ISOLATION AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* FROM STAGNANT WATER AND ITS SUSCEPTIBILITY TO METHANOLIC EXTRACTS OF CARROT (*Daucus carota* L.) ROOT AND LEAF IN VITRO

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BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN EDUCATIONAL BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
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ZARIA, NIGERIA.

NOVEMBER, 2015.

DECLARATION

I declare that the work in this dissertation entitled, **"ISOLATION AND CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS FROM STAGNANT WATER AND ITS SUSCEPTIBILITY TO METHANOLIC EXTRACT OF CARROT(Daucus carota L.) ROOT AND LEAF IN VITRO"** has been carried out by me in the Department of Biological Sciences. The information derived from 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.

Amina YAKUBU _____ _____

Signature Date

CERTIFICATION

This dissertation entitled, **"ISOLATION AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* FROM STAGNANT WATER AND ITS SUSCEPTIBILITY TO METHANOLIC EXTRACT OF CARROT (*Daucus carota* L.) ROOT AND LEAF IN VITRO"** by Amina YAKUBU, meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. S. P. Bako Chairman, Supervisory committee Department of Biological Sciences, Ahmadu Bello University, Zaria	_____ Signature	_____ Date
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DEDICATION

This work is dedicated to the Almighty Allah, my parents, husband, children and my entire family.

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Foremost, I would like to express my sincere gratitude to Almighty Allah for his loving kindness, grace and mercy, and for making this a reality.

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ABSTRACT

The present study describes the susceptibility of stagnant water isolates of *Staphylococcus aureus* in terms of antimicrobial activity. *Staphylococcus aureus* was isolated from stagnant water bodies located at four different sites i.e. Anguwan Alkali, Kofan Gayan, Kwarbai, and Magajiya areas of Zaria city and the characterization was done using conventional method and Microgen® identification kit. The study demonstrated that *S. aureus* is present along with *S. hominis* and *S. heamolyticus* in stagnant Waters of Zaria city, Nigeria. Fresh roots and leaves of carrot were obtained from local vegetable farmers in markets of Zaria city. The phytochemical screening shows the presence of some phytoconstituents in both the root as well as the leaf extract. The antibacterial potency was initially determined by agar well diffusion method followed by qualitative evaluation of antibacterial activity by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. The result showed that methanolic extracts of carrot root was more effective than the methanolic extract of carrot leaf which shows no activity. Methanolic extract of carrot root isolates had the highest antibacterial activity with mean inhibition zone diameter ranging from 6.00 ± 8.49 to 31.00 ± 1.41 mm lower than the control antibiotic, Ciprofloxacin (10µg) with the highest mean inhibition zone diameter of 40.00 ± 0.00mm along the columns and rows. This study has demonstrated the effectiveness of carrot root antibacterial activity in inhibiting *S. aureus* pathogenic bacteria from stagnant water.

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ABBREVIATIONS

ANOVA=Analyses of variance

BaC l_2=Barium Chloride

$BaSO_4$=Barium Sulphate

CLSI=Clinical Laboratory Standard Institute

$CO_{2=}$Carbon dioxide

DAHP= Deoxy-d-arabinoheptulosonate 7-phosphate

DIZ= Diameter Inhibition Zone

DMRT=Duncan Multiple Range Test

DMSO=Dimethyl sulphuroxide

DPPH=2-diphenyl-1-picrylhydrazyl

$H_2S0_{4=}$ Hydrogen tetraoxosulphate (VI) acid

MBC=Minimum Bacteriocidal Concentration

MIC=Minimum Inhibitory Concentration

MID=Microgen Identification System Software

$NaCl_2$=Sodium Chloride

PCR=Polymerase Chain Reaction

PYR=Pyrrolidonylartylamidase

S.A.S=Statistical Analyses System

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Medicinal plants are those whose extracts could be used directly or indirectly for the treatment of different diseases. The use of traditional medicine and medicinal plants in most developing countries as a basis for the maintenance of good health has to be investigated.The potential of plant extracts for curative and preventive properties was increasing being realized by the scientific community and the focus of present day research is mostly on the use of such herbs and spices and other materials for medicinal purpose(Sofowora, 1986). The realization that the effective potency of antibiotic is limited and indiscriminate usage of antibiotics is quite rise to emergence of bacterial resistance is another reason for undertaking such research investigations.

