *et al*., 2009), although among the topically applied drugs, azoles are more effective than nystatin. Workowski and Berman. (2006) summarizes the intravaginal treatment options recommended by the Center for Disease Control and Prevention (CDC, 2002) and the Infectious Diseases Society of America (IDSA). As an oral agent, fluconazole at 150 mg as a one-time dose is recommended for uncomplicated VVC (Workowski and Berman, 2006; Pappas *et al*., 2009). Because oral and topical antimycotics have shown to achieve equivalent results for the treatment of VVC (Watson *et al*., 2012), both fluconazole given orally and topical agents have received the same recommendation in the IDSA guidelines (A-1), and no preference is given to either treatment (Pappas *et al*., 2009).

2.15.2 Treatment of complicated vulvolvaginal candidiasis and recurrent vulvolvaginal candidiasis.

Complicated VVC with azole-susceptible strains requires topical therapy administered intravaginally daily for at least 7 days or multiple doses of oral fluconazole, 150 mg every 72 h for three doses (Sobel, 1997; Sobel *et al*., 2001). In cases of RVVC, this regimen followed by long-term weekly treatment with fluconazole at 150 mg orally has shown to significantly reduce recurrence rates compared to three doses of fluconazole alone (Sobel *et al*., 2004). Long-term suppressive therapy with oral fluconazole is the most convenient and well-tolerated regimen among other options and was shown to be

effective in over 90% of patients with RVVC. Against expectations, patients on suppressive therapy with fluconazole have shown little evidence of developing fluconazole resistance in *C. albicans* isolates or super infection with non-*C. albicans* species (Sobel *et al*., 2004; Shahid and Sobel, 2009). However, species identification and minimum inhibitory concentration (MIC) testing should be performed in women experiencing breakthrough or refractory infection. Other oral treatment options that have been shown to be effective for RVVC with azole-susceptible strains include suppressive therapy with ketoconazole (100 mg daily) (Sobel, 2006) and with itraconazole, 200 mg twice daily for one day each month (Witt *et al*., 2009). However, because of liver toxicity associated with oral ketoconazole (Lewis *et al*., 1999), other regimens are now preferred as maintenance therapy. For women with RVVC who prefer topical to oral drugs, clotrimazole (500-mg suppositories weekly or 200-mg suppositories twice weekly) is recommended (Pappas *et al*., 2009), although the 500-mg formulation is no longer available in the United States. Alternatively, other forms of topical maintenance therapy can be considered, without data supporting the use of a specific topical formulation. Patients on no maintenance therapy have a recurrence rate of over 70% within the first 6 months after successful treatment of VVC (Sobel, 2007), while a 40% to 50% recurrence rate after a 12-month cessation of maintenance therapy has to be anticipated (Shahid and Sobel, 2009). Non- *C. albicans*-related disease is less likely to respond to azole therapy (Nyirjesy *et al*., 1995). Vaginal boric acid, administered in a gelatin capsule at a dosage of 600 mg daily for 14 days, cures up to 70% of *C. glabrata* infections (Sobel *et al*., 2003). Treatment with AmB suppositories (50 mg nightly for 14 days) is another option with minimal side effects that has shown to be successful in 70% of women with non-*C. albicans* VVC, mostly due to *C. glabrata*, that did not respond to azole treatment (Philips, 2005). Other alternatives

include topical 17% flucytosine cream alone or in combination with 3% AmB cream administered daily for 14 days, albeit at considerable expense due to the high cost of flucytosine (Pappas *et al*., 2009). Of note is that all the treatment options for non-*C. albicans* VVC need to be compounded.

In 1990, the first topical treatment for VCC was approved by the Food and Drug Administration for OTC use, and since then the combined antifungal prescription and OTC sales have almost doubled (Lipsky and Waters, 1999). In 1995 alone, the annual cost of VVC was estimated to be $1.8 billion, with approximately half of this amount consisting of charges for doctors visits (Foxman *et al*., 2000). Furthermore, industry sources report that in 1995 OTC sales of vaginal antifungals were the largest component of the feminine health care sales in drug stores, generating nearly 60% of the category's sales and resulting in approximately $290 million (Duerr *et al*., 2003).

Considerations in choosing antifungal therapy include causative organisms, adverse effects, drug interactions, severity and duration of symptoms and patient preferences. The topical and oral azoles (imidazoles and triazoles) currently available are associated with high cure rates (ranging from approximately 85 to 90%) in most patients with infrequent episodes of vulvovaginal candidiasis (Jones *et al*., 2004). Some differences in spectrum of activity against certain yeast species, however, may affect drug efficacy For example, *C. glabrata* and *C. tropicalis* have been shown to be less sensitive than C. *albicans* to standard imidazole antifungal agents such as miconazole and clotrimazole (Galgiani, 1993; Demuri and Hostetter, 1995). The minimum inhibitory concentration (MIC) of fluconazole against *C. glabrata* and *C. krusei* has been shown to be higher than the MIC against *C. albicans* in comparison to the *in-vitro* studies, and studies assessing ketoconazole and clotrimazole have shown decreased *in-vitro* sensitivities of

C. glabrata to these agents (Sobel *et al*., 1993). In a study assessing response to therapy of vulvovaginal candidiasis patients from whom *C. glabrata* was isolated, the MIC50 and MIQQ of *C.glabrata* were generally two to four times higher than those of the *C. albicans* isolates for most drugs tested (Del Palacio, 1992), although they still fell within the sensitive range. Topical agents are the standard first-line therapy for vulvovaginitis in diabetic women. These agents have been shown to be safe and relatively free of untoward effects, the most common of which are headache and/or abdominal cramps (0.2% for both), and local burning, itching or discomfort (0.9-6.0%) (Reef *et al*., 1993). The oral antimycotics have been reported to potentiate the hypoglycemic response to sulfonylureas (Reef *et al*., 1993) and several cases of severe hypoglycemic episodes have been reported in patients taking both types of agents (Abbott *et al*., 1991, Neuhaus *et al*., 1991). In three cross over studies involving healthy volunteers, administration of fluconazole, 100 mg/day for 7 days, resulted in significantly increased plasma concentrations and areas under the concentration- time curve of tolbutamide, glipizide and glyburide. Several patients had hypoglycemic symptoms (except in the tolbutamide study), and some in the glyburide trial required oral glucose administration (Neuhaus *et al*., 1991). Itraconazole has also caused elevated sulfonylurea levels leading to hypoglycemia, and ketoconazole has been shown to decrease tolbutamide clearance, resulting in increased levels of oral hypoglycemic agents and hypoglycemia (Hay, 1993). The adverse effects most frequently reported with oral antifungal agents are gastrointestinal symptoms (5-12.5%) and headache (~13%). There have also been rare reports of angioedema (Lee *et al*., 1992), anaphylaxis (Sobel, 1993), hepatotoxicity with oral ketoconazole therapy (Sobel, 1993), and an increased incidence of abnormal results of liver function tests with oral fluconazole (Thai and Hart, 1993). Furthermore, because data on teratogenicity are

limited, oral agents should be avoided in pregnant women and those contemplating pregnancy. Patients preference is an important consideration for these women, whose daily routines are already complicated by their diabetes management. Shorter-course therapies are preferred by patients and help assure compliance, which has been shown to decline with longer treatment. The single-day therapies that have become available require minimal effort and have been shown to be as effective as short-course 3-to 7-day regimens for acute episodes of vulvovaginal candidiasis (Fong, 1992). Studies evaluating such regimens (e.g., tioconazole 6.5% ointment, clotrimazole 500-mg vaginal suppositories and fluconazole 150-mg oral tablets) have shown that after a single administration, therapeutic drug concentrations remain in the vagina for as long as 5 days, thus providing prolonged therapeutic effect (Gabbe *et al*., 1991; Inman *et al*., 1994). Furthermore, tioconazole has documented *in-vitro* activity against *C. albicans* and other *Candida* species, including *C. glabrata* (Pursley *et al*., 1996). In a multicenter comparative study of single-dose tioconazole 6.5% ointment and terconazole 0.8% vaginal cream used for 3 days, the mycologic and clinical cure rates and adverse experiences were similar for the two groups. However, a significantly greater number of tioconazole patients ($P < 0.01$) used all of the drug therapy (98.4 vs. 86.8%) and several parameters of patient acceptance favoured tioconazole over terconazole (Sobe *et al*., 1993). In a trial that assessed treatment efficacy in diabetic patients, 32 women with acute vulvovaginal candidiasis received oral ketoconazole 400 mg once daily for 5 days, which according to the authors, resulted in low relapse rates (Pursley *et al*., 1996). On the 5^{th} day, cultures were negative for *Candida* organisms in 18 patients and 28 patients reported improved symptoms. One month later, cultures were negative for *Candida* organisms in 15 patients, and 24 patient reported improved symptoms. The eight patients with symptoms repeated the treatment course, after which six patients had

cultures negative for *Candida* organisms and two patients were refractory (Pursley *et al*., 1996). Treatment of VVC treatment recommendations for VVC is separated into treatment of uncomplicated VVC caused by *C. albicans* and of complicated VVC, which includes RVVC, severe VVC, VVC caused by non-*C.albicans* species and VVC in immunocompromised hosts (Pappas *et al*., 2009; Workowski and Berman, 2006). In cases of complicated VVC, contributing factors such as diabetes or behavioural factors should be controlled or avoided. Treatment should not differ on the basis of HIV infection (Pappas *et al*., 2009). Treatment of uncomplicated VVC is achieved with single-dose or short-course therapy in over 90% of cases. Several topical and oral drugs are available, without evidence for superiority of any agent or route of administration (Pappas *et al*., 2009), although among the topically applied drugs, azoles are more effective than nystatin (Workowski and Berman, 2006). As an oral agent, fluconazole at 150 mg as a one-time dose is recommended for uncomplicated VVC (Pappas *et al*., 2009; Workowski and Berman, 2006). Because oral and topical antimycotics have shown to achieve equivalent results for the treatment of VVC (Watson *et al*., 2012). Most probiotics, in oral or topical formulation, contain lactobacilli, which are felt to inhibit or reduce the growth of *Candida* in the vaginal tract. While some clinical trials support the effectiveness of certain lactobacilli, others do not, and most are limited by methodological problems (Falagas *et al*., 2006). There are a number of other nonconventional methods available, but these have not been assessed in well-designed randomized clinical trials (Watson and Calabretto, 2007). One recently published randomized clinical trial showed that monthly oral itraconazole treatment was significantly more effective in preventing recurrent episodes of VVC than a homeopathic regimen and that an *L. acidophilus*-containing agent applied intravaginally did not add any benefit to itraconazole treatment alone (Witt *et al*., 2009).

Candida albicans is the major yeast implicated in most cases of vaginitis (80%-90%). Other *Candida* species, including *Candida torulopsis* and *Candida glabrata*, can also be associated with vaginitis. Azoles, which are fungistatic and act by inhibiting cell wall metabolism, are the mainstay of treatment for vaginal candidiasis. They are available over the counter and by prescription. They are available in topical and oral forms. Cure rates of 80% to 90% can be achieved with all the azole agents. Topical nystatin is less effective with cure rates of 50% to 80%. The choice between oral and topical therapies should be based on patient preference as well as factors such as compliance, cost, ability to insert vaginal preparations, history of response or adverse reactions to prior treatments and duration of therapy. The main disadvantage of the topical route is localized burning due to irritant or allergic reactions. Oral therapy with a single dose of fluconazole is as effective as a 3-day course of topical terconazole and a 7-day course of clotrimazole. Systemic side effects are mild, infrequent and self-limited. They include gastrointestinal intolerance, headache and rash. Persistent symptoms should be re-evaluated and vaginal yeast cultures should be obtained. *C. glabrata* is less likely to respond to azole medications. Boric acid 600 mg topically daily for 14 days is an effective treatment option. During pregnancy, topical therapy is preferred to limit drug exposure in the fetus. Fluconazole has been categorized as class C during pregnancy. These anti-fungal medications, which are available in over-the-counter form, are generally used to treat yeast infections. Treatment may last anywhere between one, three or seven days (Anukam *et al*., 2006).

Before treatment for RVVC, a vaginal culture to confirm the diagnosis and determine the species is strongly advisable. Maintenance antifungal suppressive therapy for 6 months with either ketoconazole 100 mg orally daily, itraconazole 50 to 100 mg orally

daily, clotrimazole 500-mg vaginal suppositories weekly, or fluconazole 150 mg orally weekly for 6 months is recommended. Approximately 50% of women have no symptoms after 6 months of treatment. Those who have recurrent symptoms after 6 months of therapy might need longer courses of maintenance therapy. Recurrent infections with *C. glabrata* can be treated with topical boric acid 600 mg daily for 2 weeks. Topical flucytosine can be used for resistant *C. glabrata* and *C. tropicalis* species. However, it is expensive and prolonged use is associated with resistance. The use of combination therapy has not been studied. Studies have also failed to demonstrate a benefit to treating a woman's partner. Other approaches, such as hormonal manipulation with depot medroxyprogesterone, eating yogurt, and desensitization to *Candida* antigen, lack sufficient data to support recommending their use.

Because asymptomatic colonization occurs in over 20% of women, treatment should be initiated for symptomatic women. Three- to seven-day intravaginal topical formulations effectively treat uncomplicated yeast vaginitis (CDC, 2002). Patients should be advised to apply the medication before going to sleep rather than upon waking to avoid leakage while upright. Topical azole drugs are more effective than nystatin, resulting in relief of symptoms and negative cultures in 80% to 90% of patients who complete therapy. Because the creams and suppositories are oil based, the patient should be advised that it could weaken latex condoms. Unnecessary or inappropriate use of OTC preparations is common and can lead to the delay of treatment of other etiologies. Use of OTC preparations does not induce or increase significantly resistance of yeast to prescription azole therapy, however, although it may be a future concern (Mathema *et al*., 2001). Fluconazole, 150 mg administered as a single dose is the only oral treatment approved

by the US Food and Drug Administration (USFDA) for the treatment of vaginal yeast infection (Pappas *et al*., 2009). This single dose is as safe and effective as traditional, 7-day, intravaginal azole therapies (Sobel *et al*., 1995), and this alternative oral treatment may be considered for adolescents who are uncomfortable with an intravaginal applicator, such as those who have never had sexual intercourse, or are averse to that method, such as those with a history of sexual assault. For patients with recurrent yeast vaginitis, an evaluation for underlying immunosuppressive disorders, such as diabetes mellitus and HIV, should be considered. Environmental changes such as eliminating nylon and tightly fitting undergarments, could have an effect (Sobel *et al*., 1998). Because only high-dose oral contraceptives have been associated with yeast vaginitis, discontinuing the low-dose formulations should be considered only in extreme cases. Empiric treatment of sexual partners is usually not indicated, but for patients with recurrent infection, it may be helpful to examine the male partner for signs of yeast balinitis and treat him accordingly. In women with recurrent yeast infections, defined as four or more symptomatic episodes per year (CDC, 2002), cultures should be obtained to look for species that are not as sensitive to conventional antifungal treatment (Nyirjesy *et al*., 1995). These patients may require longer treatment for up to 2 weeks with intravaginal antifungal medication or with oral fluconazole. In general, patients diagnosed and treated for uncomplicated yeast. Vaginitis does not require follow up unless symptoms persist (CDC, 2002).

2.16 *Candida* Infection

Candida spp are the most common cause of fungal infections (Richards *et al*., 2000), leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Among *Candida* spp, *Candida albicans* is the most common infectious agent.

This dimorphic yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. Non-*C. albicans* species are emerging pathogens and can also colonize human mucocutaneous surfaces (Sobel, 2006). Vaginal colonization by non-*albicans* species is more common in immunodeficient women. However, an increased frequency of infections by non-*albicans Candida* species, particularly *C. glabrata*, *C. parapsilosis* and *C. tropicalis* has been reported in healthy women (Horowitz *et al*., 1992).

2.17 Antifungal Resistance in *Candida* Isolates

In-vitro susceptibility testing for fluconazole by the former National Committee for Clinical Laboratory Standards (NCCLS) now the Clinical Laboratory Standards Institute (CLSI) revealed that 21.1% of vaginal isolates were resistant to fluconazole (Bauters *et al*., 2002). A second large study of 593 vaginal yeast isolates concluded that resistance to fluconazole and flucytosine was observed in frequently (3.7% and 3.0%, respectively), and the more resistant non-*C. albicans* species were more frequently isolated from women with RVVC (Richter *et al*., 2005). Among the different species, elevated fluconazole MICs (≥16 μg/ml) were observed only in *C. glabrata* (15.2% resistant and 51.8% susceptible-dose dependent) and *C. krusei* (41.7%) resistant (considered intrinsically fluconazole resistant) and 50% susceptible-dose dependent). Resistance to itraconazole was observed among *C. glabrata* (74.1%), *C. krusei* (58.3%), *S. cerevisiae* (55.6%) and *C. parapsilosis* (3.4%). These results support the use of azoles for empirical therapy of uncomplicated VVC. Recurrent episodes are more often caused by non-*C. albicans* species, for which azole agents are less likely to be effective (Richter *et al*., 2005). However, *in-vitro* susceptibility testing of *Candida* isolates from women with complicated VVC showed that fluconazole resistance correlated poorly

with clinical response, despite a trend to higher mycological failure rates (Sobel *et al*., 2003). The reasons for clinical improvement of VVC in patients on fluconazole treatment despite isolation of resistant or relatively resistant *Candida* strains remain unclear but could be related to a partial reduction in the vaginal fungal burden. Thus, susceptibility testing is rarely used in the management of VVC. Nevertheless, in patients with refractory VVC or breakthrough episodes, susceptibility testing is imperative to optimize treatment (Shahid and Sobel, 2009). Susceptibilities demonstrated that significant numbers of *C. glabrata* strains are resistant to fluconazole. It is noteworthy that antifungal resistance determination is done on yeast cells in suspension. Patients with indwelling Foley catheters, however, are infected with *Candida* embedded in biofilms. CLSI antifungal testing is done on planktonic logarithmically growing yeast cell populations. In modified antifungal susceptibility testing with *Candida* cells that are biofilm associated and attached to a plastic surface, one can demonstrate that these biofilms are usually resistant to azoles, and a high percentage are even resistant to amphotericin B (AmB), whereas the planktonic phenotype of the same strain remains sensitive to azoles. This may explain persistent or relapsing candiduria in patients without the presence or emergence of antifungal resistance (Sobel *et al*., 2000).

2.18 Bacterial Vaginosis

There are three vaginal infections that are typically classified as vaginitis. The most common of these is bacterial vaginosis. Bacterial vaginosis is caused by an overgrowth of one of several organisms, or bacteria, that are usually present in the vagina. Normally, the "good" bacteria in the vagina outnumber the "bad" bacteria. If, however,

the bad bacteria become too abundant, they can upset the bacterial balance or pH of the vagina, resulting in bacterial vaginosis (Barousse *et al*., 2003).

Bacterial vaginosis (BV) can be thought of as a disturbance in this vaginal ecosystem in which the lactobacilli are replaced by an overgrowth of vaginal commensal organisms. It may be transient or become persistent. It is recognized as the most common cause of abnormal vaginal discharge in women of childbearing age. The symptoms of a thin, white or yellow discharge accompanied by a fishy smell are so characteristic that it is surprising that BV was not widely recognized until described as nonspecific vaginitis (Gardner and Dukes, 1995). Although bacterial vaginosis accounts for more cases, it is less understood than yeast infection or trichomoniasis, the other common type of vaginitis. Left untreated, bacterial vaginosis can lead to the following significant health complications (Barousse *et al*., 2003):

- **Pelvic inflammatory disease**. This is also known as pelvic inflammatory disease (PID), this an infection of the upper genital tract which may lead to infertility.
- **Surgical complications**. Bacterial vaginosis can lead to complications following surgeries such as abortion, hysterectomy and other procedures.

While further study is needed, experts also believe that bacterial vaginosis may be associated with increased susceptibility to human immunodeficiency virus. This virus, often abbreviated as HIV, is the virus that causes acquired immune deficiency syndrome (AIDS). According to the U S Center for Disease Control and Prevention (CDC), bacterial vaginosis affects up to 16 percent of expectant mothers in the United States,

although many are not even aware they have the condition. Pregnant women with bacterial vaginosis are at increased risk of the following:

- Premature delivery
- Postpartum infection
- Post-surgical complications after a cesarean section

Although bacterial vaginosis can be transmitted through sexual intercourse, it is not generally considered a sexually-transmitted disease. Approximately one-quarter of women treated for bacterial vaginosis will have a recurrence within one month (Workowski and Berman, 2006)

2.19 Epidemiology of Bacterial Vaginosis

In many women, the vaginal ecosystem is in a state of flux, changing at different stages of the menstrual cycle. Four studies have looked at self-collected Gram-stained vaginal smears to examine the natural history of vaginal flora. Schwebke and colleagues monitored 51 women considered to be at low risk for sexually- transmitted infection for up to 6 weeks (Schwebke *et al*., 1997). Only 11 of these women had normal flora throughout, with 25 having an intermediate pattern at some point and 13 developing BV. There were significant associations between developing BV and prior BV (44 versus 12%), mean number of lifetime partners (13.4 versus 7.15), and a higher mean number of episodes of receptive cunnilingus (3.6 versus 1.4). Of these, only cunnilingus remained significant in a multivariate analysis. Changes in flora were also associated with menses and use of vaginal medication or spermicide.

Increased abnormalities of vaginal flora occur during the first 9 days of the cycle, and studies have concluded that abnormal flora occurs in most women at some time and that we might therefore need to revise our concept of what is normal. One study examined daily smears from 18 women with recurrent BV (Hay *et al*., 1997). Again, BV usually develop spontaneously early in the menstrual cycle and resolve spontaneously in the second half of the cycle, if it did resolve. Interestingly, in some women, the onset followed episodes of vaginal candidiasis and if anything, BV was more likely to resolve than to appear after unprotected sex with the regular partner. In some women, the onset in resolution of BV occurs within 2 or 3 days, usually after an intermediate stage (Ilkit and Guzel, 2011). The association with resolution of candidiasis is interesting in light of an *in-vitro* study, which demonstrated inhibition of candidal growth by putrescine and cadaverine (Rodriques *et al*., 1999). These amines are produced by *Gardnerella* and other organisms found in BV. An earlier study also reported an association between *Candida* and recurrent BV (Redondo-Lopez *et al*., 1990). There are changes in the vaginal flora over a period of 3 months in a woman with recurrent BV (Hay *et al*., 1997). Another woman in a study had frequent symptomatic relapses of BV over a 10-month period (Hay *et al*., 1997). She received standard treatments for BV and one course of doxycycline and metronidazole for pelvic inflammatory disease during this time. She has had no relapses in the following 4 years. It is not clear what happened to prevent BV from recurring again. During pregnancy, the vagina is not subjected to the frequent changes in hormone levels associated with menstrual cycles. Few observational studies have been performed during pregnancy, but it appears that BV resolves spontaneously in approximately 50% of women in whom it is present at around 16 weeks gestation and may develop in 2 to 3% of those who did not have it at 16 weeks gestation (Hay *et al*., 1994).

Most studies have found an increased prevalence of BV in women of black race compared to those of white race and in those who report cunnilingus, smoking and use of an intrauterine contraceptive device (Sobel, 2007). In community-based studies, BV is more common in women with chlamydial infection and also those undergoing termination of pregnancy. It has also been associated with changing sex partners and high-risk lifestyle (Nilsson *et al*., 1997). These associations suggest that it behaves as a sexually-transmitted disease (STD), but the early study by Bump and Buesching found no difference in the prevalence between virgin and non virgin adolescent women, with clue cells detected in 9% of both groups (Bump and Buesching, 1988). Thus, BV appears to be associated with sexual intercourse and risk of STDs but appears not to be an STD itself. Moreover, BV seems to be common in lesbians, a group at low risk for most STDs. In one study, there was a trend for both members of couples to have either normal flora or BV, suggesting transmission of an etiological agent (Berger *et al*., 1995). Development of BV was associated with smoking, douching and no contraceptive use. In the multivariate analysis, independent risk factors were nonwhite race, intermediate flora on Gram's stain, a lack of H_2O_2-producing lactobacilli, two or more sex partners in the previous 4 months and intercourse more than 3 times/week.

2.20 Pathogenesis of Bacterial Vaginosis

The pathophysiology of BV involves the replacement or reduction of the normal hydrogen peroxide producing *Lactobacillus* species in the vagina. This environmental change allows growth of higher-than-normal concentrations of mixed anaerobic bacteria, including *Gardnerella vaginalis*, *Peptostreptococcus* spp, *Prevotella* spp, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Mobiluncus* spp (Hill, 1993; Schwebke and Lawing, 2001; Mardh, 2004). The cause of BV is not well understood,

but significant associations and predisposing factors have been hypothesized. For example, BV is usually diagnosed more frequently in sexually active reproductive-aged patients. Despite this association, partner treatment is not effective, but the use of condoms during intercourse tends to reduce the symptoms. Because BV is also diagnosed occasionally in virgins, it is not considered to be a sexually-transmitted infection. Women may be more predisposed to developing BV if they lack normal lactobacilli. Douching and use of other chemical ‘‘hygiene’’ products can also alter the vaginal ecosystem and result in BV (Nyirjesy *et al.*, 1995).

2.21 Clinical Presentation of Bacterial Vaginosis

A patient with BV typically presents with a complaint of vaginal discharge with a fishy odour. There are otherwise no complaints of systemic symptoms, such as pain or rashes. Although not required for the diagnosis, there is typically a history of sexual activity (Neal, 2008).

2.22 Diagnosis of Bacterial Vaginosis

A combination of clinical and laboratory findings can be used to diagnose BV. Upon speculum examination, a characteristic thin, gray or white, homogenous discharge can be seen coating the vaginal walls. Because of the lack of lactic acid and hydrogen peroxide production, the vaginal pH is elevated above 4.5. There is no pain associated therefore, the bimanual examination is normal with this condition. The saline wet preparation reveals a lack of lactobacilli and presence of clue cells, which are vaginal epithelial cells that have a stippled or foamy appearance from being studded with bacteria. To classify as a true clue cell, the bacteria should cover the surface of the epithelial cell and spread past the cell boundary, giving a ‘‘shaggy’’ appearance

(Metzger, 1998). Because BV produces a non inflammatory discharge, a normal amount of white blood cells (fewer than 7– 10 per high-power field) is observed. The amines putracine and cadaveracine, volatized from the wet mount sample when potassium hydroxide is added, give a characteristic fishy odour (a positive whiff). BV can be diagnosed by the use of clinical or Gram's stain criteria. Clinical criteria first described by Amsel *et al.* (1983) and recommended by CDC (2002) require three of the four following symptoms or signs:

- Positive whiff
- Greater than 20% per high-power field of clue cells
- A thin, gray or white, non inflammatory, homogenous vaginal discharge that coats the walls
- Vaginal pH above 4.5

Gram-stained vaginal smears as a way of diagnosing BV also have been described (Speigel *et al*., 1983). Because the sensitivity is low, a modification of grading Gram stains based on *Lactobacillus* and other bacterial morphotypes into three grades was developed by Nugent (Nugent *et al*., 1991). Grade I is considered normal, grade II is intermediate and grade III is equated with BV. Grades II and III show strong correlation with the clinical criteria, with sensitivities of 37% and 92% respectively (Taylor-Robinson *et al*., 2003). The use of Papanicolaou-stained smears to diagnose BV also has been described, but is less reliable, is inconvenient because of a minimum 2- to 3-weeks' reporting time, and requires a confirmatory test if positive (Davis *et al*.,1997; Lamont *et al*.,1999). Newer, commercially available tests, such as the Fem Exam test card (Cooper Surgical, Shelton, Connecticut) and Pip Activity Test Card (Litmus Concepts, Inc., Santa Clara, California), detect increased pH and amines and may be helpful for the diagnosis of BV (CDC, 2002).

Diagnosis is made with microscopy mostly by vaginal wet mount and culture of the discharge after a careful history and physical examination have been completed. The colour, consistency, acidity and other characteristics of the discharge may be predictive of the causative agent. Determining the agent is especially important because women may have more than one infection, or have symptoms that overlap those of another infection, which dictates different treatment processes to cure the infection (Vujic *et al*., 2013). For example, women often self-diagnose for yeast infections but due to the 89% misdiagnosis rate, self-diagnoses of vaginal infections are highly discouraged (Anukam *et al*., 2006).

2.23 Treatment of Bacterial vaginosis

Bacterial vaginosis can be difficult to treat, with recurrences as high as 30% to 40% (Briseiden and Hilleier, 1990). The purpose of the current therapy is to decrease the amount of anaerobic bacteria so that the normal *Lactobacillus* has a chance to regrow and stabilize the vaginal environment. The most successful therapy to date for non pregnant women is metronidazole. This drug can be given orally in a dosage of 500 mg twice a day for 7 days (CDC, 2002). Metronidazole is also available as an intravaginal preparation. Unlike trichomoniasis, for BV metronidazole gel is equally efficacious as the oral form. Patients should be advised to avoid alcohol consumption during treatment and 24 hours thereafter to avoid the disulfiram-like reaction. Clindamycin cream and ovules also are used to treat BV, but are less efficacious compared with the metronidazole regimens and should not be used as first line treatment. Again, clinicians should warn patients that the use of oil-based medications may weaken latex condoms. The recommendation for pregnant women with BV is metronidazole, 250mg, three times a day for 7 days (CDC, 2002). Environmental changes, such as avoidance of

douching or other feminine hygiene products and use of barrier contraception, are helpful. Other therapies, such as oral and intravaginal *Lactobacillus* as a way of recolonizing the vagina, have shown varying successes (Van kessel *et al*., 2003). Partner treatment does not appear to affect the disease process in women, and thus is not recommended. Follow-up usually is not necessary for BV, although if it is, one should allow at least 1 month to diagnosis treatment failure or reoccurrence. Long-term maintenance with any regimen is not recommended (Wokowski and Berman, 2006)

The most commonly used antibiotics are metronidazole, available in both pill and gel forms, and clindamycin available in both pill and cream forms (Bradshaw *et al*., 2012). Standard regimens can achieve cure rates between 70% and 90% for confirmed cases of bacterial vaginosis. Oral and topical routes have equal efficacy, and nitroimidazole and clindamycin regimens are also equally efficacious (Bradshaw *et al*., 2012). Vaginal routes have the main advantage of reduced gastrointestinal symptoms, but they have the disadvantage of being inconvenient and being associated with a high risk of vaginal candidiasis. Asymptomatic bacterial vaginosis should only be treated in pregnant women and before elective gynaecologic surgery. Bacterial vaginosis in pregnant women is associated with an increased risk of preterm prelabour rupture of membranes and preterm delivery as well as endometritis and wound infections after cesarean delivery (Foxman *et al*., 2000). Despite the association between bacterial vaginosis and preterm birth, most studies in general obstetric populations have not found that treatment of asymptomatic infection reduced the incidence of preterm labour or delivery (Sobel, 1993). Based on these data, screening and treating all pregnant women who have asymptomatic bacterial vaginosis to prevent preterm birth and its consequences are not recommended by the United States Preventive Services Task Force (USPSTF).

Screening and treatment of bacterial vaginosis could be considered in women with a previous preterm birth so as to lower the rate of preterm prelabour rupture of membranes and low birth weight, because these conditions are associated with maternal and neonatal morbidity (Mahesh and Satish, 2008)

For most women, bacterial vaginosis is simply a nuisance, and the goal of treatment is to relieve symptoms. Doctors commonly treat bacterial vaginosis with metronidazole (Flagyl or MetroGel-Vaginal) or clindamycin (Cleocin). Either can be taken by mouth or applied as a vaginal cream or gel. However, the U S Center for Disease Control and Prevention (CDC, 2002) recommends that all pregnant women with symptoms be treated with oral medications because the medications are safe and work better than vaginal creams or gels. Studies show that a seven-day treatment with oral metronidazole or a five-day treatment with metronidazole vaginal gel is equally effective in non-pregnant women. Clindamycin vaginal cream is slightly less effective than either type of metronidazole.

All women with symptoms of bacterial vaginosis should be treated. Some women also should be screened for bacterial vaginosis even if they don't have symptoms. Pregnant women who are at high risk of preterm labour and delivery should be tested for bacterial vaginosis and considered for treatment if it is detected. Some physicians also recommend that women undergoing certain gynaecological procedures be tested for bacterial vaginosis, and treated even if symptoms are not present. This is because bacterial vaginosis has been associated with the development of pelvic inflammatory disease and other infections after endometrial biopsy, surgical abortion, hysterectomy, intrauterine device placement, caesarean section and uterine curettage (Sobel, 2005).

After therapy, approximately 30% of patients with initial responses have a recurrence of symptoms within 3 months. The reasons include re-infection or, more likely, relapse due to failure to eradicate the organism or failure to re-establish the normal protective vaginal flora dominated by lactobacilli. Resistance has been seen with clindamycin, but metronidazole resistance has not yet been reported. Management of symptomatic relapsed patients should involve prolonged therapy for 10 to 14 days. Vaginal *Lactobacillus* replacement is still considered a research endeavour, and commercially available *Lactobacillus* preparations are not recommended. Maintenance regimen of vaginal metronidazole gel, 0.75%, has shown efficacy in excess of 70% but has a high rate of relapse on cessation of the suppressive therapy. Despite evidence of sexual transmission, no study has demonstrated prevention of recurrence with treatment of male sexual partners (Sobel, 2007)

The reason that some women get BV frequently and others either never gets BV or get it infrequently has yet to be fully defined. Treatment with antibiotics to suppress the anaerobic overgrowth is usually successful in eradicating BV but not necessarily in eliminating the underlying disturbance which allowed it to develop in the first place. In some women, BV may relapse within 2 to 3 weeks of antibiotic treatment. Women with BV have an increased risk of many obstetric and gynaecological complications. These include second-trimester miscarriage and preterm birth, early failure of *in-vitro* fertilization, an increased risk of upper genital tract infection following termination of pregnancy, and an increased risk of infective complications after hysterectomy. In addition, in prospective studies, BV has emerged as a risk factor for acquisition of sexually-transmitted infection, including human immunodeficiency virus (HIV) infection (Hillier, 1998).

2.24 *Gomphrena celosioides*

Gomphrena species is an edible, commercial ornamental plant commonly known as Globe Amaranth or Bachelor Button, belongs to the family Amaranthaceae. It comprises approximately of 120 species found in the Antartica and Indo-Malaysia and hence they are believed to have better compatibility in America, 46 species are found in Brazil (Dosunmu *et al*., 2010). The chemical and medicinal constituents present in the herbal medicine or plants are drugs with pharmacological potency, the major part of the physiological functions of living flora.

2.24.1 Morphology of *Gomphrena celosioides*

The Amaranthaceae is a wide spread family which occurs at disturbed, arid or saline areas; one of the characteristics that ensure its survival in adverse environments is the operation of C4 pathway of photosynthesis (Bao and Bacon, 2004). It is erect or ascending herb to 50cm. leaves spiral, deltoid to elliptic ovate. Inflorescence is axillary, slender, thyrsiform spike. Flowering mostly occurs in June to December. Globe amaranth (*Gomphrena globosa L*) might be one of the plants that can use atmospheric sulfides for its growth (Andrade *et al*., 2012; Wang *et al*., 2009).

2.24.2 Description of *Gomphrena celosioides*

It is an annual or short-lived perennial weed, mainly prostrate, with a deep taproot. Leaves opposite on very short, hairy petioles, elliptical, entire, pubescent, 3–4 cm long, about 1 cm wide. The inflorescences are dense terminal spikes, initially round but lengthening in maturity up to 4 cm long and 1 cm thick. The individual flowers are whitish or pink, 5–6 mm long on a densely woolly receptacle. Fruit one-seeded, the seed 1.5 mm long, lenticular, brown and glossy. *Gomphrena celosioides* C. Mart

(Amaranthaceae) is an annual or perennial herb with erect stem ascending to 1-2 dm long, rather sparsely branched, appressed pilose with white silky hair. The leaves are oblong-Ianceolate to oblong obovate, 1.5-4.5 cm long and 0.5-3 wide. The flowers are terminal and axillary, cylindrical spikes 1-2cm long.1cm in diameter (Wagner *et al*., 1999). It is well distributed from South America to Asia, East and West Africa.

2.24.3 Taxonomy and classification *Gomphrena celosioides*

The amranthaceae family, class Magioliopsida and order Caryophyllales, was established by A. L. Jussieu in 1789 and includes approximately 176 genera and 2400 species (Dias *et al*., 2004). The members of amaranthaceae family are divided into four tribes; Celosieae, Achyrantheae, Brylineae and Gomphreneae. The species in Brazil are distributed in Celosieae, Achyrantheae and Gomphreneae tribes (Salvador *et al*., 2002).

2.24.4 Classification of *Gomphrena celosioides*

Kingdom	Plantae
Sub kingdom	Tracheobionta
Superdivision	Spermatophyte
Division	Mangioliophyta
Class	Mangioliopsida
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Amaranthaceae
Genus	Gomphreneae



Plate I: *Gomphrena celosioides*

2.24.5 Distribution of *Gomphrena celosioides*

Gomphrena celosioides is native in the Americas-Argentina, Bolivia, Brazil, Paraguay Uruguay. It has naturalized in Asia (Bhutan, Indonesia, Philippines, Singapore, Sri Lanka, Papua New Guinea, Taiwan and Thailand), Africa (Botswana, Ghana, Lesotho, Namibia, Swaziland, (Zimbabwe), and Australia (Reed, 1977; Holm *et al*., 1979; Grierson and Long, 1984; Kostermans *et al*., 1987; Moody, 1989; NGRP, 2002).

2.24.6 Biology and ecology *Gomphrena celosioides*

In Indonesia, *Gomphrena celosioides* is a plant that occurs along grassy roadsides and upland rice fields in high-rainfall areas up to 1,300 m elevation (Kostermans *et al*., 1987). In Thailand it is described as preferring dry conditions (Noda *et al*., 1985). The seeds are distributed by ants.

The Amaranthaceae family comprises many species with biological activities, which are used in nutrition and alternative medicine (Ahmad *et al*., 1998). This family includes approximately 65 genera and 1000 species and many species of Gomphreneae tribe

have been shown antimicrobial activity, such as *Blutaparon portulacoides* (Salvador *et al.*, 2002); *Gomphrena martiana*, *G. boliviana* (Pomilio *et al*.,1992, Pomilio *et al*.,1994).

Gomphrena celosioides belongs to the Amaranthacea family and over 120 species of the family are found in America, Australia and Indo-Malaysia, while 46 species occur in Brazil. Few species occur in the East and West of Africa (Vieira *et al*., 1994). *G. celos ioides* is an annual documbent ascending herb up to 30cm tall, branches clothes with shaggy white hairs, is used as antimalarial against *Plasmodium falciparum* in traditional medicine system of Ghana (Kohler *et al*., 2002). Alcohol extracts of *G. celosioides* is reported to be diuretic (Dhawan *et al*., 1997) and antimicrobial (De Moura *et al*., 2004). *Gomphrena* species in different parts of the world are used for various folkloric medicinal purposes. In Brazil, some species are employed in the treatment of bronchial infections, diarrhea and malaria fever, while others had found application as analgesic, tonic/carminative and diuretics (Gessler *et al*., 1994; Vieira *et al.*, 1994).

2.24.7 Uses of *Gomphrena celosioides*

Gomphrena celosioides is used in ethnomedical practice in Nigeria for treatment of various skin diseases, worms' infections and infectious diseases. In South America, the plant is utilized as an abortifacient (Burkill, 1985). A decoction of the whole plant and a related species *Gomphrena globosa* is applied to gangrenous wound. *G. martiana* and *G. boliviana* are employed as antimicrobial agents by the natives (Arenac and Azorearo, 1977).

Gomphrena martiana and *Gomphrena boliviana* which have folk medicinal usage had been proved to have pronounced antimicrobial activities (Pomilio *et al.*, 1992).

Flavones isolated from the plants were responsible for the antimicrobial activity. In South America, it is used as abortive (Burkill, 1985). In Nigeria, it is used for the treatment of dermatological problems (Onocha *et al*., 2005). In Benin, traditional healers use this plant in the treatment of many diseases including liver diseases, malaria and dysmenorrheal (Adjanohoun *et al*., 1989). Vieira *et al*. (1994) has demonstrated the analgesic, tonic, carminative and diuretic properties of this plant. Recently, Dosumu *et al.* (2010) reported its antimicrobial and anti-helminthic properties. The work of Botha and Gerristsma-Vander-Vijer. (1986) revealed the presence of saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids in this plant. These results were confirmed by De moura *et al*. (2004). *Gomphrena* species are employed in folk medicine for the treatment of several diseases and for their nutritive value. These species and its biological activities, including antimicrobial activity, used to treat gastrointestinal and respiratory disorders as well as urinary tract infection (Salvador *et al*., 2002).

2.24.8 Phytochemical properties of *Gomphrena celosioides*

Dosumu *et al*. (2010) isolated the bioactive compounds like saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids from the methanol extract of *G. celosioides.* The ethyl acetate and methanol extracts of *Gomphrena celosioides* was phytochemically examined by (Dosunmu *et al*., 2010) and revealed the presence of secondary metabolites. These metabolites include alkaloids, tannins, saponins, steroids, glycosides and reducing sugars. *Gomphrena celosioides* revealed the presence of saponins, steroids, amino acids and non reducing sugars in all the plant parts, phenols and flavonoids in leaves, inflorescence and stem. Betacyanins occurred only in the stem;

reducing sugars in the inflorescence, while ketoses were reported found in the root and stem (Botha and Gerritma-Vander-Vijer, 1986).

2.24.9 Antibacterial activity of *Gomphrena celosioides*

The ethyl acetate and methanolic extracts of *G. celosioides* displayed inhibition activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The methanol extract was active against *Candida albicans*, *Aspergillus niger* and *Trichophyton* spp with diameter zones of inhibition between 14mm and 20mm. The methanolic extracts of the leaf, stem and root of *G. celosioides* were investigated by Sharma and vijayrergia (Sharma and Vijayresia, 2011) for antibacterial activity and were found to be most effective against the three tested bacteria, *S. aureus*, *P. aeruginosa* and *E. coli*. (Abalaka and Damisa, 2013) carried out the antifungal activities of *G. celosioides* extracts at different concentrations against *A. niger*, *C. albicans* and *T. rubrum*. The methanol extracts had a fungicidal effect on the fungal isolates at a concentration of 2000mg/ml. The minimum in hibition concentration ranged from 2000mg/ml to 1500mg/ml Dias *et al*. (2004). Dosumu *et al*. (2010) screened ethanolic extract and pure compounds of this plant for antimicrobial activity using Kirby-Bauer method. Their results showed significant activity against *S. aureus* and *S. typhi*. Dosumu *et al*. (2010) found that ethyl acetate and methanol extracts exhibited antihelminthic activities against *Pheretimia pasthuma*, *Fasciola gigantic* and *Taenia solium*. Methanol extract exhibited pronounced antifungal activitiy.

2.25 *Vernonia*

Vernonia (Asteraceae) is the largest genus in the tribe Vernoniae with close to1000 species (Keeley and Jones, 1979). The genus, *Vernonia*, is named after William Vernon,

an English botanist who collected and identified this genus in Maryland in the late 1600s before his death in 1711 (Quattrocchi, 1999). The genus is distributed both in the new and Old Worlds although it is to be found mostly in the tropical regions. *Vernonia* species grow in a wide range of habitats of broad ecological diversity and climatic conditions including tropical forest, marshes and wet areas, dry plains, tropical savannahs, desert xeric or dry sites and even frosty regions of eastern North America (Gleason, 1923; Keeley and Jones, 1979). The genus is morphologically made up of annuals, herbaceous perennials, lianas, shrub, and trees. The genus *Vernonia*, is known for having several species with food, medicinal and industrial uses. For example, *Vernonia amygdalina* and *Vernonia colorata*, is eaten as leafy vegetables (Burkill, 1985; Iwu, 1993). *Vernonia* species frequently used in ethno-medicine include *Vernonia amygdalina*, *Vernonia condensata*, *Vernonia cineria*, *Vernonia guineensis* and *Vernonia conferta*. *Vernonia galamensis* is used industrially for its seed oil contents. *Vernonia amygdalina* is the most studied member of the *Vernonia* genus as well as one of the most studied plants in Africa (Ijeh and Ejike, 2011). *Vernonia amygdalina* is a shrub of the savannah as well as forest areas throughout tropical Africa (Burkill, 1985). This species and *Vernonia colorata* are very similar in appearance and are not easily distinguished. *Vernonia amygdalina* leaves have a characteristic odour and a bitter taste, which can be reduced by boiling and discarding the water or soaking and washing in several changes of water before consumption. Analysis showed that the plant is rich in nutrients including amino acids, minerals and vitamins (Alabi *et al*., 2011; Ejoh *et al*., 2007; Eleyinmi *et al*., 2008)

2.25.1 Ethnopharmacognostic relevance of *Vernonia* species

A total of 109 *Vernonia* species were reported in the literature to have medicinal properties. One hundred and five plants were linked to the treatment or management of 44 human health conditions (Adjanohoun *et al*., 1989). Plants of the genus also feature in ethnoveterinary and zoopharmacognostic practices. A total of 12 *vernonia* species were identified to be used in ethnoveterinary medicine while 2 species are used in self medication practices by chimpanzees and gorillas (Ngeh and Toyang, 2013). One hundred and three bioactive compounds isolated from various *Vernonia* species were also identified (Burkill, 1985). *Vernonia amygdalina* was identified as the most frequently used member of the *Vernonia* genus. The Vernolides, a class of sesquiterpene lactone were identified as the most studied compounds from the genus and show interesting bioactivity in antiplasmodial, antileishmanial, antischistosomial, cytotoxicity, antimicrobial and anti-inflammatory assays (Ngeh and Toyang, 2013).

2.25.2 Description of *Vernonia perrottetii*

Annual herb of 20–100 cm tall. Stems simple, usually branching only at the apex but sometimes with numerous short branches, densely leafy, finely-ribbed, appressed-pubescent; the hairs are flagelliform; branches ascending, 4–25 cm long, less densely leafy than the stem. Leaves crowded, appressed-ascending, overlapping, up to 7 cm long, usually shorter, filiform to linear, revolute, scabridulous (Burkill, 1985).



Plate II: Picture of *Vernonia perrottetii*

2.25.3 Uses of *Vernonia perrottetii*

This plant is used as purgative in West Africa (Burkill, 1985). Also used in dyes and tannins as medicinal plants spices and condiments vegetables (Burkill, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Background Information on Study Area

The study area included Ahmadu Bello University Health Service (Sick Bay) and Samaru Clinic, Zaria, Kaduna State, Nigeria. Ahmadu Bello University Health Service was established in 1968 and serves staff, students and neighbouring communities (Paul and James, 1977). Present location of the sick bay is adjacent to Suleiman hall and covers a total of over 2600 square meters. Samaru Clinic is a community-based and Primary Health Center owned by Kaduna State Government mainly for antenatal patients and other basic needs.

3.2 Study Design

This study captured women with presentation of vulvovaginal itching and volunteers. The volunteers were randomly selected for sampling with special reference to vaginitis caused by *Candida* species and *Staphylococcus* species among women attending Ahmadu Bello University Sick bay and Samaru Clinic, Zaria, Kaduna State, Nigeria. The high vagina (HVS) samples were collected between the months of May and August, 2012. Ethical clearance was granted by the University's Health Service management and informed consents from the patients were obtained prior to sampling.

3.3 Questionnaire Administration

Three hundred questionnaires were administered to 300 patients attending Ahmadu Bello University Health Service and Samaru Clinic to get information about the socio-

demographic behavours of patients towards these diseases. The information obtained was strictly for academic purpose.

3.4 Study Population

The study was carried out among women attending Ahmadu Bello University Health Service (Sick Bay) and Samaru Clinic, Zaria. The study targeted women with complains and volunteers between the age goups of 15-50 years.

3.5 Determination of Sample Size

The sample size was determined using the equation described by Naing *et al. (*2006).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

n= number of sample

p= prevalence rate of contamination of previous study (21.5% prevalence by Usanga *et al*., 2010).

z= standard normal distribution at 95% confidence limit=1.96

d= absolute desired precision of 5% =0.05

$$n = \frac{1.96^2 (0.215) (1-0.215)}{0.05^2}$$

n = 259.3.

However, 300 high vaginal swabs from 300 patients were collected.

3.6 Plant Collection and Authentication

The plant materials were obtained from the Botanical Garden and environment of the main campus of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The plants

were brought to the Department of Biological Sciences for identification. The whole plants of *Gomphrena celosioides* with a voucher number of (864) and *Vernonia perrottetii* with a voucher number of (1648) were air dried for 2-3 weeks in the shade and powdered using a wooden mortal and pistle. The powdered materials were stored in an air tight container for future use.

3.7 Extraction of Plant Materials

The extraction of the whole plant material of *Gomphrena celosioides* and *Vernonia perrotteetii* was extracted using standard procedures (Harborne, 1984). The powdered materials were exhaustively extracted using distilled water and methanol. A total of 100g powdered sample of the whole plant each were separately macerated in 1000ml distilled water for 24hrs and 70% methanol for 3 days, respectively, to obtain aqueous and methanol extracts of each plant for use in the analysis. Each extract was filtered and solvent evaporated under reduced pressure in a rotary evaporator and weighed.

3.8 Phytochemical Screening of the aqueous and methanolic extracts of *Gomphrena celosioides* and *Vernonia perrottetii*

Phytochemical screening of aqueous and methanol extracts of *Gomphrena celosioides* and *Vernonia perrottetii* were carried out using standard phytochemical procedure of (Ayoola *et al*., 2008; Sangetha *et al*., 2008; Mikail, 2010). The following tests were conducted to identify the chemical constituents.

3.8.1 Test for carbohydrates

Exactly 2ml of Molish's reagent and 2ml of concentrated sulphuric acid (H_2SO_4) were added to 2ml boiling methanolic extract and aqueous extract of *V. perrottetii* and *G. celosioides.* A reddish ring indicated the presence of carbohydrates.

3.8.2 Test for reducing sugars

Exactly 2ml of methanolic extract and aqueous extract of *V. perrottetii* and *G. celosioides* were added to boiling Fehling's solution for 5 minutes. A brick-red precipitate indicated the presence of reducing sugars.

3.8.3 Test for Tannins

Exactly 2ml of methanolic extract and aqueous extract of *V. perrottetii* and *G. celosioides*, as well as 1ml of ferric chloride ($FeCl_3$) were added and blue-black precipitate indicated the presence of tannins.

3.8.4 Test for saponins

Exactly 2ml of methanolic and aqueous extract of *V. perrottetii* and *G. celosioides*, as well as 5ml of distilled water were added and the solution shaken vigorously for 30 seconds. Stable persistent frothing indicated the presence of saponins.

3.8.5 Test for flavonoids

Magnesium ribbon and few drops of concentrated HCl were added to 2ml of methanolic and aqueous extract of *V. perrottetii* and *G. celosioides*, Pink colour indicated the presence of flavonoids.

3.8.6 Test for alkaloids

Exactly 10ml of ammoniacal chloroform solution were added to 2ml of methanolic and aqueous extract of *V. perrottetii* and *G. celosioides*. The extract was then treated with 10 drops of 10% sulphuric acid and tested with Meyer's reagent. Formation of white precipitate indicated the presence of alkaloids.

3.8.7 Test for phenols

Exactly 2ml of methanolic and aqueous extract of *V. perrottetii* and *G. celosioides* 0.5ml of Folin-cicocalteau reagent and 2ml of 20% of Na_2CO_3 were added. The presence of bluish colour indicated the presence of phenols.

3.8.8 Test for anthraquinones

Exactly 2ml of methanolic extract of methanolic and aqueous extract *V. perrottetii* and *G. celosioides*. As well as 2ml of 10% NH_4OH were added. A bright pink colour indicated the presence of anthraquinones.

3.8.9 Test for steroids

Exactly 2ml of methanolic and aqueous extract of *V. perrottetii* and *G. celosioides* were dissolved in sterile distilled water. As well as 2ml of chloroform, acetic acid and 1ml of concentrated H_2SO_4 were added. A blue-green indicated the presence of steroids.

3.9 Preparation of Culture Media

3.9.1 Preparation of Sabouraud Dextrose Agar

Sabouraud Dextrose Agar was prepared by dissolving 47g of the powdered medium in 1L of distilled water by heating. The medium was then sterilized by autoclaving at 121^0C for 15 minutes. After which 15ml of the sterile medium was dispensed into sterile Petri-dishes and allowed to set for 15mins after which the plates were stored at 4^0 C in the refrigerator for future use (NCCLS, 2000).

3.9.2 Preparation of Blood Agar

Blood agar was prepared by dissolving 14g of nutrient agar in 480ml of distilled water, after which it was autoclaved at 121^{o}C for 15 minutes. The medium was allowed to cool to about 50^{0}C after which 20ml of human blood was added aseptically and stirred gently to mix aseptically and therefore dispensed into petri-dishes (NCCLS, 2000)

3.9.3 Sample collection and isolation

Commercially available sterile swab sticks were used to obtain high vaginal swabs from women attending the selected hospitals Zaria, Nigeria. This was achieved with the assistance of medical personnel in the hospitals and transported immediately in a cool box to the Department of Microbiology laboratory, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna, State Nigeria. The swabs were inoculated onto the surface of Sabouraud Dextrose Agar plates (SDA) for *Candida* species as well as on Mannitol Salt Agar for the isolation of *Staphylococcus* species and incubated at 29^{0}C for 3 days for *Candida* spp and at 37^{0}C for the *Staphylococccus* spp. Distinctive colonies that was creamy white with fermentative odours were further subcultured on to sabouraud dextrose agar media as well as the yellow to golden and dirty white colour on nutrient agar to further purify them.

3.9.4 Gram's staining

At the end of incubation, colonies were stained by Gram's staining techniques and viewed under the microscope for yeast like organisms and a grape-like, singly, pairs and clusters appearance organism for *Staphylococu*s species. Smears were prepared for suspected *Candida* species as well as the *Staphylococcus* species, respectively, and fixed by flaming. Then, the smear were stained with crystal violet for 1minute, rinsed

with water, it was then flooded with Gram's iodine for another 1 minute and then rinsed with water. The smear was decolourized with acetone for 10 seconds and rinsed again with water. Finally, the slide was flooded with safrannin for 1second and then rinsed with water, allowed to air dry after which it was observed under microscope at ×40 objective for the detection of strongly Gram-positive budding yeast cells and at ×100 objective, for a coagulase-positive and negative *Staphylococcus* sp using the procedure of Fox and Behets, (1995).

3.9.5 Identification and characterization of *Candida* and *Staphylococcus* isolates

Candida isolates were identified using API 20C System (Analytab Products, France) according to earlier procedures (Espinel-Ingroff *et al*., 1998). Germ tube test was also performed for the confirmation of *Candida albicans*, while *Staphylococcus* species isolates were confirmed by inoculating the suspected organisms onto a blood agar plate for haemolytic reaction, coagulase test for detection of coagulase positive *Staphylococcus* spp, catalase test (ability of the organism for conversion of hydrogen peroxide to water and oxygen) as well as DNase test mainly for *S. aureus* (Harrigan, 1998).

3.9.6 Confirmatory test (germ tube formation)

Approximately 0.5ml of sheep serum was dispensed in to a micro titer plates and each well was labeled and inoculated with loopful of light suspension of the yeast isolates and incubated at 37^0C for 3hrs after which a loopful of the inoculated serum was place on a clean glass slide and observed at x100 objective. Germ tubes formation by some of the isolates confirmed *Candida albicans* (Cheesbrough, 1984)

3.9.7 Haemolysis of blood using blood agar

A quantity of 20ml whole blood was added aseptically onto a prepared nutrient agar medium. The content was gently mixed together before pouring into sterile Petri dishes. The plates were allowed to stand for 15mins, the plates were labeled appropriately. A 24hrs culture of each suspected *Staphylococcus* sp was gently streaked onto blood agar plate. Clear zones around the colonies with appearance of green colour confirmed the organism to be alpha- haemolytic while clear zones around the colonies without green appearance confirmed the organisms to be beta- haemolytic *Staphylococcus* spp (Harrigan, 1998)

3.9.8 Coagulase test

Coagulase test was done by placing 0.5ml of human serum on a clean glass slide. An overnight culture of the suspected organism was mixed with the serum using a sterile wire loop. The presence of clotting confirmed the organism to be coagulase positive and absence of clotting confirmsed coagulase-negative *Staphylococcus* spp.

3.9.9 Catalase test

Catalase test was done by placing 2 drops of hydrogen peroxide (H_20_2) on a clean glass slide. An overnight culture of the suspected organism was mixed with the H_20_2 using a wooden applicator. Gas formation in form of bubbles confirmed the organism to be catalase positive while absence of gas formation confirmed catalase negative organism (NCCLS, 2000).

3.9.10 DNase test

DNase Agar was prepared according to the Manufacturer's instruction after which it was aseptically poured into sterile petri dishes and allowed to set before the suspected *Staphylococcus* sp was gently streaked onto the agar plate and incubated at 37^0C for 24hrs after which it was flooded with 3% hydrochloric acid. A clear zone around the streak confirmed the organism to be DNase-positive while no clear zone confirmed the organism to be DNase-negative (NCCLS, 2000)

3.10 Preparation of MacFarland Standard

This was done by preparing 1% $Bacl_2$ and 1% H_2SO_4. Exactly 1g of $BaCl_2$ was dissolved in 100ml of distilled water to make 1% $BaCl_2$. Exactly 1ml of H_2SO_4 was dispensed in 99ml of distilled water to make 1% H_2SO_4. Thereafter, an 18hr culture of the organism was inoculated into a sterile distilled water to compare with the standard (NCCLS, 2002).

3.10.1 Antimicrobial susceptibility testing of the isolates

This was done using agar well diffusion method of the National Committee for Clinical Laboratory Standards NCCLS (2002). The washed overnight broth culture were diluted appropriately using sterile distilled water to 1.0 McFarland scale (1.0×10^8cfu/ml) for fungi as well as 0.5×10^6cfu/ml MacFarland Standard for bacteria. Sabouraud Dextrose Agar (15 ml) was poured into sterile Petri dishes and allowed to set. Nutrient agar plates in the case of *Staphylococcus* spp was used. The sterile Sabouraud Dextrose Agar and Nutrient Agar plates were each flooded with 0.1ml of the standardized isolates of *Candida* and *Staphylococcus* isolates. These were spread uniformly using spread plate method. Wells of 6mm diameter were bored on the agar media using a sterile cork borer

(No. 1). Aqueous solution of five standard antifungal drugs namely; nystatin, ketoconazole, fluconazole, mycotene and itraconazole and three antibacterial drugs namely; ciprofloxacin, erythromycin and amoxicillin (Sweetman, 2005) were prepared, which served as positive controls. Exactly 0.1ml of the different concentrations of the standard antimicrobial drugs was placed in each well. One of the most recommended drugs in the hospitals were selected to compare with the extracts.

Exactly 0.1ml of the different concentrations (1000mg/ml, 500mg/ml, 250mg/ml and 125mg/ml) of the extract was placed in each well in the agar medium containing the culture singly and in combination using a sterile Pasteur pipette. The plates were allowed to stand for one hour at room temperature to allow diffusion of the substrates to proceed before the growth of the organisms commenced. The plate was finally incubated at room temperature (29^0C) for 48hours for *Candida* spp and at 37^0C for *Staphylococcus* spp. The presence of zone of inhibition around the hole containing the extract as well as the antimicrobial drugs indicates the antimicrobial activity against the test organism. This was measured using a transparent ruler and expressed in terms of zones of inhibition (mm) (Ahmad and Agil, 2007).

3.10.2 Determination of Minimum inhibitory concentration and minimum bactericidal concentration of the plant extracts.

The least concentration that showed zones of inhibition from susceptibility testing was used to determine the minimum inhibitory concentration of the extracts. The least concentration that showed zone of inhibition for sensitivity was at 500mg/ml. From this concentration, serial dilution was carried out further for the MIC. These concentrations in sterile Meuller- Hinton Broth were prepared in a test tube using double dilution method. Exactly 1ml each of the standardized organisms was taken and inoculated into

a prepared Meuller-Hinton Broth in a test tube and the inoculum was allowed to diffuse into the broth agar test tubes for 30 mins after which it was incubated at 37^0C for 24hrs. The lowest concentration of the extract in the test tube that showed clear zone of inhibition was considered as the MIC of the extract against the *Staphylococcus* species (NCCLS, 2000)

3.10.3 Determination of Minimum bactericidal concentration of the extracts

A loopful of the broth culture from the MIC test tube was inoculated onto Nutrient Agar plate. The plates were incubated at 37^0C for 24hr, after which they were examined for colony growth. Absence of growth indicates that the plant extract was bactericidal while presence of growth indicated that it was bacteriostatic (NCCLS, 2000)

3.11 Statistical Analysis

Chi square was used to test for the association between the risk factors and vaginitis (SPSS, version 11.0)

One way ANOVA was also used to compare the mean zones of inhibition due to the extracts.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical characteristics of the plant extracts

The phytochemical screening of methanol and aqueous extraction of the plant extracts showed that carboghydrates, reducing sugars, tannins, saponins, flavonoids, alkaloids and cardiac glycosides was present in the plants, while phenols and anthraquinones was absent. Steroid and triterpenes was present only in *Vernonia perrottetii* crude extracts (both methanol and aqueous) while absent in *Gomphrena celosioides* (in both methanol and aqueous extracts). Reducing sugars of both methanol and aqueous extracts of *G. celosioides* and the combination were more compared to *V. perrotteetii*. Tannins were also more in *V. perrottetii* compared to *G. celosioides* both singles and in combination of the *G. celosioides* and *V. perrottetii*. Saponins were more in *V. perrottetii* and in combination compared to *G. celosioides*. Alkaloids were more in *G. celosioides* and in the combination of the two plant extracts compared to *V. perrotteetii*. (Table 4.1)

4.2 Distribution and relationship between age and vulvovaginitis caused by *Candida* spp and *Staphylococcus* spp

Out of the 300 high vagina swabs samples collected, 153(51%) were positive for both *Candida* spp and *Staphylococcus* spp. A breakdown of the prevalence showed that *Candida* species was isolated from 79 (26.3%), while *Staphylococcus* species was isolated from 74 (24.7%). There was a significant ($p<0.05$) association between age and vaginitis caused by *Candida* spp and *Staphylococcus* spp. The highest prevalence was observed in the age group of 21-30 years of age (69.44%), followed by 15-20 (45.45%) and 31-40 (43.33%) (Table 4. 2).