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist world-wide leading to many investigations being conducted on medicinal plants (Falodun*et al.,* 2006). These investigations are carried out with the objective of finding out phytochemical constituents that exert beneficial pharmacological effects on the body (Faleyimu *et al*., 2010). The phytochemical constituents includes tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, minerals and vitamins which are synthesized and accumulated as secondary metabolites in plants (Girish and Satish, 2008).Plant secondary metabolites, such as essential oils extracts are studied for their antimicrobial activities and most essential oils and extracts derived from plants are known to possess insecticidal, antifungal, antibacterial and cytotoxic activities (Celiktas *et al*., 2007).

Carrot (*Daucuscarota*L.) is a plant belonging to the family *Apiaceae* and is grown throughout the temperate regions for its roots that are consumed raw or cooked. Carrots originated in present day Afghanistan about 5000 years ago (Babic*et al*., 1994). Over the ensuing centuries, orange coloured carrots came to dominate and carrots of other colours were only preserved by growers in remote regions of the world (Babic*et al*., 1994). Carrot was mentioned by Greek and Latin writers by various names, but it was Galen (circa second century A.D.) who called it *Daucus*to distinguish the Carrot from the Parsnip (Babic *et al*., 1994). The carrot is reported to be endowed with medicinal properties, i.e. hypertensive, diuretic, carminative, stomachic and antilipemic (Gilani*et al*., 2000; Nicolle *et al*., 2004; Kumarasamy*et al*., 2005).

Modern research has shown that there are two distinct groups of cultivated carrots from which the modern orange carrot derives; these are distinguished by their root colours and features of the leaves and flowers (Babic *et al*., 1994). Firstly, theEastern carrot (anthocyanin) is identified by its characteristic purple and/or yellow branched root, grey green leaves which are poorly dissected and an early flowering habit. Secondly, the Western carrot (carotene) is identified by its yellow, orange, white or red unbranched root and yellowish green leaves more clearly dissected and slightly hairy. It is thought that Western carrots originated later in Asia Minor, around Turkey and could have formed from a mutant which removed the anthocyanin (purple colour), (Babic *et al*., 1994).

Citrus fruitsand their juice or industrially processed juices, contain mostly flavonoids (Kawaii*et al*., 2000). However, flavonoids can function as direct antioxidants and free radical scavengers, as well as exhibiting the capacity to modulate enzymatic activities and inhibit cell proliferation. Thus in plants, they appear to play a defensive role

against invading pathogens, including bacteria, fungi and viruses (Sohn*et al*., 2004). Farmer's markets and some specialty stores display carrots in a range of colors like purple, yellow, and red - that contain a variety of antioxidants lending them their color (such as anthocyanin in purple carrots and lycopene in red carrots).Many different ways of approach may be followed in the search for plant and plant products of pharmaceutical and chemical interest. A very small percentage of the world's flora has been studied chemically in details). The significance of research in to medicinal plants is that it extends the knowledge of their biological constituent, their pharmaceutical activities and their therapeutic value, so that they can be used effectively in the treatment and prevention of diseases.

The Staphylococcus genus is a common bacterial genus that is widely distributed throughout the world. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive, spherical, and facultative anaerobic bacterium that occurs in clusters and usually catalase positive. It appears as small round cocci and can stains blue or purple colour. *S. aureus* is a coagulase positive organism which differentiates it from other species of the genus Staphylococcus(Jawetz and Andelberg, 1998).The presence of *S. aureus* does not always indicate infection. It can survive from hours to weeks or even months on dry environmental surfaces depending on strain.(*Cimolai*, *2008).Staphylococcus aureus* infections include pharyngitis, endocarditis, impetigo, toxic shock syndrome, scalded skin syndrome, osteomyelitis, laryngitis, pneumonia, bronchitis, boils, meningitis and urinary tract infections (Nester *et al.*, 2004).*S. aureus* is the most common species of Staphylococcus to cause Staph infections and is a successful pathogen due to a combination of nasal carriage and bacterial immunoevasive strategies (Kluytmans*etal*., 1997; Cole *etal.*, 2001).

Coagulase positive strains of *Staphylococcus aureus* have pathogenic capacity due to the combined effect of extracellular factors and toxins, together with the invasive properties of the strains (Prescott *et al*., 2005).