4.1 Phytochemical characteristics of the methanolic and aqueous extracts of *G. celosioides* and *V. perrottetii*

	Methanol extracts			Aqueous extracts		
Phytochemicals	*Gomphrea celosioides*	*Vernon*ia *Perrotetti*	Combination of *G.celosioides* and *V.perrotetti*	*Gomphrena celosioides*	*Vernon*ia *perrottetti*	Combination of *G.celosioides* and *V. perrottetti*
Carbohydarate	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Phenols	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Steroids and Triterpenes	-	+	-	-	+	-
Cardiac glycoside	+	+	+	+	+	+

Key: + = positive - = Negative

Table 4.2 Distribution and relationship between age and vulvovaginitis caused by *Candida* spp and *Staphylococcus* spp in women attending A.B.U sick bay and Samaru clinic, Zaria, Nigeria

Age (years)	Total no sampled	No of positive %
15-20	101	46(45.54)
21-30	108	75(69.44)
31-40	60	26(43.33)
41-50	31	6(19.35)
Total	300	153(51.0)

$\chi^2 = 29.74$ $p < 0.0001$

4.3 Distribution and Relationship between Age and Vaginitis Caused by *Candida* spp

The distribution and relationship between age and vaginitis caused by *Candida* spp showed no significant ($p>0.05$) association. The highest prevalence (50%) was observed in the age group of 21-30 years, followed closely by those aged 31-40 years (40%) (Table 4.3)

4.4 The distribution and relationship between age and vaginitis caused by *Staphylococcus* spp

Significant ($p<0.05$) association occurred between the age of the respondents and prevalence of vaginitis caused by *Staphylococcus* spp. The highest prevalence (95%) was observed in the age groups of 15-20 years and 31-40 years (95%), followed by 21-30 (93.75%) (Table 4.4)

4.5 Relationship between risk factors and vaginitis

There was no significant ($p>0.05$) association between marital status and vaginitis. The highest prevalence was observed among the married women (51%) while single had (50.31%). There was significant ($p<0.05$) association in the use of birth control pills and vaginitis. The highest prevalence was, however, observed among those who used birth control pills (91.11%) and lowest prevalence was observed among those who did not use birth control pills (33.81%). There was also a significant ($p<0.05$) association among patients wearing tight clothing and vaginitis. The highest prevalence was observed among patients who wear tight clothing (79.31%) while those who did not wear tight clothing (44.21%). There was significant ($p<0.05$) association among those who used tight underwear (67.80%) while those who did not wear tight underwear

(46.89%). There was also a strong association between pregnancy status and vaginitis. The highest prevalence was observed among pregnant women (74.23%) and the lowest was observed in non-pregnant women (39.90%). There was no significant ($p>0.05$) association in the used of vagina cream and vaginitis. Highest prevalence occurred among those that use vaginal cream (61.54%) while those that did not use cream had 48.79%. There was no significant ($p>0.05$) association in the use of antibiotics and vaginitis. The highest prevalence was observed among those who did not use antibiotics (52.84%) compared to those that used antibiotics (48.39%) (Table 4.5).

4.6 Gram's reaction behaviours, Germ Tube, and Arthrospore Formation of *Candida* species on Corn Meal Agar

A total of 97(32.335%) isolates of *Candida* spp were obtained from the 300 samples, 79(26.33%) were pure for yeast after subculture for further purification. Six species were present for suspected *C. albicans* [6(7.6%)] and 33 produced arthrospore 33(41.8%) as represented in (Table 4.6).

4.7 Distribution of *Candida* species based on Biochemical Characterization Using API 20c Aux

A total of 50 suspected yeast isolates were subjected to biochemical characterization for further confirmation using API 20 C AUX. The most prevalent yeast isolates was *Candida tropicalis* with (4.33% 26%) out of 13 isolates, followed by *Candida albicans* and *Candida glabrata* (1%/6%) out 3 isolates and (1%/6%) out of 3 isolates respectively. *Candida parasiplosis* and *Candida lambica* had the same number of isolates (2%/0.33%) out of 1 isolate and (2%/0.33%) out of 1 isolate respectively. The results are presented in Table 4.7.

Table 4.3 Distribution and Relationship between Age and Vaginitis Caused by *Candida* spp in women attending A.B.U sick bay and Samaru Clinic, Zaria, Nigeria

Age (years)	Total no sampled	No of positive (%)
15-20	81	27(33.33)
21-30	60	30(50)
31-40	40	16(40)
41-50	19	6(31.58)
TOTAL	200	79(39.50)

χ^2 =4.560 P = 0.2070 df = 3

Table 4.4 Distribution and relationship between age and vaginitis caused by *Staphylococcus* spp in women attending attending Samaru Clinic

Age (Years)	Total no sampled	No of positive %
15-20	20	19(95)
21-30	48	45(93.75)
31-40	20	10(95)
41-50	12	0(0.0)
Total	100	74(74)

$\chi^2 = 54.46$ $p < 0.0001$

Table 4.5 Relationship between Risk factors and vaginitis in women attending A.B.U sick bay and Samaru clinic, Zaria, Nigeria

Factor		No examined	No /%positive
Marital status	Single	161	81(50.31)
	Married	139	72(51.80)
Total		300	153(51.0)
$\chi^2 = 0.06609$	p = 0.7971	Df =1	
Use of perfume	Spray	7	0(0)
	Don't spray	293	153(52.22)
Total		300	153(51.0)
Use of birth control pill	Use	90	82(91.11)
	Don't use	210	71(33.81)
Total		300	153(51.0)
$\chi^2 = 82.78$	p< 0.0001	OR =20.07	CI =9.192 to 43.79
Use of tight clothing	Wear	58	46(79.31)
	Don't wear	242	107(44.21)
Total		300	153(51.0)
$\chi^2 = 23.06$	p<0.0001	OR = 4.836	CI=2.440 to 9.587
Use of tight underwear	Wear	59	40(67.80)
	Don't wear	241	113(46.89)
Total		300	153(51.0)
χ^2=8.292	p = 0.004	OR= 2.385	1.306
Total			
Pregnancy	Pregnant	97	72(74.23)
	Not pregnant	203	81(39.90)
Total		300	153(51.0)
χ^2= 30.95	p< 0.0001	OR = 4.338	CI = 2.541 to 7.405
Use of cream	Use	52	32(61.54)
	Don't use	248	121(48.79)
Total		300	153(51.0)
χ^2= 2.796	p = 0.0945	OR = 1.679	CI =0.9108 to 3.096
Use of antibiotics	Use	124	60(48.39)
	Don't use	176	93(52.84)

Total		300	153(51.00
χ^2= 0.5774	p = 0.4473	OR = 0.8367	CI = 0.5281 to 1.326

Table 4.6 Gram's reaction behavior Arthrospores and Germ Tube Formation in *Candida* species isolated from women attending A.B.U sick bay and Samaru clinic, Zaria, Nigeria

	Total No	% of n=79**	% of n=300***
Germ tube formation	6	7.6	6
Arthrospore formation	33	41.8	11

Key: *= Total number of Germ tube formation, **= Total number of isolates, ***= Total number of samples.

Table 4.7 Distribution of *Candida* Species (Using API 20c Aux) isolated from women attending A.B.U sick bay and Samaru clinic, Zaria, Nigeria

	Total isolates		No of isolates	%
Organism isolated	Sick bay	n=50*	n=79**	n=300***
Candida tropicalis	13		26	4.33
Candida glabrata	3		6	1
Candida albicans	3		6	1
Candida parasiplosis	1		2	0.33
Candida lambica	1		2	0.33

Key: *= Total *Candida* sp subjected for biochemical characterization

**= Total sample positive for yeast

***= Total samples collected.

4.8 Distribution of *Staphylococcuss* species based on Biochemical Characterization

The biochemical characterization of the *Staphylococcus* isolates showed that (56%) of the isolates were α- haemolytic and (9%) were βhaemolytic. Similarly, (41%) were coagulase positive while (33%) were coagulase negative. (71%) produced catalase positive and (35%) of the isolates were DNase positive (Table 4.8).

4.9 Biochemical Distribution of Ten Randomly- Selected *Staphylococcus* Species (Using Microgen Kit)

Ten *Staphylococcus* spp were randomly selected following biochemical characterization for further confirmation using Microgen kit. Three (3) isolates were positive for *Staphylococcus aureus*, (3%) for *Staphylococcus xylosus*, (3%) for unidentified *Staphylococcus* spp, and (1%) for *Staphylococcus warneri*, respectively. The results are presented in Table 4.9.

4.10 Antifungal Pattern of *Candida* isolated from women attending A.B.U (sick bay) and Samaru clinic, Zaria, Nigeria

The antifungal susceptibility results showed that *Candida glabrata*, *C. parasiplosis* and *C. lambica* were all (100%) sensitive to Nystatin while *C. tropicalis* was 85% sensitivity to Nystatin. Similarly, *C. parasiplosis* and *C. lambica* showed 100% susceptible, followed by *C. glabrata* with 67% sensitivity to mycotene. However, *Candida* spp isolates showed multiple resistances to ketoconazole. *C. parasiplosis* showed 100% sensitivity to Itraconazole followed by *C. tropicalis* with sensitivity of 62%. *C. albicans* exhibited multiple resistance to all the antifungal agents used. *C. glabrata* and *C. lambica* showed 100% sensitivity to fluconazole followed by *C. tropicalis* with 54% sensitivity. The results are presented in Table 4.10.

Table 4.8 Biochemical distribution of *Staphylococcus* species isolated from women attending A.B.U (sick bay) and Samaru Clinic, Zaria, Nigeria

	No of isolates Positive (%)		Negative (%)	Total no
Haemolysis reaction	α= 56	β= 9	9	74
Coagulase test	41		33	74
Catalase test	71		3	74
DNase test	35		39	74

Key: α = Alpha, β = Beta

Table 4.9 Biochemical distribution of Ten Randomly Selected *Staphylococcus* Species (Using Microgen Kit) from women attending A.B.U (sick bay) and Samaru clinic, Zaria, Nigeria

Staphylococcus species	Total isolates	No of isolated organism		%
	Samaru n=10**	n=74*	n=300***	
Staphylococcus aureus	3	30		1
Staphylococcus xylosus	3	30		1
Staphylococcus warneri	1	10		0.3
Staphylococcus spp	3	30		1

Key: * = Number of Staphylococcus species positive

** = Nunber of Staphylococuss species slected for biochemical characteristics

*** = Total number of sample collected

Table 4.10 Antibiotic Sensitivity Pattern of *Candida* Species Isolated from women attending A.B.U (sick bay) and Samaru clinic, Zaria, Nigeria

Antifungal agent (Concentration)	*C. tropicalis* (n=13) Percentages		*C. albicans* (n=3)		*C. glabrata* (n=3)		*C. parasiplosis* (n=1)		*C. lambica* (n=1)	
	S	R	S	R	S	R	S	R	S	R
Nystatin (100 iu)	85	15	0	100	100	0	100	0	100	0
Mycotene (100mg)	54	31	0	100	67	33	100	0	100	0
Ketoconazole (200mg)	0	100	0	100	0	100	0	100	0	100
Itraconazole (100mg)	62	23	0	100	33	67	100	0	0	100
Fluconazole (500mg)	54	38	0	100	100	0	0	100	100	0

Key: n = number of isolates, S = Sensitive, R = Resistant

4.11 Sensitivity Pattern of *Candida* species to aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii*

The plant extracts *V. perrottetii* (aqueous) and its methanol extracts both single and in combination showed a weak activity to few of the *Candida* spp at 2000mg/ml. However, when the concentration of the extracts was increased further, there was no activity at all. Zones of inhibition of 5mm, 9mm and 10mm were observed at 2000mg/ml of *V. perrottetii* (aq) and *V. perrottetii* (methanol) in few of the *C. tropicalis* isolates (Table 4.11).

At 1000mg/ml, none of the extracts showed activity both singly and in combination. At 2000mg/ml, methanol extracts of *Vernnonia perrottetii* showed weak antibacterial with a zone of inhibition of 5mm to *C. tropicalis* isolates. At the same 2000mg/ml, methanolic extracts of the combination of *G. celosioides* and *V. perrottetii* showed zone diameter of inhibitions of 10mm for another *C. tropicalis* isolates out of 13 species isolated. Again at 2000mg/ml *V. perrottetii* methanol extracts showed diameter zones of inhibition at 8mm for another *C. tropicalis* isolates (Table 4.11)

Also at 2000mg/ml, diammeter zone of inhibition for one of the *C. albicans* isolates is 10mm for *V. perrottetii* methanol extracts. *C. parasiplosis* had 10mm for *V. perrottetii* aqueous extracts. Lastly, at 2000mg/ml, *C. parasiplosis* had zone diammeter of inhibition of 5mm for *V. perrottetii* methanol extracts and 5mm as well for one of the *C. glabrata* isolates. When the concentration was increased to 3000mg/ml and 4000mg/ml, none of the extracts showed activity against all of the isolates (Table 4.12).

Only seven isolates showed susceptibility at 2000mg/ml with a very weak susceptibility, none at 1000mg/ml, 3000mg/ml and 4000mg/ml (Table 4.11).

4.12 Pattern sensitivity of *Candida glabrata, C. albicans, C. parasiplosis* and *C. lambica* against aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii*.

Sensitivity of the the plant extracts against *C. glabrata* showed that only *Vernonia perrottetii* (ME) at 200mg/ml has activity with a very weak at 5mm. Methanol extracts of the combination showed activity against one of the *C. albicans* [10mm, 8mm for V.p (aq) and 10mm for V.p (ME)] at 2000mg/ml. All the plant extracts showed no activity against *C. lambica*. V.p (aq) and V.p (ME) showed activity at 2000mg/ml for *C. parasiplosis* with zone of inhibition of 10mm and 5mm respectively (Table 4.12).

4.13 Antibacterial Sensitivity Pattern of *Staphylococcus* species Isolates

Staphylococcus species susceptibility to Ciprofloxacin were hundred percent. Only *Staphylococcus aureus*(c) and unidentified *Staphylococcus* spp(c) were resistanct to amoxicillin while the rest of the test organisms were 100% sensitive (Table 4.13).

4.14 Sensitivity Pattern of *Staphylococcus* species to Aqueous Extract of *G. celosioides* and *V. perrottetii* at Different Concentration

The aqueous extracts activity against *Staphylococcus* species at 500mg/ml (Table 4.14) and 1000mg/ml (Table 4.15). *S. aureus* (a) produced zone of inhibition of 20.0±0.0 at 500mg/ml of the aqueous combination of the plant extract, which was lower than the

Table 4.11 Susceptibility (mm) pattern of *Candida tropicalis* to various concentrations of the queous and methanolic extracts of *G. Celosioide*s and *V. perrottetii*

Plant extract	Concentration(mg/ml)	Organism												
		C.t	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*	*C.t*
GC(ME)	1000	R	R	R	R	R	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R	R	R	R	R	R
CM(ME)	1000	R	R	R	R	R	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R	R	R	R	R	R
VP(AQ)	1000	R	R	R	R	R	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R	R	R	R	R	9
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R	R	R	R	R	R
VP(ME)	1000	R	R	R	R	R	R	R	R	R	R	R	R	10
	2000	R	R	5	R	R	10	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R	R	R	R	R	R
CM(AQ)	1000	R	R	R	R	R	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R

	4000	R	R	R	R	R	R	R	R	R	R	R	R	R
GC(AQ)	1000	R	R	R	R	R	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: GC (ME) = *Gomphrena celosioides,* CM (ME) = Combination (Methanol), VP (AQ) = *Vernonia perrottetii* (Aqueous), CM (AQ) = Combination (Aqueous), GC (AQ) = *G. celosioides* (Aqueous), C.t = *Candida tropicalis*, R = Resistant

Table 4.12 Plant extract sensitivity of *Candida glabrata*, *C. albicans*, *C. parasiplosis* and *C. lambica*

Plant extract	Concentration(mg/ml)	Organism							
		C.g	*C.g*	*C.g*	*C.a*	*C.a*	*C. a*	*C.p*	*C.*l
GC(ME)	1000	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R
CM(ME)	1000	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	10	R	R
	3000	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R
VP(AQ)	1000	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	10	R
	3000	R	R	R	R	R	8	R	R
	4000	R	R	R	R	R	R	R	R
VP(ME)	1000	R	R	R	R	R	R	R	R
	2000	R	R	5	R	R	R	5	R
	3000	R	R	R	R	R	10	R	R
	4000	R	R	R	R	R	R	R	R
CM(AQ)	1000	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R
GC(AQ)	1000	R	R	R	R	R	R	R	R
	2000	R	R	R	R	R	R	R	R
	3000	R	R	R	R	R	R	R	R
	4000	R	R	R	R	R	R	R	R

Key: GC (ME) = *Gomphrena celosioides*, CM (ME) = Combination (Methanol), VP (AQ) = *Vernonia perrotetii* (Aqueous), CM (AQ) = Combination (Aqueous), GC(AQ) = *G. celosioides*(Aqueous), C.g = *Candida glabrata*, C.a = *Candida albicans*, C.p = *Candida parasiplosis*, C.l = *Candida lambica*, R = Resistant

Table 4.13 Antibacterial Sensitivity Pattern of *Staphylococcus* species Isolates

Test organism	Antibacterial agents					
	Ciprofloxacin (500mg)		Amoxycillin(500mg)		Erythromycin(500mg)	
	S	R	S	R	S	R
S. aureus(a)	100	0	100	0	100	0
S .xylosus(a)	100	0	100	0	100	0
S .aureus(b)	100	0	100	0	100	0
S .warneri	100	0	100	0	100	0
S .aureus(c)	100	0	0	100	100	0
S .xylosus(b)	100	0	100	0	100	0
S.xylosus(c)	100	0	100	0	100	0
Staph spp(a)	100	0	100	0	100	0
Staph sp(b)	100	0	100	0	100	0
Staph spp(c)	100	0	0	100	100	0

Key: = n = number of samples, S = Sensitive, R = Resistant, Staph spp = Unidentified *Staphylococcus* species

Table 4.14 Zones of Inhibition (mm) of aqueous Plant extracts (500mg/ml against *Staphylococcus* Species

Test organism	GC (AQ)	VP (AQ)	CM(AQ)	Antibiotic sensitivity	Antibiotics	
					Amoxycillin	
					S	R
S. aureus(a)	0.0±0.0	0.0±0.0	20.0±0.0	35	100	0
S .xylosus(a)	0.0±0.0	20.67±0.33	13.0±0.0	20	100	0
S .aureus(b)	0.0±0.0	6.0±0.0	10.0±0.0	40	100	0
S .warneri	0.0±0.0	12.00±1.0	11.67±0.33	45	100	0
S .aureus(c)	0.0±0.0	18.33±0.33	19.00±0.0	-	0	100
S .xylosus(b)	0.0±0.0	12.67±0.67	0.0±0.0	45	100	0
S.xylosus(c)	0.0±0.0	19.33±0.33	10.33±0.33	50	100	0
Staph spp(a)	0.0±0.0	10.00±0.0	11.33±0.67	46	100	0
Staph spp(b)	0.0±0.0	12.00±1.00	10.00±0.0	45	100	0
Staph spp(c)	3.33±3.33	20.00±0.0	18.00±0.0	-	0	100

Key: VP (AQ) = *Vernonia perrottetii* (Aqueous), GC (AQ) = *Gomphrena celosioides* (Aqueous), CM (AQ) = Combination (Aqueous), S = Sensitive, R = Resistant

Table 4.15 Zones of Inhibition of Aqueous Plant Extract (1000mg/ml) against *Staphylococcus* species

Test organism	GC (AQ)	VP (AQ)	CM(AQ	Antibiotic sensitivity	Antibiotic Amoxylin	
					S	R
S. aureus(a)	0.0±0.0	0.0±0.0	0.0±0.0	35	100	0
S .xylosus(a)	10.0±0.0	26.67±0.33	13.0±0.0	20	100	0
S .aureus(b)	0.0 ±0.0	12.33±0.33	15.0 0.0	40	100	0
S .warneri	0.0 ±0.0	16.33±0.33	14.67±0.33	45	100	0
S .aureus(c)	0.0 ±0.0	19.33±0.33	20.0 ±0.0	-	0	100
S .xylosus(b)	0.0±0.0	20.00±0.0	10.00±0.0	45	100	0
S.xylosus(c)	10.33±0.0	22.33±0.33	15.33±0.33	50	100	0
Staph spp(a)	0.0 ±0.0	14.67±0.33	14.67±0.33	46	100	0
Staph spp(b)	0.0 ±0.0	16.00±0.0	13.67±0.33	45	100	0
Staph spp(c)	5.00±5.00	22.33±0.33	20.00±0.0	-	0	100

Key: VP (AQ) = *Vernonia perrottetii* (Aqueous), GC (AQ) = *Gomphrena celosioides* (Aqueous), CM (AQ) = Combination (Aqueous), S = Sensitive, R = Resistant, - = No zones of inhibition produced

35mm observed in the standard antibiotic, amoxycillin.

S. xylosus (a) had zone of inhibition of 13.0 ±0.0 mm for aqueous combination of the extracts, 20.67±0.33 mm for VP (aq) at 500mg/ml, 10.0±0.0 mm 13.0±0.0 mm and 26.67±0.33 for GC (aq), CM (aq) and VP (aq) respectively (Table 4.14, 4.15).

S. aureus (b) had zones of inhibition of 6.0±0.0 and 10.0±0.0 mm for VP (aq) and CM (aq) at 500mg/ml as well as 12.33±0.33 mm, 15.00±0.0 mm for VP (aq) and CM (aq) at 1000mg/ml respectively. GC (aq) at both 500mg/ml and 1000mg/ml produced no zone of inhibition. The zones produced were much lower compared to the 40mm produced by the standard antibiotic amoxicillin (Table 4.14, 4.15).

S. warneri had zones of inhibition of 11.67±0.33 mm and 12.00±1.0 mm, 14.67±0.33 mm, and 16.33± 0.33 mm at 500mg/ml and 1000mg/ml respectively for CM (aq) and VP (aq) which was very low compared to the 45mm produced by the standard antibiotic (amoxicillin). GC (aq) however, produced no zone of inhibition at both concentrations (Table 4.14, 4.15).

S. aureus (c) had zones of inhibition of 18.33±0.33 mm and 19.00±0.0 mm and 19.00±0.33 mm, 20.0±0.0 mm at 500mg/ml and 1000mg/ml respectively for VP (aq) and Cm (aq). No zone of inhibition was produced by GC (aq) both at 500mg/ml and 1000mg/ml. The combination did better than the singles in the case of this organism compared to the standard antibiotic (amoxicillin), in which the drug was 100% resistant (Table 4.14, 4.15)

S. xylosus (b) had zone of inhibition of 12.67±0.67 mm at 500mg/ml, CM (aq) produced 10.00±0.0 mm and 20.00±0.0 mm for VP (aq), while GC (aq) produced no zone of

inhibition at both 500mg/ml and 1000mg/ml. The zones of inhibition produced by the extracts were lower compared to the standard antibiotic, amoxylin which produced zone of inhibition of 45mm (Table 4.14, 4.15)

S. xylosus (c) had zones of inhibition of 10.33±0.33 mm and 19.33±0.33 mm at 500mg/ml for Cm (aq) and Vp(aq) extract, while at 1000mg/ml. CM (aq) and VP (aq) showed activity from weak to moderate to strong, (10.33±0.0, 15.33±0.33 and 22.33±0.33). However, their sensitivity was lower compared to the 50mm produced by the standard antibiotic (amoxicillin) (Table 4.14, 4.15)

Staphylococcus spp (a) had zones of inhibition of 10.00±0.0 mm and 11.33±0.67 mm for VP (aq) and CM (aq), respectively at 500mg/ml. The zones of inhibition produced at 1000mg/ml for VP (aq) and CM (aq) were the same (14.67±0.33 mm and 14.67±0.33 mm) which were much lower than the 46mm produced by the standard antibiotic (amoxycillin) (Table 4.14, 4.15)

Staphylococcus spp (b) had zones of inhibition 10.00±0.0 mm and 12.00±1.00 mm for CM (aq) and VP (aq) respectively as well as at 500mg/ml 13.67±0.33 mm and 16.00±0.0 mm at 1000mg/ml for CM (aq) and VP (aq) respectively, which were quite low compared to the 45mm produced by standard antibiotic (amoxycillin). No zone of inhibition was observed for GC (aq) at both 500mg/ml and 1000mg/ml (Table 4.14, 4.15)

Staphylococcus spp(c), had zones of inhibition for all the extracts varying from weak to moderate to strong (3.33±3.33 mm, 18.00±0.0 mm, 20.00±0.0 mm and 5.00±5.00 mm, 20.00±0.0 mm and 22.33±0.33 mm respectively). However, the organism was 100% resistant to the standard antibiotic (amoxicillin) (Table 4.14, 4.15)

4.15 Sensitivity pattern of methanolic extracts of *G. celosioides* and *V. perrottetii* against *Staphylococcus* spp at Different Concentration

Antibacterial activity of the methanolic extracts against *Staphylococcus* species at 500mg/ml is shown Table 4.16 and also at 1000mg/ml Table 4.17 showed that *S. aureus* (a) had zone of inhibition of 23.00±0.0 mm for *Vernonia perrottetii* (me) only at 500mg/ml which was low compared to the 35mm produced by the standard drug (Table 4.16, 4.17)

S xylosus (a) had varying zones of inhibition (8.67±0.33 mm, 14.33±0.33 mm, 15.33±0.33 mm and 11.33±0.33 mm and 15.33±0.33 mm and 20.00±0.0 mm) for *Gomphrena celosioides* (me), Combination (me) and VP (me) at 500mg/ml and 1000mg/ml respectively. VP (me) at 1000mg/ml has the same zone of inhibition as the standard drug (amoxicillin) (Table 4.16, 4.17)

S aureus (b) had zones of inhibition of 5.0±0.0 mm, 16.67±0.33 mm at 500mg/ml for GC (me) and VP (me) while CM (me) produced no zone of inhibition. At 1000mg/ml, all the plant extracts showed activity from moderate to strong (10.0±0.0 mm, 10.00±0.0 mm and 19.33±0.33 mm for GC (me), CM (me) and VP (me) respectively. However, their sensitivity was low compared to the 40mm produced by the standard antibiotic (amoxicillin) (Table 4.16, 4.17)

Table 4.16 Sensitivity pattern of methanolic extracts of *G. celosioides* and *V. perrottetii* against *Staphylococcus* spp (500mg/ml)

Test organisms	GC (ME) (mm)	VP (ME (mm)	CM(ME) (mm)	Antibiotic zones of inhibition(mm)	Antibiotics	
					Amoxycillin	
					S	R
S. aureus(a)	0.0±0.0	0.0±0.0	0.0±0.0	35	100	0
S .xylosus(a)	8.67±0.33	15.33±0.33	14.33±0.33	20	100	0
S .aureus(b)	5.0±0.0	16.67±0.33	0.0±0.0	40	100	0
S .warneri	12.00±0.0	19.00±0.0	11.33±0.67	45	100	0
S .aureus(c)	0.0 ±0.0	0.0 ±0.0	15.33±0.33	-	0	100
S .xylosus(b)	0.0±0.0	12.00±0.0	10.00±0.0	45	100	0
S.xylosus(c)	12.33±0.33	10.00±0.0	15.00±0.0	50	100	0
Staph spp(a)	10.00±0.0	18.00±0.0	5.00±0.0	46	100	0
Staph spp(b)	12.00±0.0	15.00±0.0	7.00±2.00	45	100	0
Staph spp(c)	15.33±0.33	20.33±0.33	15.00±0.0	-	0	100

Key: GC (ME) = *Gomphrena celosioides* (Methanol), VP (ME) = *Vernonia perrottetii* (Methanol), CM (ME) = Combination (Methanol), S = Sensitive, R = Resistant

Table 4.17 Sensitivity pattern of *Staphylococcus* species against methanolic extracts (1000mg/ml) of *G. celosioides* and *V. perrottetii*

Test organism	GC (ME)	VP (ME)	CM(ME)	Antibiotic zones of inhibition (mm)	Antibiotics	
					Amoxylin	
					S	R
S. aureus(a)	0.0±0.0	23.00±0.0	0.0±0.0	35	100	0
S .xylosus(a)	11.33±0.33	20.0±0.0	15.33±0.33	20	100	0
S .aureus(b)	10.0 ±0.0	19.33±0.33	10.00 ± 0.0	40	100	0
S .warneri	13.33±0.67	20.0 ±0.0	16.00± 0.0	45	100	0
S .aureus(c)	20.0 ±0.0	0.0 ±0.0	17.67±0.33	-	0	100
S .xylosus(b)	0.0±0.0	21.33±0.67	10.00 ±0.0	45	100	0
S.xylosus(c)	14.00±0.0	15.00 ±0.0	19.00 ±0.0	50	100	0
Staph spp(a)	13.00±0.0	20.00±0.0	8.33 ±1.67	46	100	0
Staph spp(b)	13.33±0.0	20.00±0.0	10.00 ±0.0	45	100	0
Staph spp(c)	19.33±0.33	25.33±0.33	20.33±0.33	-	0	100

Key: GC (ME) = *Gomphrena celosioides* (Methanol), VP (ME) = *Vernonia perrottetii* (Methanol), CM (ME) = Combination (Methanol), S = Sensitive, R = Resistant

S. warneri was susceptible to all the plant extracts which had moderate to strong activity at both 500mg/ml and 1000mg/ml (11.33±0.67 mm, 12.00±0.0 mm, 19.00±0.0 mm and13.33±0.67 mm, 16.00±0.0 mm, 20.0±0.0 mm for Combination (me), *Gomphrena celosioides* (me) and *Vernonia perrottettii* (me) at 500mg/ml and 1000mg/ml respectively). However, the zones of inhibition were lower than that produced by the standard antibiotic (amoxicillin) with 45mm (Table 4.16, 4.17)

For *S. aureus* (c), only CM(me) and GC(me) produced zone of inhibition (15.33±0 mm) at 500mg/ml. CM(me) and GC(me) produced zones of inhibition of 17.67±0.33 mm and 20.0±0.0 mm at 1000mg/ml. VP(me) produced no zone of inhibition at both 500mg/ml and 100mg/m. However, the plant extracts did better compared to the standard antibiotic (amoxicillin), which did not produce any zone of inhibition (Table 4.16, 4.17)

Combination (me) and *V. perrottettii* (me) had zones of inhibition of 10.00±0.0 mm and 20.00±0.0 mm at 500mg/ml and also 0.00±0.0 mm and 21.33±0.67 mm, respectively at 1000mg/ml to *S. xylosus* which were very low compared to the 45mm produced by the standard antibiotic, amoxycillin. GC (me) produced no zone of inhibition at both 500mg/ml and 1000mg/m (Table 4.16, 4.17)

The plant extracts, both singles and in combination had 10.00±0.0 mm, 12.33±0.33 mm, 15.00±0.0 mm, 14.00±0.0 mm, 15.00±0.0 mm and 19.00±0.0 mm for VP (me), GC (me), CM(me) at 500mg/ml as well as 1000mg/ml, respectively produced zones of inhibition to *S. xylosus* (c). The combination of the extracts seems to perform better compared to the individual plant extracts, though the sensitivity was very low compared to the 500mm zone of inhibition produced by the standard drug (amoxicillin) (Table 4.16, 4.17)

All the extracts; 5.00±0.0 mm, 10.00±0.0 mm and 18.00±0.0 mm at 500mg/ml, 8.33±1.67 mm, 13.00±0.0 mm and 20.00±0.0 mm at 1000mg/ml for CM (me), GC (me) and VP (me), respectively had zones of inhibition of *Staphylococcus* spp (a). The combination here produced a very weak activity when compared to the 46mm produced by the standard antibiotic (amoxicillin) (Table 4.16, 4.17)

All the plant extracts, 7.00±2.00 mm, 12.00±0.0 mm and 15.00±0.0 mm at 500mg/ml, 10.00±0.0 mm, 13.33±0.0 mm and 20.00±0.0 mm at 1000mg/ml for Combination (me), *G.celosioides* (me) and *V. perrottettii* (me) had zones of inhibition to *Staphylococcus* spp (b). Similarly, the activity was very low compared to the 45mm produced by the standard antibiotic used.

Lastly, plant extracts has antibacterial activity against *Staphylococcus* spp (c), 15.00±0.0 mm, 15.33±0.33 mm and 20.33±0.33 mm at 500mg/ml for Combination (me), *G. celosioides* (me) and *V. perrottettii* (me) respectively. 19.33±0.33 mm, 20.33±0.33 mm and 25.33±0.33 mm for *G. celosioides* (me), Combination (me) and *V. perrottettii* (me) at 1000mg/ml. Both plant extracts showed moderate to strong activity compared to the standard drug used (amoxicillin), which did not produce any zone of inhibition (Table 4.16, 4.17)

4.16 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Plant Extracts

The minimum inhibitory concentration and bactericidal concentration of the extracts showed that *Staphylococcus aureus* (a) MIC_{125} and MBC_{250}, *S. xylosus* (a) MIC_{250} and MBC_{500}, *S. aureus* (b) MIC_{250} and MBC_{500} and *S. xylosus*(b) MIC_{125}, MBC_{250}. *V.*

perrottetii aqueous extracts showed bacteriostatic activity at MIC_{500}/MBC_{1000} for only *S. aureus* (c)Only *S.* aureus (c) (Table 4.18).

Table 4.18 Minimum Inhibitory Concentrations of aqueous extract of *V. perrottetii* against *Staphylococcus* species

Organism	MIC(mg/ml	MBC(mg/ml)
S. aureus (a)	125	250
S .xylosus (a)	250	500
S .aureus (b)	250	500
S. aureus (c)	500	>1000
S .xylosus (b)	125	250

4.17 Minimum Inhibitory Concentrations of *Vernonia perrottetii* Methanol extracts

Table 4.19 below showed MIC/MBC of V.P (me) methanol at 500mg/ml. The plant extracts exhibited bacteriostatic activity to all the five *Staphylococcus* species; *S. warneri*, *S .xylosus* (b), *Staphylococcus* spp (a), *Staphylococcus* spp (b), *Staphylococcus* spp (c) with MIC_{500}, $MBC_{>1000}$ respectively.

Minimum Inhibitory Concentration of combination of aqueous extracts at 250mg/ml and 500mg/ml showed that MIC/MBC for aqueous combination of the extracts was bacteriostatic to *S. aureus* (c) with MIC_{250}, MBC_{500}. Minimum Inhibitory Concentration of Combination methanolic extracts at 500mg/ml showed bacteriostatic activity to only *S. aureus* (c) species ($MIC_{500}/MBC>_{1000}$ (Table 4.19).

Table 4.19 Minimum Inhibitory Concentration and Minimum bactericidal concentrations of methanol extracts of *Vernonia perrottetii* (500mg/ml) against *Staphylococccuss* species

Organism	MIC(mg/ml)	MBC(mg/ml)
S .warneri	500	>1000
S .xylosus (b)	500	>1000
Staph spp (a)	500	>1000
Staph spp (b)	500	>1000
Staph spp (c)	500	>1000

CHAPTER FIVE

5.0 DISCUSSION

Overall prevalence of vulvovaginitis in this study was 51%(n=153) which was higher than the previous prevalence of 30% reported by Ross *et al*. (1995), also much higher than 42% reported by Yar'zever and Ibrahim. (2013) in a study of prevalence and causes of vaginitis in women attending Aminu Kano Teaching Hospital, Kano, Nigeria, with 24%(121) having candidiasis and 9.60% (48) having Staphylococcal infection. The prevalence of the present study was also higher than the report of Bello *et al*. (2012) in Nicaragua, (41%) among women with vaginitis. It was also much higher than the one reported by Oyelese *et al*. (2005) in Ile Ife, Nigeria, who reported a prevalence of 24% for *C. albicans* among sexually transmitted diseases (STD) patients over a 10 years period. The result showed that one out of every Nigerian women in the study area had vulvovaginitis. Since one of the means of acquiring this illiness is through sexual activity, it could be an in dication of high sexual promiscuity among the population. It must also be noted that, the religious belief of the community that encourages polygamy family, could also be a contributory factors. This will certainly increase the spread of transmission. However, it was much lower than the one reported by Akerele *et al*. (2002) in Benin City from a study on ante- natal women which established *C. albicans* in 65% of women and as the commonest genital infectious agent encountered.

The highest prevalence of candidiasis here occurred among the age goup of 21-30 years with 30 positive patients (50%). This dis agrees with the report of Edmund *et al* (1992) who showed that *C. albicans* infections are more common among sexually active women aged 15-20 years. However, a similar report by Msuya *et al*. (2002) in Moshi, Tanzania, where the peak of STD among the women studied was slightly above 30

years, further strengthening the belief that sexual activity could contribute to a large extent, the spread of the disease (Ononge *et al*., 2005). The result therefore showed that younger women were particularly vulnerable as they belonged to an age group where sexual activity is at its peak, as observed by Sogbetun *et al*. (1977) and Osoba *et al*. (1989).

There was a significant association between the use of birth control pills and vaginitis (Hans, 1983). It was also observed by), that oral contraceptive pills contain hormones known as oestrogens, which was found to increase the level of glycogen in the vaginal area and allow *Candida* to flourish. This hormone as observed by Hans (1983) increases incidence of vaginal candidiasis by 20-40%, even among women in the range of 15-34 years. This finding agrees with that of the present study. The relationship between *C. albicans* and the use of contraceptive has been well established in the past, when it was reported that the administration of gestrogenic contraceptives pills exacerbates symptoms of vaginal candidiasis. Bourg *et al*. (1989) was the first to observe that progestational steroids cause changes similar to those that occur in pregnancy and could be responsible for the increase in the incidence of candidiasis. These steroids are known to alter carbohydrate metabolism through pituitary hypothalamic axis (Pincus, 1973).

Oriel *et al*. (1972) reported a high rate of prevalence of candidiasis among contraceptive users than non- contraceptive users. Milson and Ferrsman (1985) earlier suggested that this could be attributed to higher oestrogen and progesterone in the contraceptives that increase glycogen in the vagina, thus exposing it to the activities of lactobacilli (Milson and Ferrsman, 1985). Lactobacilli are believed to play a role in the conversion of glycogen to lactic acid, thus decreasing pH of the vagina. The decreased pH reduces activities of the bacterial biota which favours the growth of yeasts including *Candida*

spp (Enweani *et al*., 2001). Vaginitis was much higher in married women compared to the singles, but there was no significant association between them, however, the religious belief of the community that encourages polygamy could be a contributing factor, since the husband has to sleep with each woman and the husband or any of the wives might acquire STDs resulting in the infection of the entire household. This may certainly increase the rate of spread of STDs.

For use of perfume spray and vaginitis, there was no significant association between the two groups. Minor skin damage induced by skin sensitizers such as vaginal sprays, douches, deodorants and perfumed tampons may predispose women to *C. albicans* infection by disrupting the normal vaginal microflora (Gupta *et al*., 2010). Significant association occurred between patients who use tight clothing and vaginal. This could be because it traps heat, which is a good avenue for fungal and bacterial growth (Sobel, 2007). Studies also show that other factors that predispose to vaginal candidiasis are the use of nylon underwear and tight clothing especially in obese women, making the skin and genital mucosal surface much more susceptible to *Candida* infections (Sobel, 2007).

For relationship between pregnancy status of respondents, the disease tends to be higher among pregnant women than non pregnant women. This could be as a result of increase in vagina pH during pregnancy and high levels of reproductive hormones, which provide an excellent carbon source for *Candida* spp, that is, by providing high glycogen content in the vagina. During pregnancy, the vagina shows increased susceptibility to infection by *Candida* spp and *Staphylococcus* spp, resulting in both higher prevalence of vaginal colonization and higher rate of symptomatic vaginitis (Funk and Kumar, 2011).

Significant association did not occur between use of antibiotics and vaginitis infections in this study. Although misuse of antibiotics are one of the major factors that predispose women to vaginitis, Jacob and Nall (1990) found out that antibiotic particularly those with broad-spectrum activity are very common precipitators of commensal bacteria, e.g., lactobacilli. After antibiotic use, the rate of vaginal yeast colonization and symptomatic episode of candidiasis increase, thus allowing *Candida* to flourish.

Prevalence of vaginal candidiasis in this study was found to be 79(26.3%). The results of the prevalence of candidiasis in this study was lower to the one reported by Okonkwo *et al*. (2007), in a prevalence study of vaginal candidiasis among pregnant women in Nnewi, Anambra State, Nigeria, found a prevalence of 30%. It was also lower to the one observed by Enweani *et al*. (2001) in a study on the prevalence and antifungal susceptibility patterns of yeast, who reported a prevalence for Candidiasis of 40.6%. Also lower the one reported by Nwosu *et al*. (2001) who obtained 34.8% as the commonest genital infection in a study on patients with AIDS. The vaginal ecosystem is subjected to a variety of hormonal changes that affected the balance between lactobacilli. It is likely that the normal lactobacilli may be overwhelmed by factors such as prolonged use of antibiotics, oral contraceptives device, vaginal douching, pregnancy, diabetes mellitus and frequent sexual intercourse which alters the pH of the vagina (Rylander *et al*., 2004)

However, it was also higher than the one reported by Jombo *et al*. (2010), who obtained 29.1% in a study on symptomatic vulvovaginal candidiasis and genital colonization by *Candida* species in Jos, Nigeria as well as Ekwempu *et al*. (1981) in Zaria, who reported a *Candida* prevalence of 20.9% among women in labour. Bello-Onaghise *et al*.

(2012) in Nicaragua reported a much higher prevalence of 41% among women with understandably vaginitis.

From the *Candida* species isolated, 79(26.3%) yielded positive results, out of which only 50% were selected for further biochemical characterization. It was observed that *C. tropicalis* was the most observed *Candida* spp (13 samples), *C. albicans* (3 samples), *C. glabrata* (3 samples), *C. parasiplosis* (1) and *C. lambica* (1). This findings disagreed with the report of Rangarag *et al*. (2003) who isolated *Candida* spp, out of which 30(61.22%) positive results for *C. albicans*. However, the species identification was close to that of present study. *C. tropicalis* (20.42%) was the second most encountered *Candida* species followed by *C. glabrata* (6.12%) (Rangarag *et al*., 2003) while in the present study, *C. tropicalis* was the most encountered species followed by *C. glabrata* and *C. albicans*. The reason for the most isolated *C. tropicalis* could be due to the lack of proper presumptive methods of isolating *Candida* species. And also the fact that *Candida tropicalis* is mostly common in the tropical areas and Nigeria is one of the tropical countries. However, the present study compared well with the one observed by Rangarag *et al*. (2003) in a study Rapid identification of commonly-encountered *Candida* spp directly from blood cultured bottles, where six different types of *Candida* spp was isolated. Rubia *et al*. (2003) in a study on the *In- vitro* activity of antifungal agents on yeasts isolated from vaginal secretions, where overall yeasts isolated was 20.15% while *C. albicans* (84%) was the most isolated, followed by *C. glabrata* (7%), *C. tropicalis* (4%) and *C. parasiplosis*(2%). The present study agrees with Bello *et al*. (2012) on the prevalence of vaginal pathogens associated with genital tract infections in Ogun State, Nigeria, where *C. tropicalis* was the highest encounterered *Candida* species. This information gives an insight of the distribution of *Candida* species that are

associated with genital tract infections aside the popular indicted *C. albicans*. However, this also agreed with Marlete *et al*. (2005) in a study of *Candida* species from vaginal microbiota of healthy canine females during estrous cycle, where *C. parasiplosis* was the most frequently encountered *Candida* species for randomly forty selected for biochemical characterization. The present study is also similar to what was isolated by Reza *et al*. (2011) where they obtained *C. albicans* 24(38.7%), *C. glabrata* 15(24.2%) and *C. parasiplosis* 6 (9.6%) in a study on the comparison of enzymatic methods of rapid yeasts plus system with RFLP-PCR for identification of isolated yeast from vulvovaginal candidiasis. *C. tropicalis* was the most encountrered in the present study and this agrees with the report of Edilson *et al*. (2012) where *C. tropicalis* was found in 13(7.5%) in a study on the *in -vitro* activity of *Sapidus saponaria* against azoles – susceptible and resistant human vaginal species. The present study also agrees with the one observed in Malaysia by Chong *et al*. (2003) where isolates of *Candida* were recovered from over 54% of patients from two groups; non- albicans species were commoners in patients with recurrent vaginal candidiasis compared to those with only one episode per year. Six yeast species were also detected, which included; *C. albicans*, *C. glabrata*, *C. lusitaniae*, *C. famata, C. krus*ei and *C. parasiplosis* in a study on symptomatic vulvovaginal candidiasis and genital colonization by *Candida* species in Nigeria (Yar'zever and Ibrahim, 2013). However, the present study disagrees with what Martins and Jones, (1940) observed, where *C. albicans* was reported to be the most encountered species.

The results of the antibiotics sensitivity testing showed that, the widely used Ketoconazole, which is a drug of choice for treating fungal infections was less effective (Table 4.10), thereby limiting its use in the management of vaginal candidiasis. This

observation has also been reported by Smith *et al*. (1992), who observed that *Candida* spp was resistant to fluconazole in HIV/ AIDS patients. All the *Candida albicans* isolates exhibited resistance to mycotene (Table 4.10). *C. lambica* resistance to itraconazole, are in concordance with the studies that demonstrated vaginal isolates of non- *C. albicans* to be less susceptible to azoles, principally *C. glabrata* (Dota *et al*., 2008; Ferrer, 2000). The *In-vitro* antifungal activity of fluconazole by the former National Committee for Clinical Laboratory Standards (NCCLS), now the Clinical Laboratory Standard Institute (CLSI), revealed that 21.1% of vaginal yeasts isolates were resistant to fluconazole (Bauters *et al*., 2002). In a study of 593 vaginal yeasts isolates concluded that resistance to fluconazole and flucytosine was observed infrequently (3.7%), and the more resistant non-albicans species were more frequently isolated from women with recurrent vaginal infection (Richter *et al*., 2005). Fluconazole has been considered the drug of choice, because it is well tolerated, has a good oral bioavailability and is efficacious against most of *Candida* species (Odds, 2006). However, microbiological studies have revealed azole *C. albicans* resistance and infections by less sensitive non-albicans *Candida* species (Ribeiro, 2000; Sobel *et al*., 2001). Vaginal yeasts prevalence among the patients is common as the organism easily colonizes mucous membranes (Moyes and Naglik, 2011) such as the vagina, urinary tract and the oral cavity. From these sites, organisms multiply to cause symptomatic infection when the physiological condition of the site is altered (Kauffman *et al*., 2011).

The most frequently isolated bacteria in this study out of the ten randomly selected suspected *Staphylococcus* spp isolated for further confirmation to species level were *S. xylosus* 3(3%) and *S. aureus* 3 (3%) , *Staphylococcus* spp 3(3%) and *S. warneri* 1 (1%) (Table 4.9) The results of the antibiotic susceptibility testing showed that *S. aureus*(c)

was resistant to amoxylin. Similarly, there was also a resistance in *Staph* spp (c). This result is in agreement with the study of Simeoni *et al*. (2008), who reported that there was a similar pattern of antibiotic resistance between coagulase-negative *Staphylococcus* spp (CNS) and *S. aureus*. However, in another study, Marino *et al*. (2010) showed that CNS were phenotypically less susceptible to antimicrobial agents than coagulase-positive *Staphylococcus* spp (CPS). Resistance in micro-organisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. The number of antibiotics with increasing capability of microbes to develop multi-drug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin. From the results obtained in this study, some of the organisms exhibited multiple resistance to the plant extracts. (Table 4.11, 4.12)

Almost all the plant extracts (both single and in combination) were not active against *C. tropicalis*, except for *V.perrottetii* (aqueous) and methanol extracts, which showed a very weak activity at 2000mg/ml(Table 4.11) This findings agree with Ruttoh (2009), in a study of the efficacy of 13 medicinal plants used by indigenous communities around Lake Victoria, Kenya, against tuberculosis, diarrhea-causing bacteria and *Candida albicans*, *Vernonnia amgydalina* had moderate to poor activity against the test culture used for general antibacterial and antifungal screening, *C. albicans* was resistant to all the tested extracts including *V. amgydalina*. The poor activity of the plant extracts to the organisms in the present study may be due to the absent of steriod and triterpene which was only present in *V. perrottetii*. The weak activity of the extracts against *C. tropicalis*, may be as a result of loss of some of the plant's active principle when drying or the inability of the solvents to dissolve some of the active principles of this plant (Ellof,

1998), and also possibly due to the antagonistic nature of other phytochemicals (Ruttoh, 2009).

Only methanol extracts of *V. perrottetii* showed a negligible activity at 2000mg/ml for one of the *C. glabrata* isolates (Table 4.12). The weak activity of the plant extracts against this organism could also be due to the antagonistic nature of the phytochemicals (Ruttoh, 2009). The rest of the *C. glabrata* was not susceptible to the plant extracts. The reason coul also be due to antagonistic nature of the phytochemicals (Ruttoh, 2009).

Methanol combination of the plant extracts, *V. perrottetii* aqueous and *V. perrotteetii* methanol extracts showed weak to moderate activity against one of the *C. albicans* isolates. This also agrees with the results obtained for *V. amgydalina* by Ruttoh. (2009), and the reason for the weak to moderate activity against this organism here could also be due to the antagonistic nature of the phytochemicals (Ruttoh, 2009).

Only aqueous of *V. perrottetii* and methanol *V. perrottetii* extracts showed weak activity against *C. parasiplosis* at 2000mg/ml (Table 4.12) Weak activity was also observed for *V. amgydalina* by Ruttoh. (2009). *C. lambica* was completely resistant to all the plant extracts (Table 4.12). This was also observed by Ruttoh. (2009) in a study where *C. albicans* was completely resistant to all the extracts tested in the study. *Gomphrena celosioides* had no effects on the *Candida* spp tested (Table 4.11, 4.12). This disagrees with the study conducted by Odebode. (2004) where methanol extracts of these plants had a pronounced activity against *Candida* spp with higher activity against *C. albicans*. The difference in the susceptibility of this *C. albicans* could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts. The antifungal susceptibility levels reported were low in many countries

(Pfaller *et al*., 2011a; Pfaller *et al*., 2011b), but the difference in the susceptibility levels in these countries support the idea that the susceptibility of yeasts to antifungal drugs needs to be monitored especially in Nigeria, where resistance levels (<=22.4%) are above what was reported (Pfaller *et al*., 2005) worldwide. *Candida* is known as opportunistic fungal pathogen as it causes infection when person becomes immunocompromised, immunosuppressed or diabetic (Jacob and Nall, 1990). In immunocompromised individuals, *Candida* causes diseases like oral thrush, intestinal candidiasis, vaginal thrush and onchomycosis (Chamaine *et al*., 2005).

The most susceptible organism [*Staphylococcus xylosus* (a)] isolated had a zone of of 26.67±0.33mm to the aqueous extracts of *V. perrottetii* as compared to the standard drug activity which was 20mm at 1000mg/ml. This could be due that aqoueus extract provides multiple antibacterial properties in its crude form thus increasing its activity on the tested organism. This also agrees with the study conducted by Odunbaku *et al*. (2012) who showed that *V. amgydalina* had inhibitory activity against the gram positive bacteria (*S. albus* and *B. substilis*) and gram negative bacteria (*K. pneumonia*e, *P. aeruginosa* and *P. mirabilis*) in a study of antimicrobial effect of ethanol leaf extract of selected medicinal plants on some human pathogenic microbes. And this also justifies the use of the plant in traditional medicine among Nupe people for ex- vaginalis and sperm expulsion (Abdullahi *et al*., 2003).

V. perrottetii aqueous extracts exhibited a zone of inhibition of 22.33±0.33 mm for *S. xylosus* (c) (Table 4.15) which was lower than the zone of inhibition produced by the standard drug. Lower inhibitory activity recorded compared to the standard drug in this study might probably be due to the metabolism of the extracts by bacteria, which reduced their activity (Cowan, 1999). Combination of the extracts showed a pronounced

activity against *S. aureus* (a) isolate, (zone of inhibition of 20.00±0.0 mm at 500mg/ml) and also 20.00±0.0 mm for *S. aureus* (c) at 1000mg/ml. From the results, it shows that the aqueous combination of the extracts had an effect on the organism; this may be due to synergistic nature of the combination (Kadar and Kumar, 2005). The susceptibility of *S. aureus* to the plant extracts where the standard antibiotic failed for *S. aureus*(c) isolate, indicated that these plants have the potential that could be exploited in the development of antimicrobial agents. In addition to the antibacterial activity of this plants extract, similar trend was observed for *C. odorata* in a study conducted by Arce and Barroga (2007), which revealed that the stem of *C. odorata* has moderate antimicrobial effect against *C. albicans* and weak activity against *S. aureus*, this was also observed for *E. coli* in a study conducted by De Moura *et al.* (2004). Metabolism of the extracts by the bacteria was adduced to be responsible for this observation. One *Staphylococcus* spp(c) isolate was susceptible to aqueous extracts of *V. perrottetii* and aqueous combination with zones of inhibition of 20.00±0.0 mm and 18.00±0.0 mm at 500mg/ml and 22.33±0.33 mm, 20.00±0.0 mm (aqueous combination) of *Staphylococcus* spp(c) isolate at 500mg/ml and 1000mg/ml respectively (Table 4.15) compared to the standard antibiotic (amoxylin). *Staphylococcus aureus* was the second most susceptible after *S. xylosus* and *Staphylococcus* spp, which supported the findings that plant extracts are usually more active against Gram positive bacteria (Lin *et al*., 1999, Palombo and Semple, 2001).

V. perrottetii extracts showed a remarkable antimicrobial potential, this agrees with the observation of previous work by (Olukoya *et al*., 1993) that plant contains substances that are antimicrobial. The methanol extracts of *V. perrpttetii* also showed antibacterial activities against *Staphylococcus* spp(c), though the antibiotic tends to be higher in

activity compared to the plant extracts against *Staphylococcus aureus*(c) for methanolic combination of *V. prrottetii* extracts where the antibiotic failed. Methanol probably releases some active constituents, which have high activity against the organism. The antibacterial effect of methanol extracts against this organism may be due to the ability of the methanol to extract some of the active chemical constituents of the plant like the phenolic compound, saponnin, bryophyllin and other secondary metabolites which have been reported to be antimicrobial (Cowan, 1999; Okwu and Josiah, 2006). The presence of flavonoids in *Vernonnia* spp are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions, thus can significantly affect the cell wall of the organism and invariably leads to the collapse of the cell wall, thus affecting the entire mechanism of the organism as reported by Nwinyi *et al*. (2009).

The zone of inhibition observed for *V. perrottetii* (Me) was the same with the zone of the antibiotic, which was used as the control (amoxycillin) for *S. xylosus* (a) at 1000mg/ml. *S. warneri* was the least susceptible organism to both aqueous and methanol extracts of the plant. The resistance of the *Staphylococcus* spp(c) to antibiotic (amoxylin) where the combination of the methanol extracts showed appreciable antimicrobial effects (Table 4.16) may be due to the synergistic nature of the plant extracts and the fact that methanol releases some phytochemical constituents of these plants. Susceptibility differences between the organisms may be due to cell wall structural differences between these classes of bacteria (Nwinyi *et al*., 2009). The relatively lower activities of the extracts compared to the standard antibacterial drug (amoxylin) for some of the isolates might probably be due to metabolism of the extracts by the bacteria, which reduced their activities (Cowan, 1999).

MBC value was found to be higher than the MIC value of the extracts tested against the micro-organisms, indicating bacteriostatic effects of the extracts. Aqueous extracts of *Vernonia perrottetii* were found to be bactericidal against *S. aureus* in two isolates, *S. xylosus* in two isolates. The bactericidal activity may be possibly due to high content of essential oils such as monoterpenes and sesquiterpenes (Nwinyi *et al*., 2009). Methanol extracts of *V. perrottetii* was found to be bacteriostatic to only S. aureus (c). Also the methanolic extracts of *V. perrottetii* was found to be bacteriostatic against *S. warneri*, S. *xylosus* (a) and *Staphylococcus* spp (a), *Staphylococcus* spp (b) and *Staphylococcus* spp (c) at MIC_{500}mg/ml/ and $MBC>_{1000}$mg/ml. *Vernonia perrottetii* methanolic extracts showed bacteriostatic activity to the entire micro-organisms tested. Aqueous extracts of the combination were found to be bacteriostatic against *S. aureus* (c) at MIC_{500}mg/ml/$MBC >_{1000}$mg/ml and bactericidal against *S. xylosus* (a), MIC_{250}/MBC_{500}. The reason may also be due to high concentration of essential oil such as monoterpenes and sesquiterpene (Nwinyi *et al*., 2009). Combination of the methanol extracts was only found to be bacteriostatic against *S. aureus* (c).

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary and Conclusion

The prevalence of vaginitis from this study was 153(51%) consisting of 79(26.3%) *Candida* and 74(24.7%) Staphylococcal vaginal infections. There was a significant association between use of contraceptives, wearing tight clothing and underwear and pregnancy with incidence of vaginal infection. One of the standard antifungal agents used (Ketoconazole) had no activity to all the *Candida* spp isolated. Aqueous extracts of *V. perrottetii* gave the highest zone of inhibition to one of the micro organism compared to the antibiotics used.The antifungal susceptibility level of the plant extracts to both singly and in combination was very low. Combination of the plant showed weak to moderate activity against some of the *Staphylococcus* species isolated. Some of the isolates exhibited resistance to the plant extracts as well as the antibiotics. Where the plant extracts had a pronounced activity on the micro organisms, the standard antibiotic used failed.

6.2 Recommendations

a) Further work should be done to explain why the combination showed low activity compared to the single plant extracts.
b) Further study should be done by using different types of solvents (different from the present study) for the extraction processes as well as fractionation to identify the bioactive components of the plants. *In-vivo* study using animal models should also be carried out to know the precise dose of the plant extracts.

c) Public enlightenment should be carried out to discourage wearing of tight clothing and underwear and the indiscriminate use of contraceptives, which are predisposing factors for vaginal infections.

REFERENCES

Abalaka, M.E. and Damisa, D. (2013). Antifungal activity of *Gomphrena celosioides* (soft khaki weed) on selected fungal isolates. *Journal of Current Research in Science*, 1(2):66-70.

Abbott, M., Hughes, D.L. and Patel, R. (1991). Angioedema after fluconazole. *Lance*t, 338:633.

Abdullahi, M., Muhammad, G. and Abdulkadir, N.U. (2003). *Medicinal and Economic Plants of Nupe land*. Publisher- Jubes –Evans, Bida, Niger State, Nigeria. P.84

Abebe, E.A., Olumide, M. and Oke, O. (2001). *A manual for Health workers on Syndromic Management of Sexually Transmitted Infections*. National AIDS and STD control program; Federal Ministry of Health Abuja. P.3-7.

Abo, K.A., Adeyemi, A. and Adeite, D.A. (2000). Ethnobotanical survey of plants used in the treatment of infertility and sexually transmitted diseases in South-West Nigeria. *African Journal of Medical Science* 29: 325-327.

Adad, S.J., de Lima, R.V., Sawan, Z.T., Silva, M. L., De Souza, M. A., Saldanha, J.C., Falco, V.A., DaCunha, A.H. and Murta, E.F. (2008). Evaluation of cytokines in endocervical secretion and vaginal. *Sao Paulo Medical Journal*, 119(6):200-205.

Adjanohoun, E.J., Adjakidjè, V., Ahyi, M.R.A., Ake, A. L., Akoegninou, A., D'Almeida, J., Apovo, F., Boukef, K., Chadare, M., Dramane, K., Eyme, J., Gassita, J.N., Gbaguidi, N., Goudoté, E., Guinko, S., Houngnon, P., Issa, L., Keita, A; Kiniffo, H; Koné-Bamba, D; Musampa, N. A; Saadou ,M; Sodogandji, T., De Souza, S., Tchabi, A., Zinsou, D.C. and Zohoun, T. (1989). Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. *Agence de Coopération Culturelle et Technique*, 895: 713-724.

Afolayan, A.J. (2003).Extract from the shoot of Arctotis arctotoides inhibit the growth of bacteria and fungi. *Pharmacology and Biotechnology*, 41(1): 22-25.

Agrawal, B., Das, S. and Pandey, A. (2011). *Boerhavia diffusa* Linn: A review on its phytochemical and pharmacological profile. *Asian Journal of Applied Science*, 4: 663-684.

Ahmad, I. and Agil, F. (2007). *In-vitro* efficacy of bioactive extracts of fifteen medicinal plants against ES_{β}-producing multidrug-resistant enteric bacteria. *Microbiology Research*, 162: 264-275.