1.2 Statement of Research Problem

Antimicrobial resistance has become a global issue. Strategies to improve the current situation include research in finding new antimicrobials. The problem of microbial resistance to antibiotics and the high cost of treatment consequent upon this resistance has become a global threat to antimicrobial chemotherapy (Silver, 2003). In Nigeria as well as in most countries of Sub Saharan Africa, traditional healers prescribe herbal preparations for the cure of many diseases such as diarrhoea, intestinal tract infections, sore throat, ear infections, fever and skin diseases with the claim that plant-derived medicine is relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment (Hostettmann *et al*., 2002). However, some of these herbal preparations have little or no scientific basis for their application and could be dangerous to human health (Iroha *et al.*, 2010).In recent years, drug resistant pathogens have commonly been reported from all over the world (Mulligen*et al*., 1993; Robin *et al*., 1998; Silver, 2003). The emergence of antibiotic resistance among pathogenic and commensal bacteria has become a serious problem worldwide. The use and overuse of antibiotics in a number of settings are contributing to the development of antibiotic-resistant microorganisms (Barlow *et al*., 2004).

*Staphylococcus aureus*and *Streptococcus pyogenes* are among thecausative agents of bothcommunity and hospital acquired infections. The proportion of young children suffering from Staphylococcal infections can be >35% in overcrowded populations(Mulligen*et al*., 1993; Tumah, 2005).Some strains of *Staphylococcus*

aureus have been resistant to antibiotics such as methicillin-resistant *Staphylococcus aureus* (MRSAs) as reported by Gerber, (1995) and Lark, (2001).

Naturally-occurring antimicrobials derived from plants, animals, and micro floras have been extensively investigated. However, many of those natural antimicrobials have a limited antimicrobial spectrum and are often effective only at very high concentrations (Sofos*et al*., 1998).With the increase of bacterial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of naturally-derived compounds, such as essential oils and plant extracts, against a range of bacteria, to develop other classes of natural antimicrobials useful for infection controland treatment of diseases(Negi*et al.,* 2005).

The versatile composition of plant essential oils and extracts and the large antimicrobial spectrum, associated with their low toxicity, make them potential natural agents for food preservation (Conner, 1993).From my personal experience, the root of carrot is commonly used to prevent dysentery when eaten raw in our local villages. The leaves are soaked in warm water to prepare concoctions which are used as antibiotics. The fact that microorganisms develop resistance to many drugs, and a situation where some of the common and less expensive anti-microbial agents are losing effectiveness has created interest in herbal remedies in several parts of the world with many of the herbs being incorporated into orthodox medicine.

1.3 Justification

Although, a large number of medicinal plants are constantly screened for their antimicrobial effects, many plant species with potent antimicrobial properties are yet to be discovered.Recent reports have shown the increased emergence of bacteria resistance to many existingantimicrobial drugs(Faleyimu*et al.,* 2010).This has prompted the need to find alternative remedies, and plant products have proven to be

vital in this search. *Daucus carota* has been reported to be active against pneumonia, dysentery and several skin infections in traditional medical practices. Plant materials can serve as new, safe and cheap alternative agents for the treatment of infections (Erdogrill, 2002). The use of herbal medicine in Nigeria represents a long history of human interaction with the environment. In Northern Nigerian traditional medicine, many indigenous plants are widely used in the treatment of various infectious diseases; such plants include *Allium sativum, Bosciasenegalensis, Citrus lemon,Commiphoraaroroba,Ficusingens, Indigoferaarrecta, Moringaoleifera, Daucuscarota, Azadirachtaindica, and Vernoniabluemoides*(Faleyimu*et al.,* 2010). In order to substantiate the claims of cure made by the traditional healers and provide scientific basis for their efficacy, the need to evaluate plant resources for therapeutic potential is important (Faleyimu*et al.*, 2010).

The development of resistance to most of the antimicrobial agents, necessitate the search for newer antimicrobial substances from other sources including plants (Erdogrill, 2002). Hence, antibiotic resistance by pathogens can be prevented through continuous and urgent discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action against infectious diseases (Rojas *et al*., 2003).The antimicrobial activity of plant extracts may reside in a variety of components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Naturally occurring combinations of these compounds can be synergistic and often result in extracts having greater antimicrobial activity than the purified individual constituents (Delaquis*et al*., 2002).