Ahmad, I., Mehomood, Z. and Mohammed, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, 62: 183-193.

Akah, P. A., Nnamani, C.E. and Nnamani, P.O. (2010). Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. *Journal of Medicine* and *Medicine Sciences*, 1(10): 447-452.

Akerele, J., Abhulimen, P. and Okonefua, F. (2002). Prevalence of asymptomatic genital infection among pregnant women in Benin-city, Nigeria. *African Journal of Reproductive Health*, 6(3): 93-97

Akinjobi, C.O., Anyanuwa, B.N.,Onyeze, G.O.C. and Ibkwe, V.I. (2004). Antibacterial activities and premilinary pytochemical screening of four medicinal plants. *Journal of Applied Science*, 7(3). 4328-4338.

Akinjogunla, O.J.,Yah, S.C., Eghafona, N.O. and Ogbemudia, F.O. (2010). Antibacterial activity of leave extracts of Nymphaea Lotus (Nymphaceae) on Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Reasistant *Staphyloccus aueus* (VRSA) isolated from clinical samples. *Animal Biological Research*, 1(2): 174-184

Alabi, A.C., Busia, K., Ekanmian, V. and Bakiono, F. (2011). The use of medicinal plants in self-care in the Agonlin region of Benin. *Journal of Ethnopharmacology*, 133: 234-243.

Albrecht, P.J., Davar, G., Eisenberg, E., Pare, M. and Rice, F.L .(2006). Is CRPS a neupenic pain syndrome. *Public Medical Journal*, 123(1-2):217

Al-Fattani, M.A. and Douglas, L.J. (2006). Biofilm matrix of *Candidaalbicans* and *Candidatropicalis*: Chemical composition and role in drug resistance. *Journal of Medical Microbiology*, 55: 999-1008

Alvarez-Lerma, F., Nolla-Salas, J., Leon, C., Palomar, M., Jorda, R., Carrasco, N. and Bobillo, F. (2003). Candiduria in critically ill patients admitted to intensive care medical units. *Intensive Care Medicine*, 29**:**1069-1076.

American College of Obstetrics and Gynecology (2006). Vaginitis. ACOG practice bulletin. No 72. *Obstetric Gynaecology*, 107:1195-1201.

Amir, L. (2002) Breastfeeding and *Staphylococcus aureus* : Three Case Reports. *Breast feeding Reviews* 10: 15-18

Amsel, R., Totten, P., Spiege,l C., Chen, K., Eschenbach, D. and Holmes, K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *American Journal of Medicine*, 74:14– 22.

Anand, T. P., Bhat, A.W., Shouche, Y.S., Roy, V., Siddlarth, J. and Sharma, S.P. (2006). Antimicrobial activity of Marine bacteria associated with sponges from water off the coast of south east. *Indian Microbiology Resistance Research*, 161:252-262.

Anchkar , J.M. and Fries, B.C (2010). *Candida* Infection of the Genitourinary Tract. *Clinical Microbiology Reviews*, (2):253-257.

Anderson, M.R., Klink, K. and Cohrssen, A. (2004). Evaluation of vaginal complaints. *Journal of American Medical Association,* 291:1368-1379.

Andrade, P.B., SilvaL,R., Valentão, P., Faria, J., Ferreres, F., Sousa, C., Gil-Izquierdo, P. and Pinho B.R (2012). Phytochemical investigations and biological potential screening with cellular and non-cellular models of globe amaranth (*Gomphrena*. *Globosa* L.) inflorescences. *Food Chemistry*, 135(2): 756-63

Anukam, K., Osazuwa, E., Ahonkhai, I., Ngwu, M. O. G., Andrew, W. and Reid, G. (2006). Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial. *Microbes and Infection*, 8 (6): 1450–1454.

Arce, M. and Barroga, W. (2007). *Antimicrobial activity of wellawel (Chromolena odorata) stem extract*. An unpublished thesis. University of Northern Phillipines

Arenac, P. and Azorearo, R. M. (1977). Plants of common use in Paraguayan folk medicine for regulating fertility. *Economic Botany*, 31:298-301.

Arias, C.A. and Murray, B. E. (2009). Antibiotic Resistant. Buggs in the 21st century- A Clinical super- challenge. *New England Journal of Medicine*, 360(5):439-443

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bella, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for Malaria therapy in Baker. *Tropical Journal of Pharmaceutical Research*, 7(3): 1019-1024

Babula, O., Lazdane, G., Kroica, J., Ledger, W.J. and Witkin, S. S. (2003). Relation between recurrent vulvovaginal candidiasis, vaginal concentrations of mannose-binding lectin, and a mannose-binding lectin gene polymorphism in Latvian women. *Clinical Infectious Diseases*, 37**:**733-737.

Baille, G.S. and Douglas, L.J. (1999). Role of dimorphism in the development of *Candida albicans* biofilm. *Journal of Medical Microbiology*, 48:671–67.

Bao, S., Jiang, R. and Kwan, W. (2011). Evaluation of next generation sequencing software in mapping and assembly. *Journal of Human Genetics*, 56(6): 406–414.

Bao, Y. and Bacon, J.R. (2004). Phytochemicals protect against hetrocyclic amine-induced DNA adduct formation. In: *Phytochemicals in health and disease*, Bao Y, Fenwick GR, eds. New York: Marcel Dekker Inc. pp. 143-162.

Barnett, J. A. (2004). A history of research on yeasts 8: taxonomy. *Yeast*, 21(14):1141-93.

Barousse, M., Van- Delpol, B. J., Fortenberry, D., Orr, D. and Fidel, P.L.Jr. (2003). Vaginal yeast Colonization, Prevalence of vaginitis and associated local immunity in adolescent. *Sexually Transmitted Infections*, 80:48-53

Bauters, T. G., Dhont, M. A., Temmerman, M. I. and Nelis, H. J. **(**2002). Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in women. *AmericanJournal of Obstetric and Gynecology*, 187**:**569-574.

Beck-Saque, C.M. and Jarvis, W. R. (1993). National nosocomial infections surveillance system, Secular Trends in the Epidemiology of Nosocomial Fungal Infections in the United States, 1980-1990, *Journal of Infectious Diseases*, 167:1247-51

Beigi, R. H., Meyn, L. A., Moore, D. M., Krohn, M. A. and. Hillier, S. L. (2004).Vaginal yeast colonization in nonpregnant women: A longitudinal study. *Journal of Obstetrics and Gynaecology*, 104:926-930.

Bello, M.D., Gonzalez, A., Barnabe, C. and Larrouy, G. (2012). First characterization of *Candida*. *albicans* by Random Amplified Polymorphic DNA method in Nicaragua and comparison of the diagnosis methods for vaginal candidiasis in Nicaraguan women. *Memorias do Instituto Oswaldo Cruz*, 97(7): 985-989

Bellomaria, B. and Kacou, P. (1995). Plants of medicine population in Agboville, *Cote D'ivorie. Fitoterapia*, 66:117-141.

Bello-Onaghise, G., Vaikosen, S.E. and Envivie, S.E. (2012). Prevalence among women with understandable vaginitis. *Nigerian Journal of Agriculture, Food and Environment*, 8(4): 37-42

Berg, A.O., Heidrich, F.E., Fihn, S.D., Bergman, J.J.,Wood, R.W. and Stamm, W.E. (1984). Establishing the cause of genitourinary symptoms in women in a family practice. Comparison of clinical examination and comprehensive microbiology. *Journal of American Medical Association*, 251:620– 625.

Berger, B. J., Kolton, S., Zenilman, J. M., Cummings, M. C., Feldman, J. and Mc-Cormack, W. M. (1995). Bacterial vaginosis in lesbians: a sexually transmitted disease. *Clinical Infectious Diseases*, 21:1402–1405.

Bernharadt, H. and Knoke, M. (2008). Something special of *Candida* biofilms. *Mycoses*, 51:33-38.

Bhat, R.B and Jacobs, T.V. (2009).Traditional herbal medicine in Transkei. *Journal of Ethnopharmacology*, 48:7-12.

Biggs, W.S. and Williams, R.M. (2009). Common Gynaecologic Infections. *Primary Care*, 36(1):33-51. Abalaka, M.E and Damisa, D (2013). Antifungal activity of *Gomphrena celosioides* (soft khaki weed) on selected fungal isolates. *Journal of Current Research in Science*, 1(2):66-70.

Bishe, B., Syed, G. H. and Field, S. J. (2012). Role of Phosphatidy linositol 4-phosphate (p14p) and its binding protein GOLPH3 in hepatic C virus secreation. *Journal of Biology and Chemistry*, 287:27637-27647

Biswas, S., Van Dijck, P. and Datta, A. (2007). Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. *Molecular Biology Reviews*, 71: 348–376.

Blackwell, A. L., Thomas, P. D., Wareham, K. and Emery, S. J. (1993). Health gains from screening for infection of the lower genital tract in women attending for termination of pregnancy. *Lancet*, 342:206–210.

Bodey, G.P. (1993). *Candidiasis. Pathogenesis, diagnosis and treatment*, 2nd ed. New York,Raven Press. P. 167–84.

Botha, S. and Gerritsma-Van der Vijer, L.M. (1986). Pharmacochemical study of *Gomphrena celosioides* (Amaranthaceae). *Suid- Afrikaanse Tydskrif vir Natuurw-etenskap en Tegnologie*, 5(1): 40-45.

Bourg, A.C, Darmendrail, E. and Ricour, J. (1989). Geochemical filtration or riverbank and migration of heavy metals between the Deule piler and the Ansecrenttles Alluinon-chalk Aquifer (Nord france). *Geodermatology*, 44: 224-244

Bradley, S. F. (2002). *Staphylococcus aureus* Infections and Antibiotic Resistance in Older Adults. *Clinical Infectious Diseases*, 34: 211-216.

Bradshaw, C. S., Pirotta, M., De Guingand, D., Hocking, J. S., Morton, A.N., Garland, S.M., Fehler, G., Morrow, A. and Walker, S. (2012). Efficacy of oral metronidazole with vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-controlled double-blind trial. *Public Library of* Science, 7 (4): 34540- 34542.

Braun, B. R. and Johnson, A. D. (2000). TUP1, CPH1 and EFG1 make independent contributions to filamentation in *Candida albicans*. *Genetics*, 157:57–67

Briselden, A. and Hillier, S. (1990). Longitudinal study of the biotypes of *Gardnerella vaginalis*. *Journal of Clinical Microbiology*, 28: 2761–2764.

Brock, M. (2009). Fungal metabolism in host niches. *Curriculum Opinion Microbiology*, 12:371–376

Bump, R. C. and Buesching, W. J. (1988). Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exclusive sexual transmission. *American Journal for Obstetrics and Gynaecology*, 58:935–939.

Burkill, H. M. (1985). *The useful plants of West Tropical Africa*, Vol 1 (pp 61-2); Vol 2 (pp 337-338). Kew: Royal Botanical Gardens.

Cabell, C. H., Jollis, J. G. and Peterson, G. E. (2002). Changing Patient Characteristics and the Effect on Mortality in Endocarditis. *Archives of Internal Medicine*, 162: 90-94.

Calvo, J., Hernandez, J. L., Farinas, M. C., Garcia-Palomo, D and Aguero, J. (2000). Osteomyelitis caused by Staphylococcus schleiferi and evidence of misidentification of this Staphylococcus species by an automated bacterial identification system. *Journal of Clinical Microbiology*, 38(10)**:**3887-3889.

Casadevall, A. and Pirofski, L.A. (2001). Host-pathogen interactions: the attributes of virulence. *Journal of Infectious Diseases*, 184:337–344

Casidharan, S., Darah, I and Jain, K. (2008). Antimicrobial activity and wound healing potential on infected rat of *Gracilaria changii* Methanolic Extract. *Pharmacology Online*, 2:661-670

Cassone, A., De Bernardis, F. and Santoni, G. (2007). Anticandidal immunity and vaginitis: novel opportunities for immune intervention. *American Journal of Obstetrics and Gynaecology*, 178**:**203-211

Cathy, J.W. and Marie, P. (2011). Recurrent vulvovaginal candidiasis - current management. *Australia Family Physician*, 40(3):149-51.

Center for Disease Control and Prevention. (2002). Sexually transmitted diseases treatment guidelines. *Morbidity and Mortality Weekly Report Recommendation*, 51(6):1-80.

Cetin, M., S. Ocak, A., Gungoren and Hakverdi, A. U. (2007). Distribution of *Candida* species in women with vulvovaginal symptoms and their association with different ages and contraceptive methods. *Scandinavian Journal of Infectious Diseases*, 39**:**584-588.

Chaffin, W. L., Lopez-Ribot, J.l., Casanova, M., Gozalbo, D. and Martinez, J. P. (1998). Cell wall and secreted proteins of *Candida albicans*: identification, function and expression. *Microbiology and Molecular Biology Reviews*, 62:130–180

Chaim, W., Foxman, B. and Sobel, J. D. (1997). Association of recurrent vaginal candidiasis and secretory ABO and Lewis phenotype. *Journal of Infectious Diseases*, 176**:**828-830.

Chakrabarti, A. (2005). Microbiology of systemic fungal infection. *Journal of Postgraduate Medicine*, 51(l1): 16–S20

Chamaine, A., Loyd, C. L., Menon, T and Umamasheshweri. K. (2005). Anticandidal activity of *Azadirachta indica*. *Indian Journal of Pharmacology* 37: 386-389.

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormik, T. and Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture and drug resistance. *Journal of Bacteriology*, 18:5385–5394

Chapel, H. M., Spickett, G. P.and Ericson, D. (2000). The Comparison of the Efficacy and safety of intravenous versus subcutaneous Immunoglobulin replacement therapy. *Journal of Clinical Immunology*, 20(2): 94-100

Cheesbrough, M. (1984). *Medical Laboratory Manual for Tropical Countries*. pp 390

Chong, P. P., Lee, Y. L. and Tan, B. C. (2003). Genetic relatedness of *Candida* strains isolated from women with vaginal candidiasis in Malaysia. *Journal of Medical Microbiology*, 52**:**657-666.

Choudhry, M. D., Niteesh, K., Avorn, J., Robert, J. M. D., Glynn, D., Elliott, M., Antman, M. D., Sebastian, S. M. D., Michele, T. D., Lonny Reisman, M.S., Joaquim, F. M. D., Claire, S. M.S., Joy, L., Lee, Ph.D., Raisa Levin, M.S., Troyen Brennan, M.S., William, H. M.P.H. and Shrank, M. S. H. S. (2011). Full Coverage for Preventive Medications after Myocardial Infection. *New England Journal of Medicine*, 365: 2088-2097

Claderone, R. A. and Fonzi, W. A. (2002).Virulence factors of *Candida albicans*. *Trends in Microbiology*, 9:327–336

Corner, B. E. and Magee, P. T. (1997).*Candida* pathogenesis: Unraveling the trends of infection. *Curriculum Biology*, 2:691-694

Corsello, S., Spinillo, A., Osnengo, G., Penna, C., Guaschino, S., Beltrame, A., Blasi, N. and Festa, A. **(**2003). *An epidemiological survey of vulvovaginal candidiasis in Italy. Europoean Journal of Obstetric and Gynecology and Reproduction Biology*, 110**:** 66-*72.*

Cotch, M. F., Hillier, S. L., Gibbs, R. S. and Eschenbach, D. A. **(**1998). Epidemiology and outcomes associated with moderate to heavy *Candida* colonization during pregnancy. Vaginal Infections and Prematurity Study Group. *American Journal of Obstetrics and Gynaecology*, 178**:**374-380

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12: 564-582.

Daniels, W., Glover, D. D., Essmann, M. and Larsen, B. **(**2001). Candidiasis during pregnancy may result from isogenic commensal strains. *Infectious Disease of Obstetrics and Gynaecology*, 9**:**65-73.

Davis, J., Connor, E., Clark, P., Wilkinson, E. and Duff, P. (1997). Correlation between cervical cytologic results and gram stain as diagnostic tests for bacterial vaginosis. *American Journal of Obstetrics and Gynaecology*, 177: 532– 5.

De Bernardis, F., Arancia, S., Morelli, L., Hube, B., Sanglard, D., Schafer, W. and Cassone, A. (1999) .Evidence that members of aspartyl proteinase gene family, in particular SAP 2, are virulence factors for *Candida* vaginitis. *Journal of Infectious Diseases*, 179:201–208

De Francesco, M. A., Ravizzola, G., Peroni, L.., Negrini, R. and Manca, N. (2007). Urinary tract infections in Brescia, Italy: Etiology of uropathogens and antimicrobial resistance of common uropathogens. *Medical Science Monitor*, 13:136-144.

De Leon, E. M., Jacober, S. J., Sobel, J. D. and Foxman, B**. (**2002). Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *Biomedical Central of Infectious Diseases*, 2**:**1

Del Palacio, A. (1992). Fungal skin and soft tissue infections. *Current Opinion in Infection Disease*s 5: 687-694.

DeMoura, R. M., Pereira, P. S., Januário, A. H., França, S. D. E C. and Dias, D. A. (2004). Antimicrobial screening and quantitative determination of benzoic acid derivative of *Gomprenacelosioides* by TLC-densitometry. *Chemical and Pharmaceutical Bulletin,* 52(11): 1342-1344.

Demuri, G. P. and Hostetter, M. K. (1995). Resistance to antifungal agents. *Pediatric Clinic of North America*, 42:665-685.

Dey, S. K., Banerjee, D., Chattapadhyay. S. and Karmakar, K. B. (2010). Antimicrobial activities of some medicinal plants of West Bengal. *International Journal of Pharmaceutical and Biological Science*s, 1: 1-10.

Dhawan, B. W., Patnaik, G. K., Rastogi, R. P., Sing, K. K. and Tandon, J. S. (1997). Screening of Indian plants for Biological Activity. Part VI. *Indian Journal of Experimental Biology*, 15 (3):208-219

Dias, D. A., DeMoura, R. M. X., Pereira, P. S., Januario, A. H. and Franca, S. C. (2004). Antomicrobial Screening and Quantitative Determination of Benzoic Acid Derivatives of G. celosioides by TLC Densitometry. *Chemical Pharmaceutical Bulletin*, 52(11):1342-1344.

Dickson, R. A., Houghton, P. J., Hylands, P. S. and Gibbons, S. (2006). Antimicrobial resistance-modifying effects, antioxidant and free radical scavenging activities of Mozoneuron bentanianu-Ball, Secumega Unosaroxo and Wlld and meroglossa Pyrafolia Lam. *Phytotheraphy Research*, 20:41-45.

Dominic, R. M., Shenoy, S. and Baliga, S. (2007). *Candida* biofilms in medical devices: Evolving trends. *Kathmandu University Medical Journal*, 5:431–436

Donbraye-Emmanuel, O. O. B., Donbraye, E., Okonko, I. O., Alli, J. A., Ojezele, M. O and Nwanze, J. C. (2010). Detection and prevalence of *Candid*a among pregnant women in Ibadan, Nigeria. *World Applied Science Journal*, 10(9): 986-991

Dosumu, O. O., Idowu, P. A., Onocha, P. A. and Ekundayo, O. (2010). Isolation of 3-(4-Hydroxyphenyl) Methylpropenoate and bioactivity evaluation of Gomphrena celosioides. *EXCLI Journal*, 9: 173-180.

Dota, K. F.D., Shinobu, C. S., Patussi, E.V., Consolaro, M. E. L. and Svidzinski, T. I. E (2008). Susceptibility to vaginal yeast in most used antifungal in Maringa, Paraná, Brazil. *Acta Bioquímica Clínica Latinoamericana*, 110:66-72

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends in Microbiology*, 11:30-36

DP Hamacher and Associates (Hamacher Resource Group). (1996). Stock Check. Feminine health care: A changing market share. *Drug Topics*, 42-47.

Drew, R. H., Arthur, R. R. and Perfect, J. R. (2005). Is it time to abandon the use of amphotericin B bladder irrigation?. *Clinical Infectious Diseases*, 40**:**1465-1470

Duerr, A., Heilig, C. M., Meikle, S. F., Cu-Uvin, S., Klein, R. S., Rompalo, A. and Sobel, J. D. (2003). Incident and persistent vulvovaginal candidiasis among human immunodeficiency virus-infected women: risk factors and severity. *Obstetrics and Gynaecology*, 101**:**548-556

Eckert, L. O. (2006). Acute vulvovaginitis. *New England Journal of Medicine*, 355**:**1244-1252.

Eckert, L. O., Hawes, S. E., Stevens, C.E., Koutsky, L. A., Eschenbach, D. A. and Holmes, K. K. (1998). Vulvovaginal candidiasis: clinical manifestations, risk factors and management algorithm. *Obstetrics and Gynaecology*, 92:757– 65.

Eckert, S. E., Sheth, C. C. and Muhlschlegel, F. A. (2007). Regulation of morphogenesis in Candia species. In: d'Enfert CH, Hube B (eds) *Candida*. *Comparative and functional genomics*. Caister Academic, Norfolk, pp 263–291

Edilson, N., Reingewertz, T. H., Gostfried, Y., Lev, T., Zuri, D. and Maniu, L. (2012). Peptides Minmicking the unique ARTS-XIAP binding site promote apoplotic cell death in cultured cancer cells. *Clinical Cancer Research*, 18:2569-2578

Edmund, F. L., Martin, J. W. and John, A. (1992). *Infectious diseases text and colour atlas.* 2nd edition lower publishing, London, pp 774-784.

Ejoh, R. A., Nkonga, D. V., Gouado, I. and Mbofung, C. M. (2007). Nutritional Components of some non-conventional leafy vegetables consumed in Cameroon. *Pakistan Journal of Nutrition*, 6:712–717.

Ekwempu, C.C., Lawande, R.V. and Egler, L.J (1981). Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. *Journal of Clinical Pathology*, 34(1): 82-83.

Eleyinmi, A. F., Sporns, P. and Bressler, D.C. (2008). Nutritional composition of *Gongro-nema latifolium* and *Vernonia*. *amygdalina*. *Nutrition and Food Science*, 38:99-109

Ellof, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60:1-8.

Elvin-Lewis, M. and Lewis. W. H. (1995). *New concepts in medical and dental Ethnobotany*. In: Schultes, R. E. and Von reis, S. eds. *Ethnobotany: Evolution of a discipline*. Dioscorides Press, Portland. Pp 303–310.

Enweani, I. B., Gugnani, H.C., Okobia, R and Ojo, S.B. (2001). Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria. *Review of Ibero Americana Micologia*, 18:171-173.

Erdemeier, C. A. J., Cinali, J., Rabenan, H., Doerr, H. W., Biber , A. and Koch, E. (1996). Antiviral and antiphlogistic activities of *Hemmalisvirginiana* bark *Planta Medica*, 62: 241-245.

Espinel-Ingroff, A., Stockman, L., Roberts, G., Pincus, D., Pollack, J. and Marler. J. (1998). Comparison of RapID Yeast Plus system with API 20C system for identification of common, new, and emerging yeast pathogens. *Journal of Clinical Microbiology*, 36**:**883-886.

Falagas, M. E., Betsi, G. I. and Athanasiou, S. (2006). Probiotics for prevention of recurrent vulvovaginal candidiasis: A review. *Journal of Antimicrobial Chemotherapy*, 58:266-272.

Fan, S. R., Liu, X. P. and Li, J. W. **(**2008). *Clinical characteristics of vulvovaginal candidiasis and antifungal susceptibilities of Candida species isolates among patients in southern China. Journal of Obsteteric and Gynaecology Research*, 34**:**561-566

Febre, N., Silva, V., Medeiros, E. A., Wey, S. B., Colombo, A. L. and Fischman, O. **(**1999). Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catheterization. *Journal of Clinical Microbiology*, 37**:**1584-1586.

Federal Ministry of Health (FMOH) (2005). *A manual of technical report on the National HIV/Syphilis sentinel survey among pregnant women attending Antenatal clinics in Nigeria*. Abuja. Nigeria. pp. 1-11.

Ferrer, J. (2000). Vaginal Candidosis: Epidemiological and Etiological factors. *International Journal of Gynaecology and Obstetrics*, 71(1):21-27.

Fidalgo, S., Vazquez, F., Mendoza, M. C., Perez, F. and Mendez, F. J. (1990**).** Bacteremia due to *Staphylococcus epidermidis*: Microbiologic, Epidemiologic, Clinical, and prognostic features. *Review Infectious Dis*eases, 12(3)**:**520-528.

Fidel, P. L. (2004). Immunity to *Candida*. *Oral Diseases*, 8: 69–75.

Fidel, P. L. Jr., Vanquez, J. A. and Sobel, J. D. (1999). *Candida glabrata*: A review of epidemiology, pathogenesis and clinical disease with comparison to *Candida albicans*. *Clinical Microbiology Reviews*, 12:80–96

Filler, S. G., Sheppard, D. C. and Edwards, J. E. Jr. (2006). *Molecular basis of fungal adherence to endothelial and epithelial cells*. In: Heitman, J., Filler, S. G., Edwards, J. E. Jr., Mitchell, A. P (eds). *Molecular principles of fungal pathogenesis*. ASM, Washington, DC, pp 187–196

Fischer, P. and Addison, L. (1985). Diagnosis of *Candida* vaginitis. *Journal of Family Practice*, 120:19–20

Fluckiger, U., Marchetti, O., Bille, J., Eggiman, P., Zimmerli, S., Imhof, A., Garbino, J., Ruef, C., Pittet, D., Tauber, M., Glauser, M. and Calandra, T. (2006). Treatment options of invasive fungal infections in adults. *Swiss Medical Weekly*, 136:447-463

Fong, I. W. (1992). The value of treating the sexual partners of women with recurrent vaginal candidiasis with ketoconazole. *Genitourinary Medical*, 68:174-176.

Fox, K. K. and Behets, F. M. (1995). Vaginal discharge. How to pinpoint the cause. *Postgraduate Medicine*, 93:87-90.

Foxman, B. (1990). The epidemiology of vulvovaginal candidiasis: Risk factors. *American Journal of Public Health,* 80**:**329-331.

Foxman, B., Barlow, R., D'Arcy, H., Gillespie, B. and Sobel, J. D. (2000). *Candida* vaginitis: Self-reported incidence and associated costs. *Sexually Transmitted Diseases*, 27:230-235.

Franklin, T. J. and Snow, G. A., (1989). *Biochemistry of Antimicrobial action*, 4th edn, Published by Chaoman Hall London, P.216.

Funk, D.J. and Kumar, A. (2011). Antimicrobial Therapy for Life - threatening Infections: *Speed is Life Critics Care Clinical*, 27: 53-76.

Gabbe, S., Niebyl, J. and Simpson, J. L. (1991). *Obstetrics: Normal and Problem Pregnancy*. 2nd ed. NewYork: Churchill Livingston. p 1097.

Galgiani, I. N. (1993). Susceptibility testing of fungi: Current status of the standardized process. *Antimicrobial Agents Chemotherapy*, 37:2517-2521.

Gantait, A., Maji, A., Barman, T., Banerji, P.,Venkatesh, P. and Mukherjee, P. K. (2011). Estimation of capsaicin through scanning densitometry and evaluation of different varieties of capsicum in India. *Natural Product Resources*, 26: 216-222.

Gardner, H. L. and Dukes, C. D. (1995). *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis. *American Journal of Obstetrics and Gynaecology*, 69:962–976

Geiger, A. and Foxman, B. (1996). Risk factors for vulvovaginal candidiasis: A case-control study among university students. *Epidemiology*, 7:182 – 1877.

Geiger, A., Foxman, B. and Gillespie, B. (1995). The epidemiology of vulvovaginal candidiasis among university students. *American Journal of Public Health*, 85:1146– 1148.

Georgiev, A., Cjukic-Ivancevic S. and Bujko, M. (2003). Bacterial vaginosis: Epidemiology and Risk Factors. *Srp Arh Celok Lek,* 128: 29-35.

Gessler, M. C., Nkunya, M. H., Mwasumbi, L. B., Heinrich, M. and Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropical*, 56:65-77.

Ghani, A., Abdulrahman, E. M. and Onaolapo, J. A. (1989). Chemical and Microbiological Evaluation of Some Nigerian Traditional Preparations, The Report, Nigeria. *Journal of Medical Microbiology*, 3:3

Ghannoum, M. A. (2000). Potential role of phospholipases in virulence and fungal pathogenesis. *Clinical Microbiology Reviews*, 13:122–143

Gilson, R. and Mindel, A. (2001). Recent advances: Sexually-Transmitted Infections. *British Medical Journal*, 322: 1160 – 1164.

Giraldo, P. C., Babula, O., Goncalves, A. K. S., Linhares, I. M., Amaral, R. L., Ledger, W. J. and Witkin, S. S. **(**2007). Mannose-binding lectin gene polymorphism, vulvovaginal candidiasis, and bacterial vaginosis. *Obstetrics and Gynaecology*, 109**:**1123-1128.

Gleason, H. A. (1923). Evolution and geographical distribution of the genus *Vernonia* in North America. *American Journal of Botany*, 10:187-202.

Gorinstein, S., Nue, J. and Arruda, P. (1991). Alcohol-soluble and total proteins from Amaranthus seeds and their comparison with other cereals. *Journal of Agriculture and Food Chemistry*, 39: 848-850.

Goswami, R., Dadhwal, V., Tejaswi, S., Datta, K., Paul, A., Haricharan, R. N., Banerjee, U. and Kochupillai, N. P. (2000). Species-specific prevalence of vaginal candidiasis among patients with diabetes mellitus and its relation to their glycaemic status. *Journal of Infections,* 41:162-166.

Gow, N. A. R., Brown, A. J. P. and Odds, F. C. (2002). Fungal morphogenesis and host invasion. *Current Opinion of Microbiology*, 5:366–371

Graybill, J. R. (1989). New antifungal agents. *European Journal of Clinical Microbiology and Infectious Diseases,* 5: 402-412.

Grierson, A. J. C. and. Long. D. G. (1984). *Flora of Bhutan including a record of plants from Sikkim and Darjeeling*. Vol. 1, Pt. 2. Royal Botanic Garden, Edinburgh. pp. 190-462

Gupta, K., Hooton, T. M., Naber, K. G., Wultts, B., Colgan, R. and Miller, L.G. (2010). International Clinical Guideline for Treatment of acute uncomplicated by the infectious disease society of America and the European Society for Microbiology and Infectious Diseases. *Clinical Infectious Diseases,* 52(5):103-120.

Haas, B. J., Salzberg, S. L., Zhu, W., Pertea, M., Allen, J. E., Orvis, J., White, O., Buell, C. R. and Wortman, J. R. (2008). Automated eukaryotic gene structure annotation using evidence Modeler and the Program to Assemble Spliced Alignments. *Genome Biology*, 9(1): 7

Hall-Stoodley, L., Costerton, J. W. and Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *National Reviews in Microbiology*, 2:95–108

Hans, R. (1983). *Superficial Fungal Diseases. In: Pharmanual. A Comprehensive guide to theapeutic use of Ticonao*. Pharmalibri NY, p.I - 21

Harborne, J. C. (1984). *Phytochemical Methods*, A guide to modern Techniques of plant analysis, 2nd ed: Chapmann and Hall Ltd, London, pp. 168-170.