Both ancient and modern men of many cultures have widely used medicinal plants for treating different ailments. Scientific discoveries have shown that plants produce a wide range of complex compounds (secondary metabolites) as part of their normal

metabolic process(Newman *et al*., 2003). Several secondary metabolites have been reported to have significant therapeutic properties. Therefore, plants are model source of medicines as they contain many chemical agents with therapeutic properties(Newman *et al*., 2003). Despite increasing advancement in the field of medicine and molecular diagnosis, reports indicate that close to 80% of the world population still dependent on plant derived pharmaceuticals. There are also reports that suggest that nearly 28% of drugs available inthe market are plant based products and its derivatives (Newman *et al*., 2003). In recent times, scientists have extensively reported on bio-assays of several plants of nutritional and medicinal values (Adebayo,*et al*., 2012).The present study reveals the antibacterial potency of leaves and root of *D. carota*. The antibacterial activity of the extracts against *S. aureus* was comparable to that of the control (Ciprofloxacin). These results seem to justify the continued use of the plant in thetreatment of microbial infections such as pneumonia, syphilis and skin diseases. In addition, the inhibition of growth of the test organism (*S. aureus)* that was known to display multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice.

1.4Aim

This work is aimed at evaluating the potency of carrot root and leaf (*Daucuscarota* L.) extracts on stagnant water isolates of *Staphylococcus aureus.*

1.5Objectives

1. To isolate and chacterise *Staphylococcus species* present in stagnant water using conventional method and Commercial identification kit.
2. To determine the phytochemical constituents of methanolic extracts ofcarrot root and leaf.
3. To determine the potency of extracts of carrot root and leaf against *Staphylococcus aureus* isolates.
4. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) concentration of carrot root and leaf extract against the isolates of *Staphylococcus aureus.*

1.6 Hypotheses

1. There isno *Staphylococcus species* present in stagnant Waters of Zaria city.
2. There are no phytochemical constituents in methanolic extracts of carrot root and leaf.
3. There is no difference between the potency of methanolic extract of carrot root and leaf on isolates of *Staphylococcus aureus.*
4. There is no difference between minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of carrot root extract and that of the leaf extract on *Staphylococcus aureus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutritional Value of Carrot

The carrot (*Daucuscarota* L.)is an important vegetable crop worldwide. Carrot is a root vegetable, usually orange, purple, red, white or yellow in color, with a crisp texture when fresh.It is a rich source of β-carotene and contains other vitamins, like thiamine, riboflavin, vitamin B-complex and minerals(Kumarasamy *et al*., 2005). Consumption of carrot can be mainly as raw, juice, salads, cooked vegetable, sweet dishes etc.The leaves of carrot are considered edible and are highly nutritive, rich in protein, minerals and vitamins(Gajewski*et al*., 2007). They contain 6 times the vitamin C of the root and are a great source of potassium and calcium. The tops of the carrots are loaded with potassium which can make them bitter, limiting their use as food(Kumarasamy *et al*., 2005).Consumption of fresh and processed carrots has increased due to the high biological value of the crop. The most important quality attributes of carrots are: low nitrate (V) content, high sugar and dry matter content and high β-carotene content (Hardisson *et al*., 1996). According to Holden *et al*., (1999), raw carrot roots contain on average 12% of dry matter, 4.5% of sugars (mainly sucrose) and 5.7 mg 100 g^{-1} of β-carotene. However, chemical composition of carrot storage roots (tap root) relates to a high degree, to genotype and agricultural practices (Cserni and Prohaszka, 1988; Brunsgaard*et al*., 1994; Gajewski*et al*., 2007; Gajewski*et al*., 2009). There are reports that growing conditions significantly affect the sugar content in carrots (Suojalat, 2000). *Daucus carota* contains carotenoids and the dominant compounds found in carrot roots are α-carotene and β-carotene, (Holden *et al*., 1999; Handelman, 2001; Kopsell and Kopsell, 2006). Also carrot roots contain amino acids, sugar, carotenoids, flavones, proteins and fibers. All these components

of carrot are rich in essential oil, caffeic acid, chlorogenic acid and gallic acid also roots of carrots contain three activities of 3-deoxy-d-arabinoheptulosonate 7-phosphate (DAHP) synthase, (Alasalvar*et al*., 2001).