Harrigan, W. F. (1998). *Laboratory Methods in Food Microbiology* (3rd ed.) Academic Press, San Diego, pp. 198

Hasan, F., Xess, I., Wang, X., Jain, N. and Fries, B. C. (2009). Biofilm formation in clinical *Candida* isolates and its association with virulence. *Microbes Infections*, 11:753-761.

Hawser, S. P. and Douglas, L. J. (1994). Biofilm formation by *Candida* species on the surface of catheter materials *in vitro*. *Infectious Immunology*, 62:915–921

Hay, P. E., Lamont, R. F., Taylor-Robinson, D., Morgan, D. J., Ison, C. and Pearson, J. (1994). Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *British Medical Journal*, 308:295–298

Hay, P. E., Ugwumadu, A. and Chowns, J. (1997). Sex, thrush and bacterial vaginosis. *International Journal of SexuallyTransmitted Disease and Aquire Immune Defeciency Syndrome*, 8:603–608.

Hay, R. J. (1993). Risk/benefit ratio of modern antifungal therapy: Focus on hepatic reactions. *Journal of American Academic Dermatology*, 29(l):50-54.

Hayens, K. A. and Westerneng, T. J. (1996). Rapid identification of *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* by species specific PCR of large subunit ribosomal DNA. *Journal of Medical Microbiology*, 44:390–396

Haynes, K. (2002) .Virulence in *Candida* species. *Trends in Microbiology*, 9:591–596

Hedayati, T. and Shafiei, G. (2010). Candidiasis in Emergence Medicine. *Medscape*. http://emedicine.medscape.com/article/781215-overview#showall.

Helbig, I., Mefford, H. C., Sharp, A. J., Guipponi, M., Fichera, M., Franke, A., Muhle, H., De Kovel, C., Baker, C., Von Spiczak, S., Kron, K. L. and Steinich, I. (2009). Micro-deletions increase risk of idiopathic generalized epilepsy. *Nature Genetics*, 41: 160-162.

Hill, G. (1993). The microbiology of bacterial vaginosis. *American Journal of Obsteteric and Gynaecology*, 169:450- 454.

Hillier, S. L. (1998). The vaginal microbial ecosystem and resistance to HIV. AIDS Res. *Human Retroviral*, 14(1):17-21.

Hitchcock, G. L., Mariano, A. J. and Rossby, T. (1993). Mesoscale pigment fields in the Gulf Stream: Observations in a meander crest and trough. *Journal of Geophysical Research*, 98: 0148-0227.

Hogan, D. A. (2006). Talking to themselves: Autoregulation and quorum sensing in fungi. *Eukaryotic Cell*, 5:613–619

Holland, J., Young, M. L., Lee, O. and Chen, C. A. S. (2003). Vulvovaginal carriage of yeasts other than *Candida albicans*. *Sexual Transmission and Infections,* 79:249-250.

Holm, L. G., Pancho, J. V., Herberger, J. P. and Plucknett, D. L. (1979). *A Geographical Atlas of World Weeds*. Wiley, New York. pp. 391

Horn, D. L., Neofytos, D., Anaissie, E. J., Fishman, J. A., Steinbach, W. J., Olyaei, A. J., Marr, K. A., Pfaller, M. A., Chang, C. H. and Webster, K. M. (2009). Epidemiology and outcomes of candidemia in 2019 patients: Data from the prospective antifungal therapy alliance registry. *Clinical Infectious Diseases*, 48:1695-1703.

Horowitz, B. J., Giaquinta, D. and Ito, S. (1992). Evolving pathogens in Vulvovaginal candidiasis: implication for patient care. *Journal of Clinical Pharmacology*, 32:248-255.

Hoyer, L. L. (2001). The ALS gene family of *Candida albicans*. *Trends in Microbiology*, 9:176–180

Hube, B. and Naglik, J. (2004). From Commensal to Pathogen: Stage and tissue specific gene expression of *Candida albicans*. *Current Opinion of Microbiology*, 7:336–341

Ibrahim, A. S., Mirbod, F., Filler, S. G.,Yoshiko, B., Cole, G., Kitajima, Y., Edwards, J. E. Jr., Nosawa, Y. and Ghannoum, M .A. (1995). Evidence implicating phospholipase as a virulence factor of *Candida albicans*. *Infectious Immunology*, 63:1993–1998.

Ijeh, I. I. and Ejike, C. E. C. C. (2011). Current perspectives on the medicinal potentials of *Vernonia amygdalina*. *Journal of Medicinal Plants Research*, 5:1051.

Ilkit, M. and Guzel, A. B. (2011). The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. *Critical Reviews in Microbiology*, 37 (3): 250–261.

Inman, W., Pearce, G. and Wilton, L. (1994). Safety of fluconazole in the treatment of vaginal candidiasis: A prescription-event monitoring study, with special reference to the outcome of 15pregnancy. *European Journal of Clinical Pharmacology*, 46:115-118.

Iwu, M. M. (1993). Handbook of African Medicinal Plants. CRC Press, London. Pp. 415

Iwu, M. W., Duncan, A. R. and Okunji. C. O. (1999). *New antimicrobials of plant origin*. *In*: Janick J, eds., *Perspectives on New Crops and New Uses*. Alexandria, VA, ASHS Press, pp. 457-462.

Jacob, P. H. and Nall, J. S. (1990). Fluconazole Vs Econazole in the treatment of vaginal candidiasis. In: *Current issues and challenges in the management of Vagina candidiasis.* Nice France, Pp. 21-23.

Jacqueline, M. A. and Bettina, C. F. (2010). *Candida* Infection of the Genitourinary Tract. *Clinical Microbiology Reviews*, 23(2):253-2743

Jain, N., Kohli, R., Cook, E., Gialanella, P., Chang, T. and Fries, B. C. **(**2007). *Biofilm formation by and antifungal susceptibility of Candida isolates from urine. Applied Environmental Microbiology*, 73**:**1697-1703.

James, W. D. and Berger, T G. (2006). *Andrews' Diseases of the Skin: Clinical Dermatology*. Saunders Elsevier. pp. 308–311.

Jombo, G. T. A., Opajobi, S.O., Egan , D. Z., Banwat, E. B. and Denenakaa, P. (2010). Symptomatic Vulvovaginal Candidiasis and Genital colonization by *Candida* species in Nigeria. *Public Health and Epidemiology*, 2(6):147-151

Jones, R. N., Bale, M. I. and Hoban, D. (2004). *In-vitro* antimicrobial activity oftioconazole and its concentrations in vaginal fluids following topical (Vagistat-l 6.5%) application. *Diagnostic Microbiology and Infectious Diseases*, 17:45-51

Kadar, A. A. and Kumar, A. (2005). Prevalence and antimicrobial susceptibility of extended spectrum beta lactamase producing *Escherischia coli* and *Klebsiella pneumonia* in a general hospital. *Annual Saudi Meeting*, 25:239-242.

Kauffman, C. A., Vazquez, J. A., Sobel, J. D., Gallis, H. A., McKinsey, D. S., Karchmer, A. W., Sugar, A. M., Sharkey, P. K., Wise, G. J., Mangi, R., Mosher, A., Lee, J. Y. and Dismukes, W. E. (2000). Prospective multicenter

surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. *Clinical Infectious Diseases*, 30:14-18.

Kauffman, C. A., Fisher, J. F., Sobel, J. D and Newman, C. A. (2011). *Candida* Urinary Tract Infections Diagnosis. *Clinical Infectious Diseases,* 52:452-456.

Kayode, A. A. A. and Kayode, O. T. (2011). Some medicinal values of *Telfairia occidentalis*: A review. *American Journal of Biochemistry and Molecular Biology*, 1: 30-38.

Keeley, S. C. and Jones, S. B. (1979). Distributionofpollentypesin Vernonia (Vernonieae: Compositae). *Systematic Botany*, 4: 195-202.

Kenneth, O. B. (2003). STDs/HIV/AIDS-Challenge of next millennium. *Nigerian Journal of Genitourinary Medicine*, 18: 18-30.

Kent, H. L. **(**1991). Epidemiology of vaginitis. *American Journal of Obsteteric and Gynaecology*, 165**:**1168-1176

King, R. D., Lee, J. and Morris, A. (1980). Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infectious Immunology*, 27:667–74.

Kohler, I., Kristina, J. S., Carola, K., Karstens, S., Daniel, A., Urich, B. and Eckart, E. (2002). Herbal remedies traditionally use against malaria in Ghana. Bioassy-Guide fraction of *Microglossa pyrifolia* (Asteraceae). *Verlag der Zeit5 chrif fur Naturfors chung*, 57c:1022-1027.

Kostermans, A. J. G. H., Wirjahardja, S. and Dekker, R. J. (1987). *The weeds: Description, ecology and control*. p 24-565 In : Soerjani, M., Kostermans, A. J. G. H. and Tjitrosoepomo, G. (eds.). *Weeds of Rice in Indonesia. Balai Pustaka, Jakarta, Indonesia*.

Kourkoumpetis, T., Manolakaki, D., Velmahos, G., Chang, Y., Alam, H.B., De Moya, M.M., Sailhamer, E.A. and Mylonakis, E. (2010). *Candida* infection and colonization among non-trauma emergency surgery patients. 1(5):359–366. *Sexually Transmitted Diseases*, 28**:**579-597

Kuleta, J. K., Kozik, M. R. and Kozik, A. (2009). Fungi pathogenic to humans: molecular basis of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochimica Polonica*, 56:211–224

Kumar, T. and Chandrashekar, K. S. (2011). *Bauhinia purpurea* Linn: A review of its ethnobotany, phytochemical and pharmacological profile. *Research Journal of Medicinal Plants,* 5: 420-431.

Kvaal, C., Lachke, S. A., Srikantha, T., Daniels, K., McCoy, J. and Soll, D. R. (1999). Misexpression of the opaque phase specific gene PEP1 (SAP1) in the white phase of *Candida albicans* confers increased virulence in a mouse model of cutaneous infection. *Infections and Immunity*, 67:6652–6662

Lamont, R., Hudson, E. and Hay, P. (1999). A comparison of the use of Papanicolaou-stained cervical cytological smears with gram-stained vaginal smears for the diagnosis of bacterial vaginosis in early pregnancy. *International Journal of Sexually-Transmitted Diseases and Aquired Immune Deficiency Syndrome*, 10: 93 -97.

Latha, M. A, Rao, K.V, Reddy, T. P and Reddy, V. D. (2006). Development of transgenic pearl millet [Pennisetum glaucium (L) R. Br] plants resistant to downg mildew. *Plant Cell Reproductive*, 25:927-935

Leberer, E., Harcus, D., Broadbent, I. D., Clark, K. L., Dignard, D., Ziegelbauer, K., Schmidt, A. and Gow, N. A. R. (1996). Dimorphism in Human Pathogenic and Apathogenic Yeasts. *Molecular Microbiology*, 19:1255-1263

Lee, B., Feinberg, M., Abraham, J. and Murthy, A. (1992). Congenital malformations in an infant born to a woman treated with fluconazole. *Paediatric Infectious Diseases*, 11:1062-1064.

Lewis, J. H., Zimmerman, H. J., Benson, G. D. and Ishak, K. G. (1999). Hepatic injury associated with ketoconazole therapy: analysis of 33 cases. *Gastroenterology*, 86:503-513.

Lin, J., Opake, A. R.., Geheeb-Keller, M., Hutchings, A. D., Terblanche, S. E and Jäger, A. K. (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti microbial activities. *Journal of Ethnopharmacology* 68: 267-274.

Linder, H. P (2008). The Tradition of the Cape Flora, Southern Africa. *Biological Reviews*, 78:597-638

Lipsky, M. S. and Waters, T. (1999). The prescription-to-OTC switch movement: Its effects on antifungal vaginitis preparations. *Archives of Family Medicine*, 8:297-300.

Liu Chang, T. and Chu, C. (2001). Expectorant Principle from *G.globosa* structure determination of a new flavones glycoside. *Yao HSush Tung.pao*, 1(6):55-56

Lundstrom, T. and Sobel, J. **(**2001). Nosocomial candiduria: a review. *Clinical Infectious Diseases*, 32**:**1602-1607

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plant and human pathogens. *World Journal of Agricultural Science*, 4: 839-843.

Mardh, P. A. (2004). The vaginal ecosystem. *American Journal of Obsteteric and Gynecology*, 165:1163–1168.

Mari, E. E and Martin, S. L. (2000). Diagnosis of Virginities. *American Family Physician*, (1):2

Marieb, N. and Elaine. (1997). *Essentials of Human anatomy and physiology*. U.S.A, Benjamin/Cummings Publishing company inc. pp 471-490.

Marino, M., Frigo, F., Bartolomeoli, I. and Maifreni, M. (2010). Safety-related properties of Staphylococci isolated from food and food environments. *Journal of Applied Microbiology*, 110:550-561.

Marlete, B. F., Alzira, P. D., Renata, O. D., Ana, R. M. M., Tatiana, D. A., Flavia, B. D., Da Silva, P. N., Marcia, D. N. and Mario, C. A. M. (2005). Isolation of *Candida* spp from vaginal microbiota of Healthy canine females during estrous cycle. *Brazilian Journal of Microbiology*, 36:201-204

Marrazzo, J. M., Handsfield, H. H. and Whittington, W. L. H. (2002). Predicting chlamydial and gonococcal cervical infection: implications for management of cervicitis. *Obstetrics and Gynaecology*, 100:579-84.

Martins, G. S. and Jones, D. M. (1940). The epidemiology of sepsis in the United States. *New England Journal of Medicine*, 348(16):1546-1554

Masoko, P. and Eloff, J. N. (2005). The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography. *African Journal of Biotechnology*, 4(12): 1425-1431.

Mathema, B., Cross, E., Dun, E., Park, S., Bedell, J. and Slade, B. (2001). Prevalence of vaginal colonization by drug-resistant *Candida* species in college-age

women with previous exposure to over-thecounter azole antifungals. *Clinical Infectious Diseases*, 33:23-27.

McClelland, R. S., Richardson, B. A., Hassan, W. M., Graham, S. M., Kiarie, J., Baeten, J. M., Mandaliya, K., Jaoko, W., Ndinya-Achola, J. O. and Holmes, K. K. **(**2009). Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *Journal of Infectious Diseases*, 199**:**1883-1890.

McGregor, J. A., French, J. I., Parker, R., Draper, D., Patterson, E., Jones, W., Thorsgard, K. and McFee, J. (1995). Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation. *American Journal of Obstetrics and Gynaecology*, 173:157-167.

Merit, D. F. (2011). Vulvovaginitis In: Kliegman, R.M., Behrman, R.E., Jenson, H.B. and Stanton, B.F, (eds.) *Nelson Text Book of Pediatrics*, 19th ed Philadelphia, pa. Saunders Elsevier, pp 543.

Metzger, G. (1998). Laboratory diagnosis of vaginal infections. *Clinical Laboratory Science*, 11: 47- 52.

Mikail, H. G. (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum (*L.) bulbs in experimental rabbits. *Journal of Medicinal Plants*, **4**: 322-326.

Miller, M. G. and Johnson, A. D. (2002). White opaque switching in *Candida albicans* is controlled by mating type locus homeodomain proteins and allows efficient mating. *Cell* , 110:293–302

Milson, I. and Ferrsman, L. (1985). Repeated candidiasis reinfection or recrudescence? A review. *American Journal of Obstetrics and Gynaecology*, 152: 956-957

Mitchell, S. J., Engelman, J. and Kent, C. K. (2007). Azithromycin-resistant syphilis infection: San Francisco, California, 2000--2004. *Clinical Infectious Diseases*, 42:337-345

Mohajer, M., Sarkhail, P., Hajarolasvadi, N., Zamani, M. J. and Khorasan, R. (2006). Anti inflammatory and analgesic effects of *Phlomis lanceolata* boiss extracts and examination of their components. *International Journal* of *Pharmacology*, 2:50-54.

Molero, G., Dies-Oreja, R., Navarro-Garcia, F., Monteoliva, L., Pla, J., Gill, C., Sanchez-Perez, M. and Nambela, C. (1998). *Candida albicans*: Genetics, dimorphism and pathogenicity. *International Journal of Microbiology*, 1:95–106

Monod, M. and Zepelin, M. B. (2002) Secreted proteinases and other virulence mechanisms of *Candida albicans*. *Chemical Immunology*, 81:114-128

Moody, K. (1989). Weeds Reported in Rice in South and Southeast Asia. International Rice Research Institute (IRRI), Manila, Philippines. pp. 442

Moore, R. D. and Chaisson, R. E. (1996). Natural history of opportunistic disease in an HIV-infected urban clinical cohort. *Annual International Medicine*, 124:633–642

Moyes, D. L and Naglik, J. R. (2011). Mucosal Immunity and *Candida*. *albicans* Infection. *Clinical and Developmental Immunology*, 1:9.

Msuya, S. E., Mbizuo, E., Stray-Pedersen, B., Sundby, J., Sam, N. E. and Hassan, A (2002). Reproductive tract infections among women attending primary health care facilities in Moshi, Tanzania. *East African Medical Journal*, 79(1): 16-21.

Mukherjee, P. K., Mukherjee, D., Maji, A. K., Rai, S. and Heinrich, M. (2009). The sacred lotus (*Nelumbo nucifera*), Phytochemical and therapeutic profile. *Journal of Pharmacy and Pharmacology*, 61: 407-422.

Mulhern, S. M., Logue, M. E. and Butler, G. (2006). *Candida albicans* transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. *Eukaryotic Cell*, 5:2001–2013

Mulligen, M. E., Murry-Leisure, K. A., Ribner, B. S., Standiford, H. C., John, J. F., Karvick, J. A., Kauffman, C. A.and Yu, V. L. (1993). Methicillin-resistant *Staphylococcus aureus*. *American Journal of Medicine*, 94:313–328.

Musyimi, D. M., Ogur, J. A. and Muema, P. M. (2008). Phytochemical compounds and antimicrobial activity of extracts of *Aspilia* plant (*Aspilia mossambicensis*) (Oliv) wild. *International Journal of Botany*, 4: 56-61.

Naglik, J.R., Albercht, A., Bader, O. and Hube, B. (2004). *Candida albicans* proteinases and host pathogen interactions. *Cellular Microbiology*, 6:915–926

Naglik, J. R., Challacombe, S. J. and Hube, B. (2003). *Candia albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiology and Molecular Biology Reviews*, 67:400–428

Naglik, J. R., Moyes, D., Makwana, J., Kanzaria, P., Tsichlaki, E., Weindl, G., Tappuni, A. R., Rodgers, C. A., Woodman, A. J., Challacombe, S. J., Schaller, M. and Hube, B. (2008). Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. *Microbiology*, 154:3266–3280

National Committee for Clinical Laboratory Science (2002). *In-vitro* Susceptibility Testing of *Geotrichum capitatum*: Comparison of the E-Test, Disk Diffusion, and Sensititre Colorimetric Methods with the NCCLS M27-A2 Broth Microdilution Reference Method. *Antimicrobial Agents Chemotherapy*, 47(12): 3985–3988

National Committee for Clinical Laboratory Science. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard Fifth Edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA. Pp 610-68.

National Germplasm Resources Program (2002). World Economic Plants in GRIN (Germplasm Resources Information Network). United States Department of Agriculture, Agricultural Resources Service, National Germplasm Resources Program (NGRP). Beltsville. pp 701

Neal, D. E. Jr. (2008). Complicted Urinary Infection. *Urologic Clinical North America*, 35(1):13-22.

Neuhaus, G., Pavic, N. and Pletscher, M. (1991). Anaphylactic reaction after oral fluconazole. *Biomedical Journal*, 302:1341

Nilsson, U., Hellberg, D., Shoubnikova, M., Nilsson, S., Mardh, P. A. (1997). Sexual behavior risk factors associated with bacterial vaginosis and Chlamydia trachomatis infection. *Sexually-Transmitted Diseases*, 24:241–246.

Njeh, J. and Toyang, R. V. (2013). A review of the Medicinal Potentials of plants of the genus *Vernonia* (Asteraceae). *Journal of Ethnopharmacology*, 146:681-723.

Noble, R. C. (1982). *In: sexually transmitted diseases*, New York; Med. Pub. Company, Inc., 1982, pp. 62.

Noda, K., Teerawatsakul, M., Prakongvongs, C. and Chaiwiratnukul, L. (1985). *Major Weeds in Thailand*. National Weed Science Research Institute, Bangkok, Thailand, pp 142

Nugent, R, P., Krohn, M. and Hillier, S. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram's stain interpretation. *Journal of Clinical Microbiology*, 29:297–301.

Nwankwo, E. O. K., Kandakai olukemi, V. T. and Shavaibu, S. A. (2010). Aetiologic agents of abnormal vaginal discharge among females of reproductive age in Kano, Nigeria. *Medicine and Biochemical Sciences*, 12:16.

Nwinyi, O; Chinedu, N. S; Ajani, O. O; Ikpo, C. O and Ogunniran, K. O. (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of Food Science*, 3(3): 77-81.

Nwosu, M. C., Nwosu, M. N., Mba, I. E. K., Opara, C. and Nwajuaku, C. (2001). Genital ulcers and Sexualy-Transmitted Diseases in Rural Nigeria. *Journal of Medical Investment Practice*, 2: 28-33

Nyirjesy, P., Seeney, S. M., Grody, M. H., Jordan, C. A. and Buckley, H. R. (1995). Chronic fungal vaginitis: The value of cultures. *American Journal of Obstetrics and Gynaecology*, 173:820-823.

Odds, F. C., Gow, N. A. R. and Brown, A. J. P. (2006). *Towards a molecular understanding of Candida albicans virulence*. In: Heitman, J., Filler, S.G., Edwards, J.E. Jr. and Mitchell, A.P. (eds). *Molecular principles of fungal pathogenesis*. ASM, Washington, DC, pp 305–319

Odebode, S. D. (2004). Acceptability of sweet potato "sparri" and its potentials for enhancing food security and economic empowerment of rural woman in southeastern Nigeria. *Nigerian Journal of Rural Sociology*, 1(2):104-112.

Odunbaku, O. A., Ilusanya, O. A. and Akasoro, K. S. (2008). Antimicrobial effects of ethanolic leaf extract of selected medicinal plants on some human pathogenic microbes. *Science Research Essay*, 3(11): 562-564

Okonkwo, K. C., Reich, K., Alabi, A. I., Umeike, N. and Nachman, S. A. (2007). An Evaluation of Awareness: Attitudes and Beliefs of Pregnant Nigerian Women toward Voluntary Counselling and Testing for HIV. *Acquire Immune Deficiency Syndrome Patient Care and Sexually- Transmitted Diseases*, 21(4):252-260.

Okulicz, J. F., Grandits, G. A., French, J. A., George, J. M., Simpson, D. M., Birbeck, G. I., Ganesan, A., Weintrob, A. C., Crum-Cianflone, N., Lalani, T. and Landrum, M. I. (2011). Virologic outcomes of HAART with concurrent use of cytochrome P450 enzyme-inducing antiepileptics: a retrospective case control study. *Aquired Immune Deficiency Syndrome Research Therapy*, 8:18.

Okwu, D. E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, 5 (4): 257-361.

Olukoya, D. K., Ndika, N. and Odugbemi, T. O. (1993). Antibacterial activity of some medicinal plants in Nigeria. *Journal of Ethnopharmacology*, 39: 69-72

Onocha, P. A; Ajaiyeoban, E. O; Dosumu, O. O. and Ekundayo, O. (2005). Phytochemical Screening and Biological Activities of *Gomphrena celosioides* (C.Mart) extracts. *Biokemstri*, 5(2): 59-65.

Ononge, S., Wondabwa, J., Kiondo, P. and Busingye, R (2005). Clinical presentation and management of alleged sexually assaulted females at Mulago hospital, Kampala, Uganda. *African Journal of Health Science*, 5(1): 50-54.

Opaneye, A. A. (1999). The Socio - *Economic Impact of Sexually Transmitted Infections and HIV/ AIDS in Developing Countries: Contemporary Issues in Reproductive Health in Nigeria*. Occasional Working Papers Series, Woman Health and Action Research Centre (WHARC), Benin City, Nigeria. pp. 26

Oriel, B., Partridge, J. D., Denny, B. M. and Coleman, K. J. J. C (1972). Genital yeast infections. *British Medical Journal*, 4: 761-764

Osoba, A. O, Johnston, N, Ogunbanjo, B. O and Ochei, J. (1989). The Susceptibility of *Neisseria Gonorrhoeae* in Nigeria to Specitnomyan. *African Journal of Clinical Microbiology*, (In Press) In: Ogunbanjo, B.O (ed). Sexually-Transmitted Diseases in Nigeria. *West African Journal of Medical Science*, 8(1): 43-45.

Oyelese, A. O., Onipede, A. O., Aboderin, A. O., Adedosu, A. N. and Onayemi, O (2005). Sexually transmitted infections in Obafemi Awolowo University Teaching Hospital Ile-Ife, Nigeria: A decade of clinic experience. *African Journal of Clinical and Experimental Microbiology*, 6(1): 64-68.

Pakshir, k., Yazdani, M. and Kimiaghalam, R. (2007). Etiology of vaginal candidiasis in Shiraz, Southern Iran. *Research Journal of Microbiology*, 2:896-700.

Palombo, E. A and Semple, S. J. (2001). Antibacterial activity of traditional Australian medicinal plants .*Journal of Ethnopharmacology*, 77: 151-157.

Pappas, P. G., Kauffman, C. A., Andes, D., Benjamin, D. K., Calandra, Jr., T. F., Edwards, J. E., Filler, Jr., S. G., Fisher, J. F., Kullberg, B. J., Ostrosky-Zeichner, L., Reboli, A. C., Rex, J. H., Walsh, T. J. and Sobel, J. D. (2009). Clinical practice guidelines for the management of candidiasis. *Clinical Infectious Diseases*, 48**:**503-535.

Pappas, P. G. (2006). "Invasive candidiasis". *Infectious Diseases and Clinical North America*, 20 (3): 485–506.

Patel, D. A., Gillespie, B., Sobel, J.D., Leaman, D., Nyirjesy, P., Weitz, M.V. and Foxman, B. (2004). Risk factors for recurrent vulvovaginal candidiasis in women receiving maintenance antifungal therapy: results of a prospective cohort study. *American Journal of Obstetrics and Gynaecology*, 190(3): 644-653

Paul, B. and James, O. (1977). History of Ahmadu Bello University, Education and Power in Nigeria, Hodder and Stoughton, pp 26-30

Paxton, L. A., Sewankambo, N., Gray, R., Serwadda, D., McNairn, D., Li, C. and Wawer, M. J. (1998). Asymptomatic non-ulcerative genital tract infections in a rural Ugandan population. *Sexually-Transmitted Infectious Diseases*, 74:421–425.

Pfaller, M. A., Andes, D., Arendrup, M. C., Diekema, D.J., Espinel-Ingroff, A., Alexander, B. D., Brown, S. D., Chaturvedi, V., Fowler, C.L., Ghannoum, M. A., Johnson, E. M., Knapp, C. C., Motyl, M. R., Ostrosky Zeichner, L and Walsh, T. J. (2011a). Clinical breakpoints for voriconazole and *Candida* spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of speciesspecific interpretive criteria. *Diagnosis and Microbiology Infectious of Diseases*, 3: 330-343.

Pfaller, M. A., Diekema, D. J., Andes, D., Arendrup, M. C., Brown, S. D., Lockhart, S. R., Motyl, M., Perlin, D. S and Testing, C. S. F. A. (2011b). Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical and microbiological data to arrive at species-specific interpretive criteria. *Drug Resistance Update*, 14: 164-176.

Pfaller, M. A., Messer, S. A., Boyken, L., Rice, C., Tendolkar, S., Hollis, R. J., Doern, G.V and Diekema, D. J. (2005). Global trends in the antifungal susceptibility of *Cryptococcus neoformans*, *Journal of Clinical Microbiology*, 43: 2163-2167.

Phillips, A. J. (2005). Treatment of non-albicans *Candida* vaginitis with amphotericin B vaginal suppositories. *American Journal of Obstetrics and Gynaecology*, 192**:**2009-2013

Piddock, K. J. V. and Wise, R. (1989). Mechanisms of resistance to quinolones and clinical perspective. *Journal of Antimicrobial Chemotherapy*, 23:475–483.

Pincus, S. H. (1973). Treatment of fox-fordyce disease. *Journal of American Medical Association*, 223:924

Pomilio, A. B., Buschi, C. A., Tomes, C. N. and Viale, A. A. (1992). Antimicrobial constituents of *Gomphrena martiana* and *Gomphrena boliviana. Journal of Ethnopharmacology*, 36: 155-161.

Pomilio, A.B., Sola, G. A., Mayer, A. M. and Rumi, L. S. (1994). Antitumor and cytotoxic screen of 5,6,7-trisubstituted flavones from *Gomphrena martiana*. *Journal of Ethnopharmacology*, 44: 25-33.

Preethi, R. M., Devanathan, V. V. and Loganathan, M. (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food-borne pathogens. *Advances in Biological Research*, 4:122-125

Prescott, M. l., Harley, J., Donald, P. and Klein, A. (2002). In: *Antimicrobial chemotherapy: Microbiology*. 2nd Edition published by C. Brown publishers, U.S.A. pp3251

Projan, S. J. and Novick, R. P. (2000).The molecular basis of pathogenicity*:* In K. B. Crossley and G. L. Archer (ed.). *The staphylococci in human disease.* Churchill Livingstone, New York, N.Y. p. 55-81.

Pursley, T. J., Blomquist, I. K. and Abraham, J. (1996). Fluconazole- induced congenital anomalies in three infants. *Clinical Infectious Diseases*, 22:336-340.

Quattrocchi, U. (1999). *CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms. Synonyms, and Etymology*, first ed. CRC Press, p. 640

Ralph, S. G., Rutherford, A. J. and Wilson, J. D. (1999). Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *British Medical Journal*, 319:220–223.