2.2 Economic Importance of Carrot

Traditional medicine has always played and will continue to play a very important role in health care delivery for a very long time to come. The use of plants in traditional medicine is as old as the history of man on earth. In other words, traditional medicine in many countries of the world can be traced back to antiquity (Sofowora, 1982).

Carrot is grown as an annual for its fleshy root, but is a true biennial requiring two years for flowering and seed production.Carrots are an excellent source of vitamin A, providing 21% of the average adult's needs for the day. They also provide 6% of vitamin C needs, 2% of calcium needs and 2% of iron needs per serving (Ahmed *etal*., 2005). It is the antioxidant beta-carotene that gives carrots their bright orange color. Beta-carotene is absorbed in the intestine and converted into vitamin A during digestion. Carrots also contain fiber, vitamin K, potassium, manganese, phosphorous, magnesium, vitamin E and zinc.Greencarrot leaves are rich in protein, minerals and vitamins (Pisani*et al*., 1986).Excess consumption of carrots leads to carotemia a condition caused by yellow skin pigmentation which is frequently seen in children but is not at all dangerous (Ahmed *et al*., 2012).Carrots are rich in alkaline elements which purify and revitalize the blood. They nourish the entire system and help in the maintenance of acid-alkaline balance in the body .It also regulates eye-sight, raw carrot (grated) can be applied as a compress to burns for a soothing effect. Its juice when taken has beneficial effect on the liver (Ahmed *et al*., 2012). An infusion of

carrot seeds (1 teaspoon per cup of boiled water) is believed to be diuretic. The dried flowers are also used as tea for remedy to dropsy. Chewing a carrot after food kills harmful germs in the mouth and prevents bleeding of gum's and tooth decay (Pisani *et al.*, 1986). Carrots are credited with many medicinal properties, they are said to cleanse the intestine and to be diuretic, remineralizing, anti-diarrheal, remedy for allergies, aneamia, and rheumatism. Carrot can be used for the treatment of disturbances and diseases of gastrointestinal tract, for constipation, carminatives and as anthelmintic. The other medical uses for roots of carrots and their extracts is the use as antiseptic for wounds and activator for gastrointestinal tract and anti-inflammatory especially in tonsillitis and as hepatoprotective and in treatment of jaundice and dermatitis (Ahmed *et al*., 2005).

2.3 Use of Carrot Root and Leaf against Microbes

Treating disease with natural foods may be the future choice of people due to various side effects of synthetic drug formulations.Much of the research on carrots has traditionally focused on carotenoids and their important antioxidant benefits. Carrots (along with pumpkin and spinach) rank high on the list of all commonly-consumed antioxidant vegetables in terms of their beta-carotene content. Many plant extracts have been shown to possess antibacterial potential against microorganisms *in vitro*, for example, garlic, clove, ginger, allium species (Indu *et al.*, 2006; Pundir *et al.*, 2010; Silva and Fernandes , 2010; Kyung, 2012).The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Katsuyama*et al.*, 2005; Niyitegeka*et al* ., 2006). Interest in

plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Abu-Safiya *et al*., 2004).

Extract of carrot root also promoted the growth of carrot root explant, tobaccostem callus and sunflower crown gall tissue. These facts seem to suggest that extracts from many different kinds of plant materials contain common growth promoting substances, which may be involved in the mechanism of induction of growth in various kinds of plant tissues (Jim *et al.,* 2005; Anwar *et al.,* 2011).The effect of the carrot and its extracts can be due to many interacting factors such as the presence of zerpinene and other active ingredient which have chemical nature capable to impair the activity or growth of the bacteria due to the effect on the internal contents of the bacteria which cause the inhibition of growth or killing of the pathogenic bacteria (Janssen *et al*., 1987; Lee *et al*., 2004).Puchooa and Ramburn (2004) showed that using of carrot juice in the medium increases the fresh weight, dry weight and moisture content of the explants with increasing concentration of carrot juice in the medium.

Different studies provide evidence that there is a relationship between the plantchemical structures and their antimicrobial activity (Cowan, 1999; Iwu*et al*., 1999).Essential seed oils of *Daucuscarota*subsp. *carota*and cultivated carrot roots were studied (assessed) for their antibacterial activity to four species of bacteria (*Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa*and *Escherichiacoli*) and two fungi species (*Aspergillus niger* and *Alternaria alternata*). The oils obtained from cultivated carrot roots were more effective against all tested organisms (Staniszewska, 2005). In another study, six components that contained sesquiterpens was isolated from root of cultivated *Daucuscarota*subsp. *carota*plants, and their effect determined on six bacteria species (*Staphylococcus aureus,*

*Streptomyces scabies, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa*and *Escherichiacoli*) (Brkovic *et al*., 2006). The components showed slight antimicrobial activity (Ahmed *et al*., 2005).Activity of *D. carota*acetone, ethanol, hexane, and methanol extracts exhibited significant activity against *Culexannulirostris* (Shaalan*et al*., 2006). The in vitro antibacterial activities of root leaf and stem unsaponifiable, methanolic and aqueous extracts of *Daucus crinitus*against the selected bacteria were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. The root, leaf and stem unsaponifiable extracts exhibited inhibitory effects against the tested bacterial pathogens. *Candida albicans*, *S. aureus* and *Bacillus cereus* were found to be the most inhibited pathogens by the unsaponifiable extracts with their respective diameter zones of inhibition of 30mm (roots, leaves and stems), 18mm (leaves) and 14mm (roots). Using the dilution agar method technique, root, leaf and stem methanolic extracts of *D. crinitus*showed antimicrobial properties with MIC values ranging from 500 to 1800 µg/ml for bacteria and 400 µg/ml for *Candida albicans*. However, the roots, leaves and stems exhibited inhibitory effects against *C.albicans*with diameters of zones of inhibition from 13mm, 16mm and 15 mm respectively, *E. coli* (14mm, 13mm and 15 mm respectively), *B. cereus* (14, 15 and 10 mm respectively) and *S.aureus* (13m, 11mm and 11 mm respectively). However, aqueous extracts exhibited no effect of antibacterial activity against of the bacterial pathogens tested.(Dib*et al.*, 2010).

According to the results of Kumarasamy*et al*., (2005), the methanol seed extract of *Daucuscarota*that is components of luteolin and 4-0-glucoside showed antibacterial effect on *Staphylococcus aureus*, *Escherichia coli*,*Cirobacterfreundii*and *Lactobacillus plantarum*.However, the results of Osclik *et al*., (2004) on examining antimicrobial characteristics of four plants that contain furanocoumarins,establish

slight antibacterial activity of ethanol extract of *D. carota* in relation to *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcusaureus.*

Out of 21 examined plants of Scotland, methanol seed extracts of 11 plants, including *Daucuscarota*, showed certain antimicrobial properties. *D. carota*showed antibacterial activity in relation to only one bacterial species (Kumarasamy*et al*., 2002). Nadia, (2009) studies on the effects of carrot extracts on *Pseudomonasaeruginosa*results revealed that the treatments with carrot extracts especially the alcoholic extract was more efficient to inhibit the growth of *Pseudomonas aeruginosa*invitro.It was found that the alcoholic extract has more effect on the growth of bacteria which explained the efficiency of carrot to reduce the contamination by *Pseudomonas aeruginosa*(Ahmed *et al*., 2005). This gave an indication that the carrot extracts have inhibitory effect on the growth of *Pseudomonasaeruginosa* invitro.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The organism (*S.aureus*) was isolated from stagnant water bodies in four different water bodies located at different areas in Zaria city,Kaduna state (i.e. Anguwan Alkali, Kofan Gayan, Kwarbai, and Magajiya, See figure 1)

3.1.1 Collection, identification and preparation of plant materials

Fresh roots and leaf of carrot (See Plates I and II) were obtained from local vegetable farmers in Zaria city Kaduna state. Zaria is located at Longitude 10°57□ 46.86□ □ N 11°6□ 44□ □ .27 E and Latitude °36□ 16.55□ □ N7°46□ 38.96□ □ E at an altitude of 686 meters above sea level. The samples were rinsed thoroughly with tap water followed by distilled water to remove all forms of dirt. The samples were then shade dried at room temperature for three weeks after which the samples were pulverized using clean mortar and pestleand were stored in sample bottles for further use.

3.2 Plant Extraction

3.2.1 Soxhlet extraction method

Extraction was done using the soxhlet extraction method as described by Akannmi and Sultanbawa (1983). 30g of the root and leaf powders were placed in a porous cellulose thimble. The thimble was inserted in an extraction chamber which was suspended above a flask and below a condenser. The flask was sealed and the

methanol was evaporated and moves up into the condenser where it was convertedinto liquid and trickles back into the extraction chamber containing the sample.