Ramage, G., Ghannoum, M. A. and Lopez-Ribot, J. L. (2006). *Fungal biofilms: agents of disease and drug resistance*. In: Hetman, J., Filler, S.G., Edwards, J.E. Jr., Mitchell, A.P. (eds). *Molecular principles of fungal pathogenesis*. ASM, Washington, DC, pp 177–185

Ramage, G., Saville, S. P., Thomas, D. P. and Lopez-Ribot, J. L. (2005). *Candid*a biofilms: An update. *Eukaryotic Cell*, 4:633–638

Rangarag S.V.B., Ajits, P. H. and Brad, T. G. (2003). Rapid Identification of Community- Encountered *Candida* spp directly from blood culture bottles. *Journal of Clinical Microbiology*, 4(12):5660-5664

Rappleye, C. A. and Goldman, W. E. (2006). Defining virulence genes in the dimorphic fungi. *Annual Reviews in Microbiology*, 60:281–303

Raskin, I[1]., Ribnicky, D. M., Komarnytsky, S., Ilic, N., Poulev, A., Brinker A, Moreno, D. A., Ripoll, C., Yakoby, N., O'Neal, J. M., Cornwell, T., Pastor, I. and Fridlender, B. (2002). Plants and human health in the twenty-first century. *Trends in Biotechnology*, 20(12):522-31

Ray, A. M., Emerson, S. L. and Lawrence, M. H (2006). Predicted taxonomic patterns in pheromone production by Long-horned beetles. *Naturwissenchaften*. 93: 543-550

Redondo-Lopez, V., Meriwether, C., Schmitt, C., Opitz, M., Cook, R. and Sobel, J. D. (1990). Vulvovaginal candidiasis complicating recurrent bacterial vaginosis. *Sexually-Transmitted Diseases*, 17:51–53.

Reed, B. D., Zazove, P., Pierson, C. L., Gorenflo, D. W. and Horrocks, J. **(**2003). *Candida* transmission and sexual behaviors as risks for a repeat episode of *Candida* vulvovaginitis. *Journal of Women Health*, 12**:**979-989.

Reed, C. F. (1977). *Economically Important Foreign Weeds: Potential Problems in the United States*. Agricultural Research Services, Animal and Plant Health Inspection Service, U.S. Dept. of Agriculture, Washington, DC. pp.746

Reef, S. E., Levine, W. C. and McNeil, M. M. (1993). Treatment options for vulvovaginal candidiasis, *Clinical Infectious Diseases*, 20(1):80-90.

Reza, S. M., Tabatabaie, R. M., Maharramov, A. and Ali, R. M. (2011). Synthesis and *in-vitro* studies of biodgeable modified chitosan nanoparticles for photodynamic treatment of cancer. *International Journal of Biomacromolecule*, 49 (5): 1059-1065

Ribaud, P., Chastang, C., Latge, J. P., BAffroy-Lafitte, C., Parquet, N., Devergie, A., Esperou, H., Selini, F., Rocha, V., Derouin,F., Socie, G. and Gluckman, E. (1999). Outcome and prognostic factors of invasive aspergillosis after allogenic bone marrow transplantation. *Clinical Infectious Diseases*, 28:322–330

Ribeiro, M. A. (2000). Susceptibility profile of vaginal yeast isolates from Brazil. *Mycopathologia Dordrecht*, 151: 5-10

Richards, M. J., Edwards, J. R., Culver, D. H. and Gaynes, R. P. **(**2000). *Nosocomial infections in combined medical-surgical intensive care units in the United States. Infectious Control Hospitals Epidemiology*, 21**:**510-515

Richardson, M. D. (2005).Changing pattern and trends in systemic fungal infections. *Journal of Antimicrobial Chemotherapy*, 56:5-11.

Richter, S. S., Galask, R. P., Messer, S. A., Hollis, R. J., Diekema, D. J. and Pfaller, M. A. (2005). Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *Journal of Clinical Microbiology*, 43:2155-2162.

Robin, E. H., Anril, W., Alexander, M., Loeto, M. and Keith, K. (1998). Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenza* Type b in children under 5 years of age in Botswana. *International Journal of Infectious Diseases*, 3 (1), 18–25.

Rodrigues, A. G., Mardh, P. A., Pina-Vaz, C., Martinez-de-Oliveira, J. and Da Fonseca, A. F. (1999). Is the lack of concurrence of bacterial vaginosis and vaginal candidosis explained by the presence of bacterial amines? *American Journal of Obstetrics and Gynaecology*, 181:367-370.

Ronnqvist, P. D., Forsgren-Brusk, U. B. and Grahn-Hakansson, E. E. **(**2006). Lactobacilli in the female genital tract in relation to other genital microbes and vaginal pH. *Acta Obstetricia and Gynaecologica Scandinavica*, 85**:**726-735.

Ross, R. A., Lee, M. T and Onderdonk, A. B. (1995). Effects of *Candida albicans* infections and clotrimazol treatment on vaginal micro flora *in -vitro*. *Obstetrics and Gynaecology*, 86: 925-930.

Rottingen, J. A., Cameron, D. W., and Garnett, G. P. **(**2001). A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? *Sexually-Transmitted Diseases*, 28**:**579-597

Roy, S. and Banerjee, D. (2010). Isolation of Antimicrobial Compound by Endophytic bacteria from *Vinca rosea*. *International Journal of Currrculum Research*, 5: 47-51.

Rubia, K., Smith, A.B., Brammer, M.J. and Taylor, E. (2003). Right inferior prefrontal cortex mediates response inhibition while mesial prefrontal cortex is responsible for error detection. *NeuroImage*, 20: 351–358.

Ruiz-Herrera, J. and Hard, T. (1997). Dimorphism in Mucor rouxii (Zygomycetes). *Publications Wissens chatlichen Film.Biologie*, 23: 17–32.

Rupp, S. (2007). Interactions of the fungal pathogen *Candida albicans* with the host. *Future Microbiology*, 2:141–151

Ruttoh, E. K. (2009). Antimicrobial *efficacy of selected plants use by traditional medical practioners in Kerio, Kenya*. Nairobi, Kenya. M.Sc. Dissertation. Kenyatta University, p. 78-87.

Rylander, E., Berglund, A. L.., Krassny, C. and Petrini, B. (2004). Vulvovaginal *Candida* in a young sexually active population: prevalence and association with oro-genital sex and frequent pain at intercourse, *Sexually Transmitted Infections*, 80(1): 54–57

Saadabi, A. M. A., Sehemi, A. G. A. L. and Zailaie, K. A. AL. (2006). *In-vitro* antimicrobial activity of some Saudi Arabian plants used in folkloric medicine. *International Journal of Bot*any, 2: 201-204.

Salvador, M. J., Ferreira, E. O., Pral, E. M. F., Alfieri, S. C., Albuquerque, S., Ito, I.Y. and Dias, D. A. (2002). Bioactivity of crude extract and some constituents of *Blutaparon portulacoides* (Amaranthaceae). *Phytomedicine*, 9: 566-571.

Samanta, A., Ganguly, S., Hashimoto, H., Devadiga, S., Vermote, E., Knyazikhin, Y., Nemani, R. R. and Myneni, R. B. (2010). Amazon forests did not

green-up during the 2005 drought. *Geophysiology Research*, 37(10):1029-2009.

Sangetha, S. N., Zuraini, Z., Sasidharan, S. and Suryani, S. (2008). Free radical scavenging activity of *Cassia spectabillis* and *Cassia fistula*. *International Journal of Experimental Sciences*, 2:111-112.

Schafer, W. M. (2006). Glycosylphosphatidylinositol anchored proteases of *Candida albicans* target proteins necessary for both cellular process and host pathogen interactions. *Journal of Biological Chemistry*, 281:668–694

Schaller, M., Hube, B., Ollert, M. W., Schafe, W., Borg-Von, Z. M., Thoma-Greber, E. and Korting, H. C. (1999). *In-vivo* expression and localization of *Candida albicans* secreted aspartyl proteinases during oral candidiasis in HIV-infected patients. *Journal of Investigation in Dermatology*, 112:383–386

Schuster, B. G. (2007). A new integrated program for natural product development and the value of an Ethnomedical Approach. *Journal of Alternative Complementary Medicine*, 7:61-72.

Schwebke, J. and Lawing, L. (2001). Prevalence of Mobiluncus spp among women with and without bacterial vaginosis as detected by polmerase chain reation. *Sexuallly -Transmitted Diseases*, 28:195– 199.

Schwebke, J. R., Morgan, S. C. and Weiss, H. L. (1997). The use of sequential self-obtained vaginal smears for detecting changes in the vaginal flora. *Sexually-Transmitted Diseases*, 24:236–239.

Senet, J. M. (1998). *Candida* adherence pheneomenon, from commensalisms to pathogenicity. *International Journal of Microbiology*, 1:117-122

Shahid, Z. and Sobel, J. D. (2009). Reduced fluconazole susceptibility of *Candida albicans* isolates in women with recurrent vulvovaginal candidiasis: effects of long-term fluconazole therapy. *Diagnosis Microbiology of Infectious Diseases*, 64**:**354-356.

Shariff, Z. U. (2001). *Modern Herbal Therapy for Common Ailments. Nature Pharmacy SeriesVolume 1*. Spectrum Books Limited, Ibadan,Nigeria in Association with Safari Books(Export) Limited, United Kingdom, pp. 9 – 84.

Sharma, N. and Vijayrergia, R. (2011). Study of primary metabolites and antimicrobial activity of *Gomphrena celosioides* Mart. *International Journal of Pharmacy and Bioscience*, 2(4): 581-586.

Sheehan, D. J., Hoelscher, A. C. and Sibley, M. C. (1999). Current and emerging azole antifungal agents. *Clinical Microbiology Research,* 12: 40-79.

Sherarrad-Stratford, M. and James, S. A. (2003). *Non-alcoholic beverages and yeasts*. In: Boelhout, T, Robert, V. (eds) *yeasts in food. Beneficial and detrimental aspects*. Behn, Hambury, pp 309-346

Shuttleworth, R., Behme, R. J, McNabb, A and Colby, W. D (1997). Human isolates of *Staphylococcus caprae*: Association with bone and joint infections. *Journal of Clinical Microbiology*, **35**(10)**:**2537-2541.

Simeoni, D., Rizzotti, L., Coccencelli, P., Gazzola, S., Dellaglio, F. and Torriani, S (2008). Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities. *Food Microbiology*, 25:196-201.

Singh, S. I. (2003). Treatment of vulvovaginal candidiasis. *Clinical Reviews*, 136 (9): 26-30.

Singh, M., Chaudhry, M. A., Yadava, J. N. S. and Sanyal, S. C. (1992). The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984– 1986 in: *Northern India Journal of Antimicrobials and Chemotherapy,* 29: 159–168.

Smith, D., Boug, F and Midgley, J. (1992). Fluconazole- resistant *Candida* in Acquired Immune Deficiency Syndrome. *Journal of infectious*. 231: 345-346

Sobel, I., Vasquez, J. and Lynch, M. (1993). Vaginitis due to Saccharomyces cerevisiae: epidemiology, clinical aspects and therapy. *Clinical Infectious Diseases*, 16:93-99.

Sobel, J. (1993). Pathogenesis and treatment of recurrent vulvovaginal candidiasis. *Clinical Infectious Diseases,* 14:148–153.

Sobel, J. D. (1997). Vaginitis. *New England Journal of Medicine*, 337**:**1896-1903.

Sobel, J. D. (2006). The emergence of non-albicans *Candida* species as causes of invasive candidiasis and candidemia. *Curriculum Infectious Diseases and Reproduction,* 8:427-433.

Sobel, J. D. (2007). Vulvovaginal candidosis. *Lancet*, 369**:**1961-1971.

Sobel, J. D., Bradshaw, S. K., Lipka, C. J. and Kartsonis, N. A. **(**2007). Caspofungin in the treatment of symptomatic candiduria. *Clininical Infectious Diseases*, 44**:**46-49

Sobel, J. D., Chaim, W., Nagappan, V. and Leaman, D. **(**2003). Treatment of vaginitis caused by *Candida glabrata*: use of topical boric acid and flucytosine. *American Journal of Obstetrics and Gynaecology*, 189**:**1297-1300.

Sobel, J. D., Faro, S., Force, R. W., Foxman, B., Ledger, W. J., Nyirjesy, P. R., Reed, B. D. and Summers, P. R. **(**1998). Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *American Journal of Obstetrics and Gynaecology*, 178**:**203-211.

Sobel, J. D., Kapernick, P. S., Zervos, M., Reed, B. D., Hooton, T., Soper, D., Nyirjesy, P., Heine, M, W., Willems, J., Panzer, H. and Wittes, H. **(**2001). Treatment of complicated *Candida* vaginitis: Comparison of single and sequential doses of fluconazole. *American Journal of Obstetrics and Gynaecology*, 185**:**363-369.

Sobel, J. D., Kauffman, C. A., McKinsey, D., Zervos, M., Vazquez, J. A., Karchmer, A. W., Lee, J., Thomas, C., Panzer, H. and Dismukes, W. E.(2000). Candiduria: A randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. *Clinical Infectious Diseases*, 30**:**19-24.

Sobel, J. D., Wiesenfeld, H. C., Martens, M., Danna, P., Hooton, T. M., Rompalo, A., Sperling, M., Livengood, C. I., Horowitz, B., Von Thron, J., Edwards, L., Panzer, H. and Chu, T. (2004). Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. *New England Journal of Medicine*, 351**:**876-883.

Sobel, J., Brooker, D. and Stein, G. (1995). Single oral dose fluconazole compared with conventional clotrimazole tropical therapy of *Candida* vaginitis. *American Journal of Obstetric Gynaecology*, 172:1263–1268.

Sobel, J. D (2005). What's new in bacterial vaginosis and trichomoniasis? *Infectious Diseases Clinical North America*, 19(2):387-406.

Sobel, J. D. (1993). Pathogenesis and treatment of recurrent vulvovaginal candidiasis. *Clinical and Infectious Diseases*, (1):I48-153.

Sofowora, A. (1993). Medical plants and traditional medicine in Africa.' Published by Spectrum books Limited, Ibadan. pp. 1-10

Sogbetun, A. O., Amusa, K.O. and Osoba, A. O. (1977). Sexually transmitted diseases in Ibadan, Nigeria. *British Journal of Veneral Diseases,* 53(1): 156-160.

Sohail, M. N., Rasul, F., Karim, A., Kanwal, U. and Attitalla, P. M. (2011). Plant as a source of natural antiviral agents. *Asian Journal of Animal and Veterinary Advance* 6: 1125-1152.

Sojakowa, M., Liptajova, D., Borousky, M. and Subik, J. (2004). Fluconazole and Itraconazole susceptibility of vaginal yeasts isolated from Slovakia. *Mycopathologia,* 157:163-169.

Soll, D. R. (1988). High-frequency switching in *Candida albicans* and its relations to vaginal candidiasis. *American Journal of Obstetrics and Gynaecology*, 158**:**997-1001.

Soll, D. R. (2002a). *Phenotypic switching*. In: Claderone, R. (ed). *Candida* and candidiasis. ASM, Washington, DC, pp 123–142

Soll, D. R. (2002b). *Candida* commensalism and virulence: the evolution of phenotypic plasticity. *Acta Tropical*, 81:101–110

Speigel, C., Amsel, R. and Holmes, K. (1983). Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. *Journal of Clinical Microbiology*, 18: 170-177.

Spence, D. (2007). *Candidiasis* (*Vuvolvaginal*)*: Clinical evidence*. http:www.clinical evidence. Com

Spinillo, A., Capuzzo, E., Acciano, S., De Santolo, A. and Zara, F. (1999). Effect of antibiotic use on the prevalence of symptomatic vulvovaginal candidiasis. *American Journal of Obstetrics and Gynaecology*, 180(1):14- 17.

Spinillo, A., Capuzzo, E., Gulminetti, R., Marone, P., Colonna, L. and Piazzi, G. (1997). Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. *American Journal of Obstetrics and Gynaecology*, 176:138-141.

Staab, J. F., Bradway, S. D., Fidel, P. L. and Sundstrom, P. (2002). Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science*, 283:1535-1538

Sweetman, S. (2005). The complete drug reference. *European Journal of Scientific Research,* 49(3):433-441.

Syed, M., Lee, S., Zheng, J. and Zhou, Z. J. (2004). Strange dependent dynamics and modulation of spontaneous waves in the developing rabbit retina. *Journal of physiology*, 560 (3): 533-549

Taylor-Robinson, D. and Hay, P. E. (1997). The pathogenesis of the clinical signs of bacterial vaginosis and possible reasons for its occurrence. *International Journal of Sexually-Transmitted Diseases and Acquired Immune Deficiency Syndrome*, 8(1):35-37.

Taylor-Robinson, D., Morgan, D., Sheehan, M., Rosenstein, I. and Lamont, R. (2003). Relation between gramstain and clinical criteria for diagnosing bacterial vaginosis with special reference to gram grade II evaluation. *International Journal of Sexually-Transmitted Diseases and Aquired Immune Deficiency Syndrome*, 14: 6–10.

Thai, L. and Hart, L. L. (1993). Boric acid vaginal suppositories. *Annual Pharmacotherapy*, 27:1355-1357.

Theiss, S., Ishdorj, G., Brenot, A., Kretschmar, M., Lan, C.Y., Nichterlein, T., Hacker, J., Nigam, S., Agabian, N. and Kohler, G. A. (2006). Inactivation of the phospholipase B gene PLB5 in wild type *Candida albicans* reduces cell associated phospholiupase A2 activity and attenuates virulence. *International Journal of Medical Microbiology*, 296:405–420

Thewes, S., Kretschmar, M., Park, H., Schaller, M., Filler, S. G. and Hube, B. (2007). *In- vivo* and *ex-vivo* comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. *Molecular Microbiology*, 63:1606–1628.

Trofa, D., Gacser, A. and Nosanchuk, J. D. (2008). *Candida parapsilosis, an emerging fungal pathogen. Clinical Microbiology Reviews*, 21**:** 606-625

Usanga, V. U., Abia-Bassey, L., Inyang-etoh, P.C. and Udoh, S. (2010). *Trichomonas vaginalis* infection among pregnant women in Calabar, Cross River State, Nigeria. *The Internet Journal of Gynaecology and Obstetrics*, 14(2): 101-107

Uzair, B; Ahmed, N., Ahmad, V. V., Mohammad, F.V. and Edward, D. H. (2008). The isolation, purification and biological activity of a novel antibacterial compound produced by pseudomonas stutzeri. *FEMS Microbiology Letter*, 279: 240-243.

Van kessel, K., Assefi, N., Marrazzo, J. and Ecker, L. (2003). Common complementary and alternative therapies for yeast vaginitis and bacterial vaginosis: A systematic review. *Obstetrics and Gynaecology Survey*, 58:351-8.

Vazquez, L., Salvarezza, R. C., Ocon, P., Herrasti, P., Vara, J. M., Arvia, A. J (1994). Self-affine fractal electrodeposited gold surfaces: Characterization by scanning tunneling microscopy. *Physiological Review*, 49: 1507-1511

Vermitsky, J. P., Self, M. J., Chadwick, S. G., Trama, J. P., Adelson, M. E., Mordechai, E. and Gygax, S. E. (2008). Survey of vaginal-flora *Candida* species isolates from women of different age groups by use of species-specific PCR detection. *Journal of Clinical Microbiology*, 46:1501-1503.

Vieira, C. C. J; Mercier, H; Chu, E. P and Figueiredo-Ribeiro, R. C. L. (1994). *Gomphrena species (globe amaranth) in-vitro culture and production of secondary metabolites. In: Medicinal and Aromatic Plants VII*. Bajaj, Y. P. S. (ed). *Biotechnology and Agricultural Forestry Journal*, 28: 257-270

Vitali, B., Pugliese, C., Biagi, E., Candela, M., Turroni, S., Bellen, G., Donders, G. G. G. and Brigidi, P. **(**2007). Dynamics of vaginal bacterial communities in women developing bacterial vaginosis, candidiasis, or no infection, analyzed by PCR. *Applied Environmental Microbiolgy*, 73(18): 5731-5741

Vujic, G. J., Knez, A., Despot, S. V. and Kuzmic, V. V. (2013). Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: A double-blind, randomized, placebo-controlled study. *European Journal of Obstetric Gynecology and Reproductive Biology*, 168 (1): 75-79.

Wagner, W. L., Herbst, D. R. and Sohmer, S. H. (1999). *Colocasia*. In: *Manual of the Flowering Plants of Hawaii*. University of Hawaii Press, Honolulu, Hawaii, pp. 1356-1357.

Waldvogel, F. A. **(**2000). *Staphylococcus aureus In: G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases. Churchill Livingstone, Philadelphia, Pa. p 2069-2092.*

Walsh, T.J. and Dixon, D.M. (1996). *Deep Mycoses*. In: Baron, S. eds. Baron's Medical Microbiology (4th ed.). University of Texas Medical Branch. Pp 109-135

Wang, C. C., McClelland, R. S., Reilly, M., Overbaugh, J., Emery, S. R., Mandaliya, K., Chohan, B., Ndinya-Achola, J., Bwayo, J. and Kreiss, J. K. **(**2009). The effect of treatment of vaginal infections on shedding of human

immunodeficiency virus type1. *Journal of Infectious Diseases*, 183**:**1017-1022.

Warrick, A. W., Myers, D. E. and Neisen, D. R. (1986). *Geostatistical Methods Applied to soil science*. P.53-82. In: A.Kluteced, *Method of soil analysis*. 2nd ed. Soil science society of America, Madison, WI.

Watson, C., and Calabretto, H. (2007). Comprehensive review of conventional and non-conventional methods of management of recurrent vulvovaginal candidiasis. *Aust. N. Z. Journal of Obstetrics and Gynaecology*, 4 7:262-272

Watson, M. C., Grimshaw, J. M., Bond, C. M., Mollison, J. and Ludbrook, A. **(**2012). *Oral versus intra-vaginal imidazole and triazole anti-fungal agents for the treatment of uncomplicated vulvovaginal candidiasis (thrush): a systematic review. An International Journal of Obstetrics and Gynaecology,* 109**:**85-*95.*

Weisberg, M. (1988). *Considerations in therapy for vulvovaginal candidiasis*: when and whom to treat. In: Sobel, J.D, (ed.*) Clinical Perspectives: Terconazole, an Advance in Vulvovaginal Candidiasis Therapy*. New York: McGraw-Hill, p1-8.

Wilton, L., Kollarova, M. and Heeley, E (2003). Relative Risk of vaginal candidiasis after use of antibiotics compared with antidepressants in women: post marketing survaliance data. *England Drugs Safety,* 26 (8):589

Wingard, J. R., Merz, W. G., Rinaldi, M. G., Miller, C. B., Karp, J. E. and Saral, R. **(**1993**).** Association of Torulopsis glabrata infections with fluconazole prophylaxis in neutropenic bone marrowtransplant patients. *Antimicrobial Agents Chemotherapy*, 37: 1847–1849

Witt, A., Kaufmann, U., Bitschnau, M., Tempfer, C., Ozbal, A., Haytouglu, E., Gregor, H. and Kiss, H. (2009). Monthly itraconazole versus classic homeopathy for the treatment of recurrent vulvovaginal candidiasis: a randomised trial. *An International Journal of Obstetrics and Gynaecology*, 116**:**1499-1505.

Workowski, K. A. and Berman, S. M. (2006). Sexually-transmitted diseases treatment guidelines, *Morbidity and Mortality Weekly Recommendation and Reproduction*, 55**:**1-94.

World Health Organisation, (2001a). *Traditional Medicine. Fact sheet number134*. Revised May, 2003. Available on http://WWW.WHO.int/media center fact sheet/fs/134.

Xess, I., Jain, N., Hasan, F., Mandal, P. and Banerjee, U. (2007). Epidemiology of candidemia in a tertiary care centre of north India: fived year study. *Infection*, 35:256-259.

Yang, Y. I. (2003). Virulence factors of *Candida* species. *Journal of Microbiology and Immunology*, 36: 223-228

Yar'zever I.S. and Ibrahim, J. (2013).Vulvovaginal candidiasis and genital colonization by *Candida* species in Nigeria. *Journal of Microbiology and Immunology Infection*, 36:223–228

Zhou, X., Westman, R., Hickey, R.., Hansmann, M. A., Kennedy, C., Osborn, T. W. and Forney, L. J. **(**2009).Vaginal microbiota of women with frequent vulvovaginal candidiasis. *Infectious Immunology*, 77**:**4130-4135.

APPENDICES

Appendix I: Ethical clearance

DEPARTMENT OF BIOLOGICAL SCIENCES
Faculty of science
AHMADU BELLO UNIVERSITY ZARIA, NIGERIA

Vice-Chancellor: Professor Abdullahi Mustapha, B.Sc. (Hons) pharm. (A.B.U), ph.D. (London), FPSN
Head of Department: Professor A.K. Adamu, B.Sc. M.Sc; ph.D. PDGE (A.B.U)

Telephone: 08035868950
Telegrams: UNIBELLO ZARIA (Biology)
E-mail: biologicalsciencesabu@yahoo.com

Your Ref:

Our Ref: MSc/Scien/8486/2010-2011

Date 20th March, 2013

The Director,
University Health Services (Sick-Bay),
Ahmadu Bello University,
Zaria.

Dear Sir,

REQUEST FOR ETHNICAL CLEARANCE

The above named Department wishes to introduce the student with registration number Esther I. Ogundana (MSc/Scien/8486/2010-2011) to your clinic (Sick Bay). She is a postgraduate Student currently undertaking a Research on the topic:"**Vaginal candidiasis**" She needs your assistance to collect swabs sample for a period of two weeks.

Anticipating your kind approval

Thank you.

Yours faithfully,

Department of Biological Sciences A. B. U. ZARIA.

Prof. A.K. Adamu
Head of Department

Appendix II: Structured questionnaire

Questionnaire on the investigation of vaginitis among women attending Ahmadu Bello University Health Care Center and Samaru Clinic Zaria, Kaduna State Nigeria.

I, Esther Iyadunni Ogundana, a master student of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria.

This questionnaire is to determine the prevalence of vaginitis among women attending Ahmadu Bello University Health Care Center (Sick Bay) and Samaru Clinic respectively. The information obtained will be used for academic purpose only and will remain absolutely confidential. I therefore solicit your honest participation in answering correctly.

1. Serial number: ……
2. Date: ……..
3. Age: …….....
4. Place of residence: …….
5. Educational status:[Primary] [Secondary][Tertiary] [None]
6. Marital status: [Single] [Married]
7. Occupation: [Student], [Staff] [house wife] [outsider].
8. Last menstrual period: ……
9. Have you taken antibiotics before based on this condition: [Yes] [No]
10. How often do you take antibiotics: [once in a month] [2ice in a month] [3 times in a month] [more than 3 times in a month]
11. Do you prescribe antibiotics for yourself or Doctor prescribed for you-State……
12. General health history of the vagina itching or discharge (how long have you being experiencing this condition and the challenges you have been passing through …….....

13. Are you sexually active: [Yes] [No]
14. Which one do you usually experience among these factors:[burning sensation] [itching of the vulva] [foul smelling] [fever] [painful sex] [painful urination].
15. Do you spray perfumes on your vulva: [Yes] [No]
16. Any use of cream to rub the vulva: [Yes] [No]
    (a) If yes- are they antibiotics cream recommended by the Doctors [ ]
    (b) Or Normal cream [ ] or the one prescribed by yourself [ ]
17. Are you aware of the health implications of lack of proper care of the vagina: [yes] [No]
18. Do you use of oral Contraceptive (Birth control measures): [yes] [No]
19. Are you pregnant? [Yes] [No], if Yes state how many months [1,2 ,3] [4, 5, 6] [6, 7, 8,9]
20. Do you wash your vagina after toilet: [Yes] [No]
21. Do you wash your vagina with soap: Yes [ ] No [ ]

22. How often did you change your pad when menstruating: [once daily] [twice daily] [3 times daily] [often].
23. Are you a Diabetic patient? [Yes] [No]
24. Nature of the illness;
    a. [having more than 1 episode of less than 14 days within 2 months]
    b. Persistent: [1 episode lasting up to 14 days]
    c. Duration: 1-4days [ ] 5-8days [ ] 9-12days [ ] 13 and above days [ ]
    d. When was the last episode: 1 week[ ] 2 weeks [ ] 3 weeks[ ] above 3 weeks:
    e. Are you on HIV drugs? [Yes] [No].
25. Did you wear tight clothing: [Yes] [No]
26. Tight underwear: [Yes] [No]

Appendix III: Biochemical characteristics of *Staphylococcus* species isolates (conventional method)

SPECIMEN CODE	HEAMOLYSIS ON BLOOD AGAR		COAGULASE TEST		CATALASE TEST		DNASE TEST	
	α HEAMOLYSIS	βHEAMOLYSIS	+VE	-VE	+VE	-VE	+VE	-VE
Y82	+		+		+		+	
Y51	+		+		+		+	
Y79	+		+		+		+	
Y53	+		-	-	+			-
Y49	-		-	-	+		+	
Y83	+		+		+		+	
Y81	+		+		+			-
Y70	+		+		+		+	
Y80	+		+		+		+	
Y84	+		+		+		+	
Y54	+		+		+		-	-
Y60	-	+	+		+		-	-
Y19	+		+		+		-	-
Y4	+	+	-	-	+		+	
Y44	-		+		+		-	-
Y45	+		+		+		-	-
Y3	+		+		+		+	
Y11	+		+		+		+	
Y92	+		-	-	+			-
Y24	+		+		+		+	
Y41	+		-		+		-	-
Y34	-		+		+		+	
Y55	+		+		+		+	
Y89	+		-	-	+		+	
Y35	+		-	-	+		-	-

Y15		+	-	-	+	+	
Y40	-	-	+		+	-	-
Y33	-	-	-	-	+	-	-
Y30	-	-	-	-	+	-	-
Y14		+	-	-	+	+	
Y42		+	+		+	+	
Y78	+	+	+		+	+	
Y73	+		+		+	+	
Y68	+		+		+	-	
Y67	+		+		+	+	
Y59	+		+		+	+	
Y72	+		+		+	+	
Y76	+		+		+	+	
Y74	+		+		+	+	
Y75	+		+		+	-	-
Y65	+		+		+	-	-
Y69	+		+		+	-	-
Y66	+		-	-	+	-	-
Y90	+		+		+	+	
Y91	+		+		+	+	
Y86	+		+		+	-	
Y77	+		+		+	+	
Y85	+		+		+	+	
Y87	+		+		+	+	
Y93	+		+		+	+	
Y99	+			-	+	-	-
Y100	-			-	+	-	-
Y94	+			-	+	+	
Y95	+			-	+	+	
Y96	-			-	+		-

Y88	+			-	-	+	
Y98	+		+		+	-	-
Y102	-		-	-	+	+	
Y39		+	+		+	+	
Y2	+		-	-	+	+	
Y13	+		+		+	+	
Y18	+		+		+	+	
Y5	+		-	-	+	-	-
Y22	+		-	-	+	+	
Y17	+		-	-	+	+	
Y23	+		+		+	-	-
Y1	+		-	-	+	-	-
Y6	+		-	-	+	+	
Y28	+		-	-	+	+	
Y38	+		-	-	+	+	
Y16	+		-	-	+	+	
Y7	-		+		+	+	
43	-		-	-	+	+	
Y32	+		-	-	+	-	-