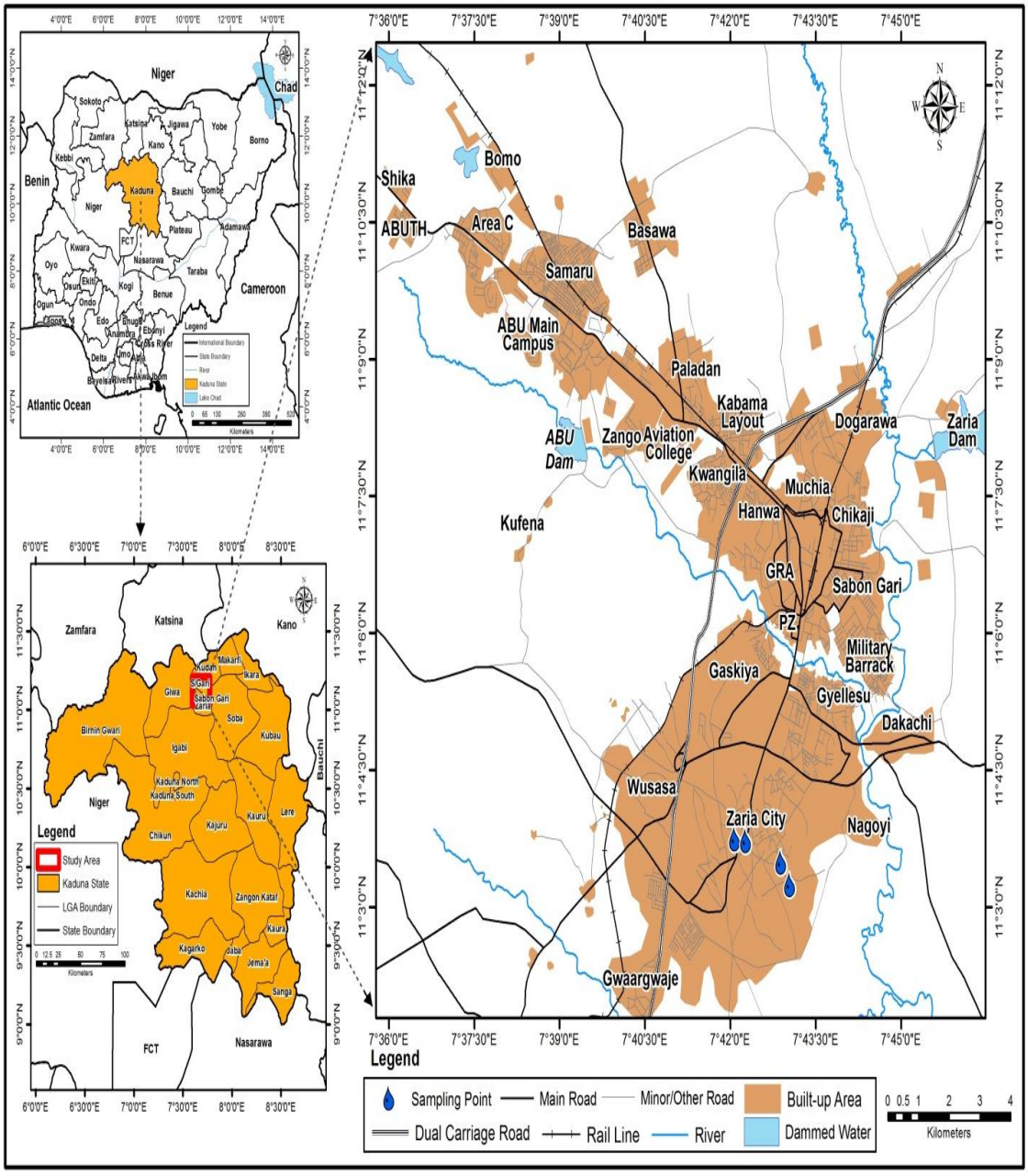

Figure 1: Map of Zaria Town showing Sampling Points
Source: Modified from the Administrative Map of Zaria

Plate I: *Daucus carota* L. (Carrot)root.

PlateII: *Daucus carota*L. (Carrot) Leaves.