KEY: +VE –POSITIVE

-VE –NEGATIVE

Appendix IV: Microgen *Staphylococcus* spp identification report

IS	L	I	N	S	T	M	N	M	T	P	β	B	U	A	PYR	%probability	Profile no	Final identification
49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	100%	77776	*S.aureus*Sub spp aureus
28	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	84.13%	26774	*S.xylosus*
13	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	98.57%	77767	*S.aureus* subpp aureus
20	-	+	+	+	+	+	-	+	-	+	+	+	+	+	-	69.18%	37376	*S.warneri*
3	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	76.21%	77676	*S.aureus* subspp aureus
39	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	99.72%	37677	*S.xylosus*
14	-	+	+	+	+	+	+	-	-	+	+	+	-	+	+	97.24%	37473	*S. xylosus*
90	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	887777	Staph spp
91	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	88777	Staph spp
89	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	88777	Staph spp

KEY

IS=Isolate No.
L= Latex agglutination test
C=Colony pigmentation
N=Nitrate
S=Sucrose
T=Trehalose
M=Mannose
T=Turanose
P=Alkaline Phosphatase
β= β- Glucosidase
β= β- Glucuronidase
U=Urease

Appendix V: Antibiotic sensitivity pattern (mm) of *Staphylococcus* species

Drug type (Conc)	Y18	Y75	Y14	Y81	Y83	Y90	Y43	Y65	Y28	Y1	Y17	Y91	Y93	Y79	Y49	Y4
Ciproflaxin	40	45	50	45	40	50	40	50	45	43	40	45	45	40	50	50
Amoxylin	35	33	50	25	40	46	30	45	37	45	35	45	40	30	35	35
Erythromycin	39	35	40	35	35	35	35	35	40	40	45	35	40	30	40	40
	Y98	Y76	Y54	Y15	Y73	Y11	Y41	Y42	Y40	Y67	Y60	Y102	Y82	Y53	Y89	Y83
Ciproflaxin	-	40	45	45	40	35	43	40	45	40	30	15	25	30	25	40
Amoxylin	-	-	36	35	28	28	30	30	35	24	20	-	35	38	-	-
Erythromycin	-	30	30	40	35	35	30	38	35	20	22	-	22	40	40	25
	Y13	Y84	Y74	Y16	Y51	Y70	Y19	Y45	Y44	Y3	Y30	Y33	Y35	Y34	Y55	Y24
Ciproflaxin	20	25	30	25	-	40	-	40	40	-	-	35	-	40	45	-
Amoxylin	28	-	30	30	-	45	-	35	25	-	-	30	-	20	40	-
Erythromycin	30	25	30	40	-	35	-	30	35	-	-	40	-	50	35	-
	Y92	Y68	Y59	Y72	Y69	Y66	Y86	Y77	Y85	Y87	Y99	Y100	Y94	Y95	Y96	Y88
Ciproflaxin	-	45	45	40	40	40	-	-	50	50	45	-	50	40	50	45
Amoxylin	-	20	23	25	45	20	-	-	45	45	50	-	50	33	35	20
Erythromycin	-	35	30	30	40	15	-	-	35	32	32	-	40	45	40	18
	Y39	Y2	Y5	Y22	Y23	Y6	Y38	Y7	Y32	Y80						
Ciproflaxin	-	-	40	50	40	45	40	50	40	40						
Amoxylin	-	-	35	32	40	40	35	30	40	20						
Erythromycin	-	-	30	25	35	30	35	30	28	35						

Appendix VI: Plant extracts sensitivity pattern (mm) of *Staphylococcus* species

Extracts type	Conc	Y87	Y72	Y82	Y13	Y19	Y76	Y95	Y51	Y77	Y94	Y49	Y6	Y7	Y43
	100	-	-	-	-	-	10	-	-	10	-	12	-	20	-
GC(AQ)															
	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	50	-	-	-	-										
	50	-	-	-	-										
GC(ME)		10	11	10	11	10	9	10	-	-	-	-	-	-	10
		9	10	-	-	-	-	-	-	-	-	-	-	-	5
		5	-	5	10										
		-	-	-	-										
VP(AQ)		14	16	12	15	16	16	26	-	20	15	-	-	-	18
		12	12	-	-	10	10	10	-	10	12	-	-	-	11
		10	10	10	11										
		-	10	-	-										
VP(ME		16	15	14	16	23	15	19	-	15	16	23	17	20	17
		16	12	-	-	20	11	16	-	12	-	20	15	20	17
		12	12	13	15										
		12	10	-	-										
CM(AQ)		13	15	12	11	10	10	10	-	12	12	-	-	-	11
		13	13	-	-	-	-	-	-	10	10	-	-	-	-
		11	11	10	10										
		10	10	-	-										
CM(ME)		12	-	12	14	-	-	-	-	-	10	-	-	-	11
		-	-	-	-	-	-	-	-	-	10	-	-	-	-

	10	-	10	11										
GC(AQ)	-	-	-	-										
	Y54	Y69	Y68	Y90	Y70	Y91	Y89	Y53	Y54	Y73	Y18	Y84	Y102	Y67
		-	10	-	-	-	15	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	10	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-
GC(ME)	10	-	11	13	14	15	19	-	12	15	10	-	-	11
	5	-	9	13	12	-	-	-	-	15	-	-	-	-
				10	12	10	15	-	10	13	5	-	-	10
				10	12	-	-	-	-	12	-	-	-	-
VP(AQ)	15	20	26	15	16	15	22	13	12	15	12	-	-	14
	10	12	21	14	16	-	-	-	-	12	12	20	-	-
				10	13	10	20	10	10	10	11	-	-	10
				10	10	-	-	-	-	10	-	10	-	-
VP(ME	15	22	20	20	20	29	25	12	13	20	19	15	-	19
	5	12	15	20	20	-	-	-	-	19	20	-	-	-
				18	19	15	10	10	10	15	14	10	-	18
				18	19	-	-	-	-	12	12	-	-	-
CM(AQ)	9	10	13	15	15	13	20	12	13	14	15	18	15	14
	-	-	13	14	14	-	-	-	-	13	14	-	-	-
				12	12	10	18	10	10	12	10	14	10	10
				11	16	-	-	-	-	12	10	-	-	-
CM(ME)	-	10	15	10	16	10	20	-	10	18	11	-	-	14
	-	10	14	10	12	-	-	-	-	12	10	-	-	-
				5	10	5	15	-	5	13	10	-	-	13
				5		-	-	-	-	12	-	-	-	-
	Y42	Y88	Y11	Y59	Y93	Y16	Y74	Y83	Y4					
GC(AQ)	-	-	-	-	-	-	-	-	-					
	-	-	-	-	-	-	-	-	-					

	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-
GC(ME)	-	13	10	12	10	-	11	20	-
	-	10	5	12	-	-	-	-	-
	-	10	10	11	5	-	10	19	-
	12	5	-	11	-	-	-	-	-
VP(AQ)	-	13	14	13	12	12	14	19	13
	-	12	13	12	-	-	-	-	-
	-	11	10	9	6	10	11	18	10
	10	10	5	9	-	-	-	-	-
VP(ME	-	18	10	16	19	15	15	-	14
	5	17	10	15	-	-	-	-	-
	-	15	5	15	16	12	14	-	10
	-	10	-	14	-	-	-	-	-
CM(AQ)	-	19	12	14	15	12	15	20	12
	-	18	10	12	-	-	-	-	-
	-	12	10	10	10	-	11	19	-
	-	10	5	10	-	-	-	-	-
CM(ME)	-	10	12	14	10	-	10	17	-
	-	10	-	-	-	-	-	-	-
	-	5	6	10	-	-	9	15	-
	-	5	-	-	-	-	-	-	-

Appendix VII: Morphological and Biochemical characteristics of *Candida* species using conventional method

	Arthropores formation		Gram reaction				
Isolate	Cell morphology	Cell arrangement	Cell morphology	Cell arrangement	Inference	Gram's reaction	Inference
Du49	Oval yeast cell	multiple	Yeast cell	scattered	+	+	*Candida*.spp
Du 66	Yeast cell	multiple	Yeast cell	Branch like	+	+	*C. albicans*
Du 17	Yeast cell	Long	Yeast cell	multiple	+	+	*Candida*.spp
Du 36	Yeast cell	multiple	Yeast cell	scattered	-	+	*Candida*.spp
Du 48	Long hyphae	overlaps	Yeast cell	Tetra	+	+	*Candida*.spp
Du 61	Oval yeast cell	Absentof hyphae	Yeast cell	Tetra	+	+	*Candida*.spp
Du 35	Oval yeast	spherical	Yeast cell	Branch like	+	+	*Candida*.spp
Du 58	Oval	spherical	Yeast cell	Tetra	-	+	*Candida*.spp
Du 30	Oval	Long filamentous	Yeast cell	Scattered	-	+	*Candida*.spp
Du 2	Oval	multiple	Yeast cell	Scattered	-	+	*Candida*.spp
Du 71	Oval	Long chain	Yeast cell	Branch like	+	+	*Candida*.spp
Du 63	pseudohyhae	Germ tube	Yeast cell	Scattered	+	-	- *C. albicans*
Du 69	Oval	Chain	Yeast cell	Tetra	+	+	*Candida*.spp
Du 67	Oval	Long chain	Yeast cell	Scattered	+	+	*Candida*.spp
Du 4	Pseudohyphae	Germ tube	Yeast cell	Scattered	+	+	*C. albicans*
Du 1	Oval	Short tube like	Yeast cell	Tetra	-	+	*Candida*.spp
Du 68	Long	Germ tube			+	+	*C. albicans*

	filamentous							
Du 10	Oval	spherical	Yeast cell	Scattered	+	+		*Candida*.spp
Du 64	Oval	scattered	Yeast cell	Branch like	+	+		*Candida*.spp
Du 7	Oval	Chains	Yeast cell	Tetra	-	-		*Candida*.spp
Du 9	Oval	Long chain	Yeast cell	Scattered	-	+		*Candida*.spp
Du 37	Pseudohypha e	germtube	Yeast cell	Scattered	-	+		*C. albicans*
Du 31	Oval	spherical	Yeast cell	Scattered	+	-	-	*Candida*.spp
Du 3	Oval	scattered	Yeast cell	Scattered	+	-	-	*Candida*.spp
Du 16	Oval	Chains	Yeast cell	Scattered	+	+	-	*Candida*.spp
Du 51	Oval	scattered	Yeast cell	Branch	-	+	-	*Candida*.spp
Du 29	Oval	scattered	Yeast cell	Scattered	-	+	-	*Candida*.spp
Du 22	Oval	Chain	Yeast cell	Scattered	+	+	-	*Candida*.spp
Du70	Oval	Spherical	Yeast cell	Tetra	-	+		*Candida*.spp
Du 6	Spores	Chains	Yeast cell	Branch	-	-	-	*Candida*.spp
Du 27	Oval	Tetra	Yeast cell	Scattered	+	+	-	*Candida*.spp
Du 24	Oval	scattered	Yeast cell	Tetra	-	-	-	*Candida*.spp
Du 28	Oval	Chains	Yeast cell	Tetra	-	+	-	*Candida*.spp
Du 39	Oval	Tetra	Yeast cell	Tetra	-	+	-	*Candida*.spp
Du 62	Oval	Tree like	Yeast cell	Scattered	+	+	-	*Candida*.spp
Du 44	Yeast cell	spherical	Yeast cell	Branch	-	+	-	*Candida*.spp
Du 56	Yeast cell	spherical	Yeast cell	Scattered	-	+	-	*Candida*.spp
Du 55	Yeast cell	spherical	Yeast cell	Scattered	-	+	-	*Candida*.spp
Eo 27	Yeast cell	scattered	Yeast cell	Scattered	+	+	-	*Candida*.spp
Eo 24	Oval	Tree like	Yeast cell	Multiple	-	+	-	*Candida*.spp
Eo 56	Spores	multiple	Yeast cell	Multiple	-	+	-	*Candida*.spp
Eo 65	Oval	Chain	Yeat cell	Branch	-	+	-	*Candida*.spp
Eo 30	Oval	Short tube like	Yeast cell	Scattered	-	+	-	*Candida*.spp
Eo 23	Oval	scattered	-	-	-	+	-	*Candida*.spp
Eo 93	Oval	Chain	-	-	-	+	-	*Candida*.spp

Eo 25	Yeast cell	Tree like	Yeast cell	Tetra	-	+	-	*Candida*.spp
Eo 59	Yeast cell	Oval	Yeast cell	Branch	+	+	-	*Candida*.spp
Eo 31	Yeast cell	Oval	-	-	-	+	-	*Candida*.spp
Eo 55	Yeast cell	Oval	Yeast cell	Branch like	+	+		*Candida*.spp
Eo 78	Yeast cell	Oval	Yeast cell	Scattered	+	-	-	*Candida*.spp
Eo 72	Yeast cell	Oval	Yeast cell	Scattered	+	-	-	*Candida*.spp
Eo 87	Yeast cell	Oval	Yeast cell	Scattered	+	+	-	*Candida*.spp
Eo 90	Yeast cell	Oval	Yeast cell	Branch	+	-	-	*Candida*.spp
Eo 15	Yeast cell	Oval	Yeast cell	Tetra	-	+	-	*Candida*.spp
Eo 26	Yeast cell	Oval	Yeast cell	Scattered	+	+	-	*Candida*.spp
Eo 20	Yeast cell	Oval	Yeast cell	Branch	-	+	-	*Candida*.spp
Eo 89	Yeast cell	Oval	Yeast cell	Branch	-	+	-	*Candida*.spp
Eo 88	Yeast cell	multiple	Yeast cell	Scattered	-	-	-	*Candida*.spp
Eo 94	Yeast cell	Tree like	Yeast cell	Branch	-	-	-	*Candida*.spp
Eo 2	Yeast cell	multiple	Yeast cell	Scattered	-	+	-	*Candida*.spp
Eo 29	Yeast cell	Oval	Yeast cells	Tetra	-	+	-	*Candida*.spp
Eo 91	Yeast cell	Oval	Yeast cell	Scattered	-	-	-	*Candida*.spp
Eo 55	Yeast cell	Oval	Yeast cell	Branch	+	+	-	*Candida*.spp
Eo 14	Yeast cell	Tree like	Yeast cell	Scattered	-	+	-	*Candida*.spp
Eo 36	Yeast cell	Oval	Yeast cell	Scattered	+	+	-	*Candida*.spp
Eo 57	Yeast cell	Tree like	Yeast cell	Tetra	-	-	-	*Candida*.spp
Eo 85	Yeast cell	Oval	Yeast cell	Branch	-	+	-	*Candida*.spp
Eo 44	Yeast cell	Oval				+	-	*Candida*.spp
Eo 73	Yeast cell	Oval	Yeast cell	Branch	-	-	-	-
Eo 87	Yeast cell	Oval	Yeast cell	scattered	+	+	-	-
Eo 86	-	-	-	-	-	-	-	-

Appendix VIII: Biochemical characteristics of *Candida* species using API 20 AUXC

		Biochemical Substrates																						
Isolate code	Hours	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF	Hyphae/Pseudohyphae	% propability	Profile No	Final identification
Eo25	48	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	99.3	2000040	*C. glabrata*
	72	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		-			
Eo87	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	95	2776773	*C.tropicalis*
	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-			
Du30		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	99	6776171	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-			
Du56		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	-	-	99	6776170	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	-	-			
Du 31		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	89	6776173	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-			
Du 69		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	84.3	6776373	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-			
Du 48		-	+	-	+	-	-	+	+	+	-	+	-	+	-	-	+	-	-	-	-	+	99	2172170	*C.albicans*
		-	+	+	+	-	-	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+			
Du 17		-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-		-	-	-	95	6776370	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+		-	-	-			
Du 44		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	99	6776171	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-			

Eo 26		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	99.3	2000040	*C.glabrata*
		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-			
Du 66		-	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	-	-	92.9	6756171	*C.parasiplosis*
		-	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	-	-			
Du 89		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	84.3	6776373	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-			
Du 86		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	99	6776171	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-			
Du 27		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93	2000000	*C.lambica*
		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Du 62		-	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	99	2776373	*C.tropicalis*
		-	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-			
Eo 36		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	84.3	6776373	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-			
Eo 27		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	-	-	99	6776170	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	-	-	-			
Du 54		-	+	+	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	95	6376173	*C.tropicalis*
		-	+	+	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-			
Eo 14		-	+	-	+	-	+	-	-	+	-	+	+	+	-	-	+	+	+	-	-	+	99	2556170	*C.albicans*
		-	+	-	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	-	+			
Eo 20		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	99.3	2000040	*C.glabrata*
		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-			
Eo 30		-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	-	99	6776171	*C.tropicalis*
		-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-			
Du 71		-	+	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+	99	2572171	*C.tropicalis*
		-	+	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+			

Appendix IX: Antibiotic sensitivity pattern (mm) of *Candida* spp isolated using antifungal agents

Organism																				
Drug type	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.g	C.g	C.g	C.a	C.a	C.a	C. p	C.l
NY	-	31	30	33	25	37	35	26	30	-	30	32	55	27	35	36	-	-	-	50	28
	-	30	29	28	28	33	26	27	29	-	30	30	39	26	35	36	-	-	-	33	30
	-	28	26	28	28	28	25	24	28	-	29	27	35	26	32	33	-	-	-	30	29
MY	-	16	-	26	15	15	11	15	26	-	20	42	25	-	25	30	-	-	-	35	27
	-	22	-	25	15	15	12	12	27	-	22	33	30	-	26	30	-	-	-	32	27
	-	17	-	20	12	21	13	22	31	-	30	42	28	-	28	28	-	-	-	30	25
KT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IT	-	15	22	21	17	-	15	15	-	30	20	26	27	-	25	-	-	-	-	31	-
	-	13	23	21	15	-	14	15	-	30	21	27	25	-	19	-	-	-	-	27	-
	-	14	25	21	17	-	17	12	-	31	21	30	28	-	20	-	-	-	-	27	-
FL	-	28	-	35	-	37	21	34	-	-	32	50	-	31	35	36	-	-	43	-	37
	-	23	-	30	-	36	22	32	-	-	29	42	-	30	25	36	-	-	35	-	32
	-	24	-	30	-	30	22	30	-	-	31	37	-	27	28	25	-	-	37	-	34

Keys:
NY-Nystatin
MY-Mycotene
KT-Ketoconazole
FL-Fluconazole
C.t-*Candida tropicalis*
C.g-*Candida glabrata*
C.a-*Candida albicans*
C.p-*Candida parasiplosis*
C.l-*Candida lambica*

Appendix X: Plant extracts sensitivity pattern (mm) of *Candida* species

Organism																					
Drug type	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.t	C.g	C.g	C.g	C.a	C.a	C.a	C.p	C.l
GC(ME)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP(AQ)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	8	10	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP(ME)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	5	-	-	10	-	-	-	-	10	-	-	-	5	-	-	10	5	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CM(AQ)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CM(ME)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GC(AQ)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C.t = *Candida.tropicalis*
C.g = *Candida.glabrata*

C. a = *Candida.albicans*
C.p = *Candida.parasiplosis*
C.l= *Candida.lambica*
GC (ME) = *Gomphrena*. *Celosioides*(Methanol)
VP (AQ) = *Vernonia.perrottetii* (aqueous)
VP (ME) = *Vernonia.Perrottetii*(Methanol)
CM (AQ) = Combination of GC/VP(aqueous)
CM (ME) = Combination of GC/VP(methanol)
GC (AQ) = *Gomphrena.celosioides* (aqueous)

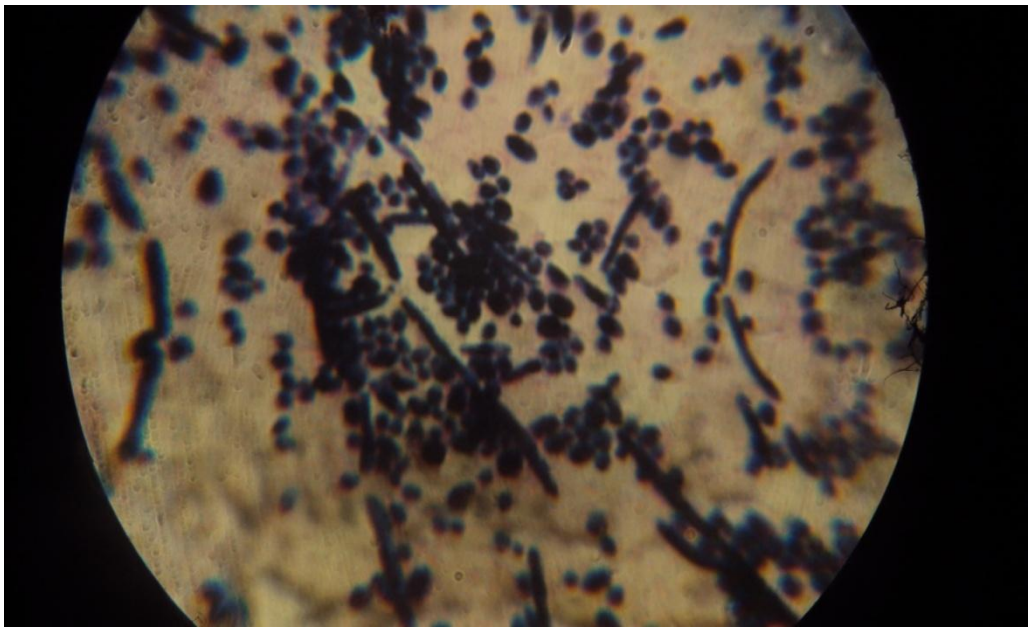


Plate I: Arthropores formation in *Candida* species grown on cornmeal agar medium



Plate II: Biochemiocal characterization of *Candida* species using API 20 AUXC

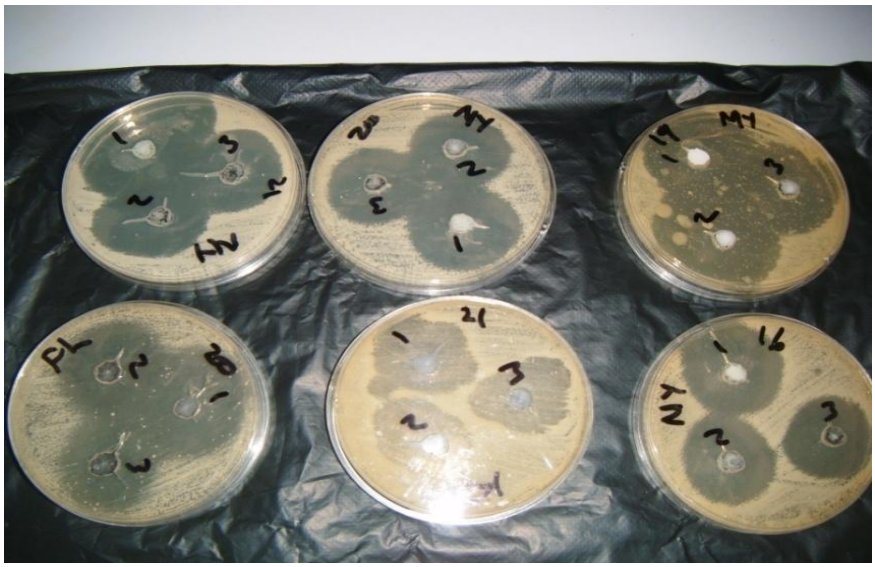


Plate III: Antibiotic sensitivity pattern of *Candida* species

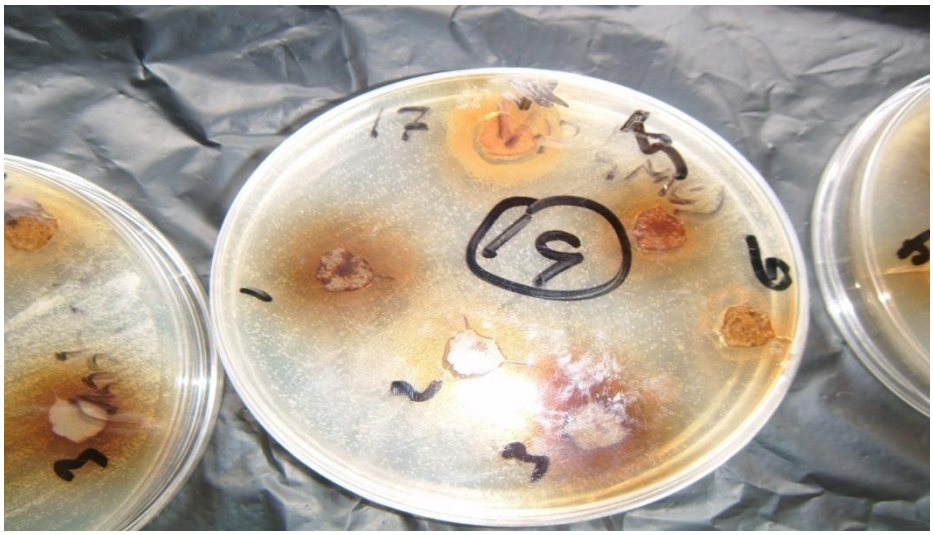


Plate IV: Sensitivity pattern of *Candida* species against aquoeus and methanolic extracts of *G. celosioides* and *V. perrottetii*

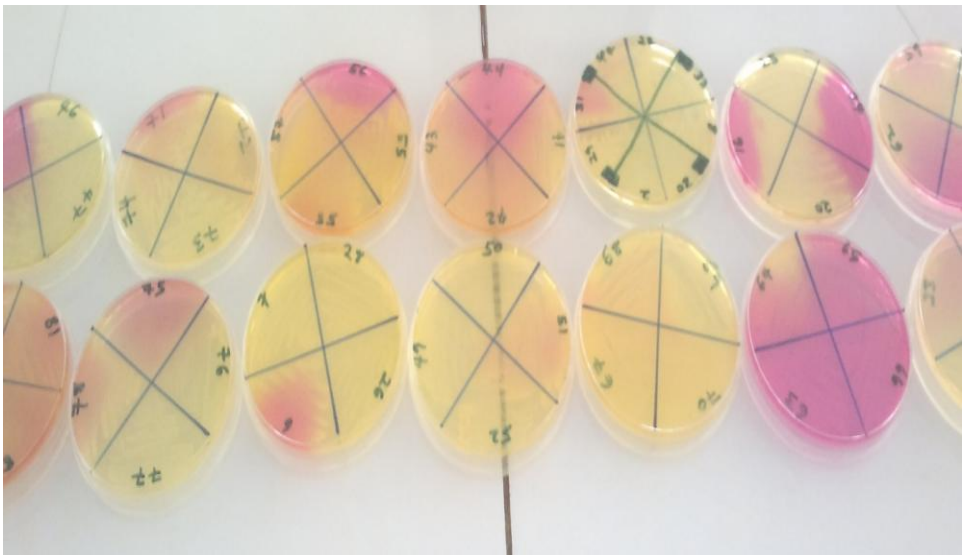


Plate V: Culture of *Staphylococcus* species growing on mannitol salt agar medium

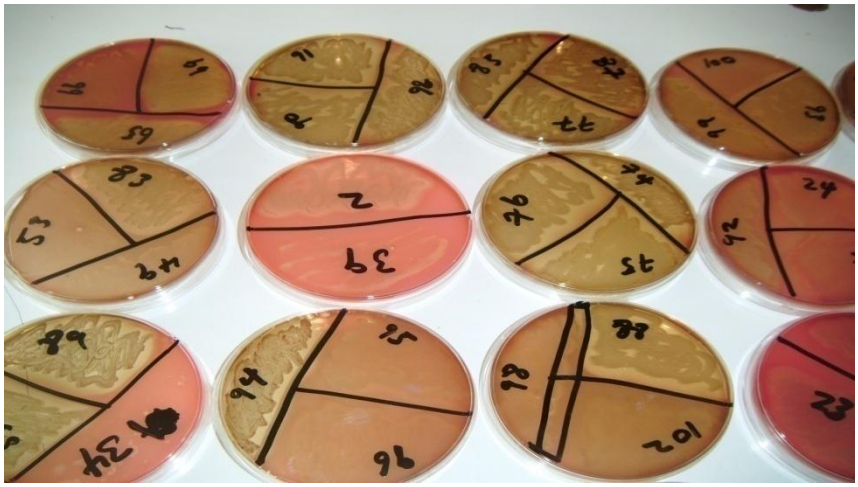


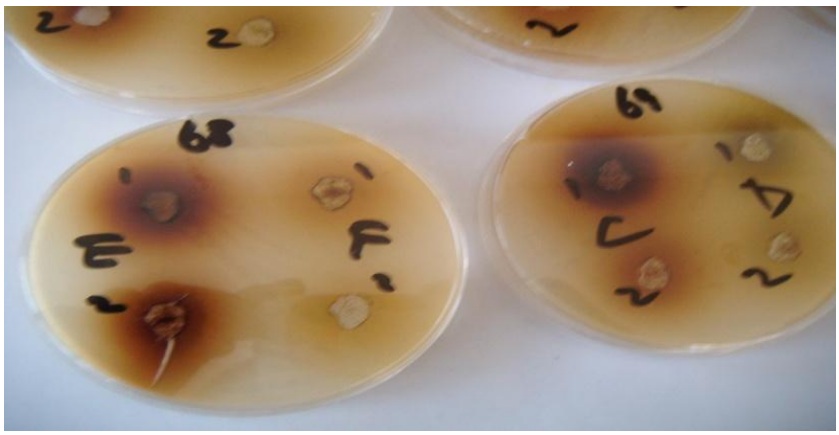
Plate VI: Haemolysis of *Staphylococcus* species on blood agar medium



Plate VII: Microgen kit identification of *Staphylococcus* species



(a)



(b)

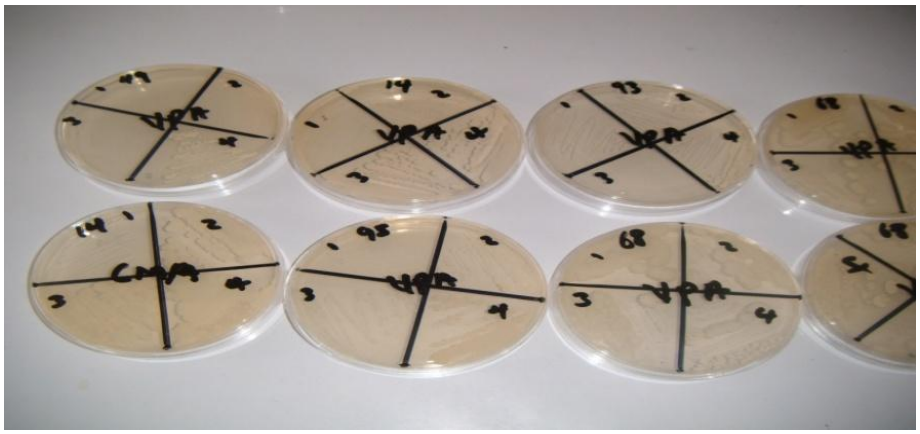
Plate VIII: Sensitivity pattern of *Staphylococcus* species against aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii*



Plate IX: MIC for *Staphylococcus* species against the aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii*



(a)



(b)

Plate X: MBC for *Staphylococcus* species against aqueous and methanolic extracts of *G. celosioides* and *V. perrottetii*