The liquid continues to accumulate inthe extractor containing the sample until it reaches a point and over flows and trickles back into the boiling flask (See Plate III).At the end of the extraction process which takes a minimum of six (6) hours and maximum 8 hours, the flask containing the extracted ingredients was removed and the solvent was recovered using a rotary evaporator, leaving behind the residue which was removed and stored in form of paste for further use.

3.3 Preliminary Phytochemical Screening

The extracts were subjected to various phytochemical screening to identify the chemical constituents present using standard methods as described by Sofowora (1993) where 1g of each extract was weighed and dissolved in 10ml of sterile distilled water and filtered using Whatman no. 1 filter paper such that 1ml each of the filtrate of different extracts dissolved, was dispensed in to various test tubes and used for the following tests;

3.3.1 Test for carbohydrates

(a) Molisch's test

To a portion of each extract in a test tube, few drops of molisch reagent were added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish coloured ring at the interphase indicates presence of carbohydrates.

3.3.2 Test for unsaturated steroid and triterpenes

(a) Liebermann-bucchard test

To a portion of each extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a

Plate III: Soxhlet extractor containing carrot root and leaf samples duringmethanolic extraction

period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour indicate the presence of triterpenes.

3.3.3 Test for cardiacglycosides

(a) Keller- kiliani test

A portion of each extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. These were then transferred in to a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. These were observed carefully at the interphase for purple-brown ring that indicates the presence of desoxy sugars and a pale green colour in the upper acetic acid layer indicating the presence of cardiac glycosides.

3.3.4 Test for saponin glycoside

(a)Frothing test

About 10ml of distilled water was added to a portion of each extract and were shaken vigorously for 30seconds. The tubes were allowed to stand in a vertical position and were observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins.

3.3.5 Test for tannins

(a) Ferric chloride test

To a portion of each extract, 3-5 drops of lead sub-acetate solution was added. A colored precipitate indicates the presence of tannins.

3.3.6Test for flavonoids

(a) Shinoda's test

A portion of the extracts were dissolved 1-2ml of 50% methanol in the heat Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red colour indicates presence of flavonoids.

3.3.7 Test for alkaloids

(a) Mayer's test

To test tubes containing 1ml of each extract, few drops of Meyer's reagent were added. A cream precipitate indicates presence of alkaloids.

(b) Dragendoff's test

To test tubes containing 1ml of each extract, few drops of Dragendorff's reagent were added. A reddish brown precipitate indicates presence of alkaloids.

(c) Wagner's test

Few drops of Wagner's reagent were added to a portion of each extract, whitish precipitate indicates presence of alkaloids.

3.3.8 Test for Free anthracene derivatives (Bontrager's test)

To a portion of the extract in a dry test tube, 5ml of chloroform was added and were shaken for at least 5 minutes. These were filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Sofowora, 1993).

3.4 Microbial Assay

3.4.1 Media preparation

Mannitol salt agar (MSA), Nutrient Agar, Mueller hinton broth (MHB), and Mueller hinton agar (MHA) were used for the isolation and characterization of the organism and these were prepared according to the manufacturer's recommendation/instructions under aseptic conditions.

3.4.2 Collection of water samples

A total of 120 water samples were collected from stagnant water bodies into clean sterile sample bottles. Each of the water source was sampled twice a week for the duration of one month. At each sampling visit, five samples were collected from different points from each water bodyi.e. from four different water bodies (i.e. Anguwan Alkali, Kofan Gayan, Kwarbai, and Magajiya areas of Zaria city, See Plates IV and V).

3.5 Isolation, Identification and Characterization of Test Organisms

3.5.1 Isolation of *Staphylococcus aureus*

Theisolation of *Staphylococcus aureus*was done by transferring 0.1ml of the serial diluted sample into a sterile plate of Mannitol salt agar (MSA) and the inoculated media was then incubated at 37°C for 24 hours. After the incubation period, colonies thatappear to be yellow were picked, Gram stained and then subcultured into sterile Nutrient agar slants which was used for the biochemical test (See Plate VI and VII).

3.5.2 Gram stain and microscopy

The Gram staining reaction is used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. The required Gram staining reagents that were used includes; Crystal violet stain,

Plate IV: Water samples prior to serial dilution

PlateV: Serial diluted samples ready for inoculation on MSA media

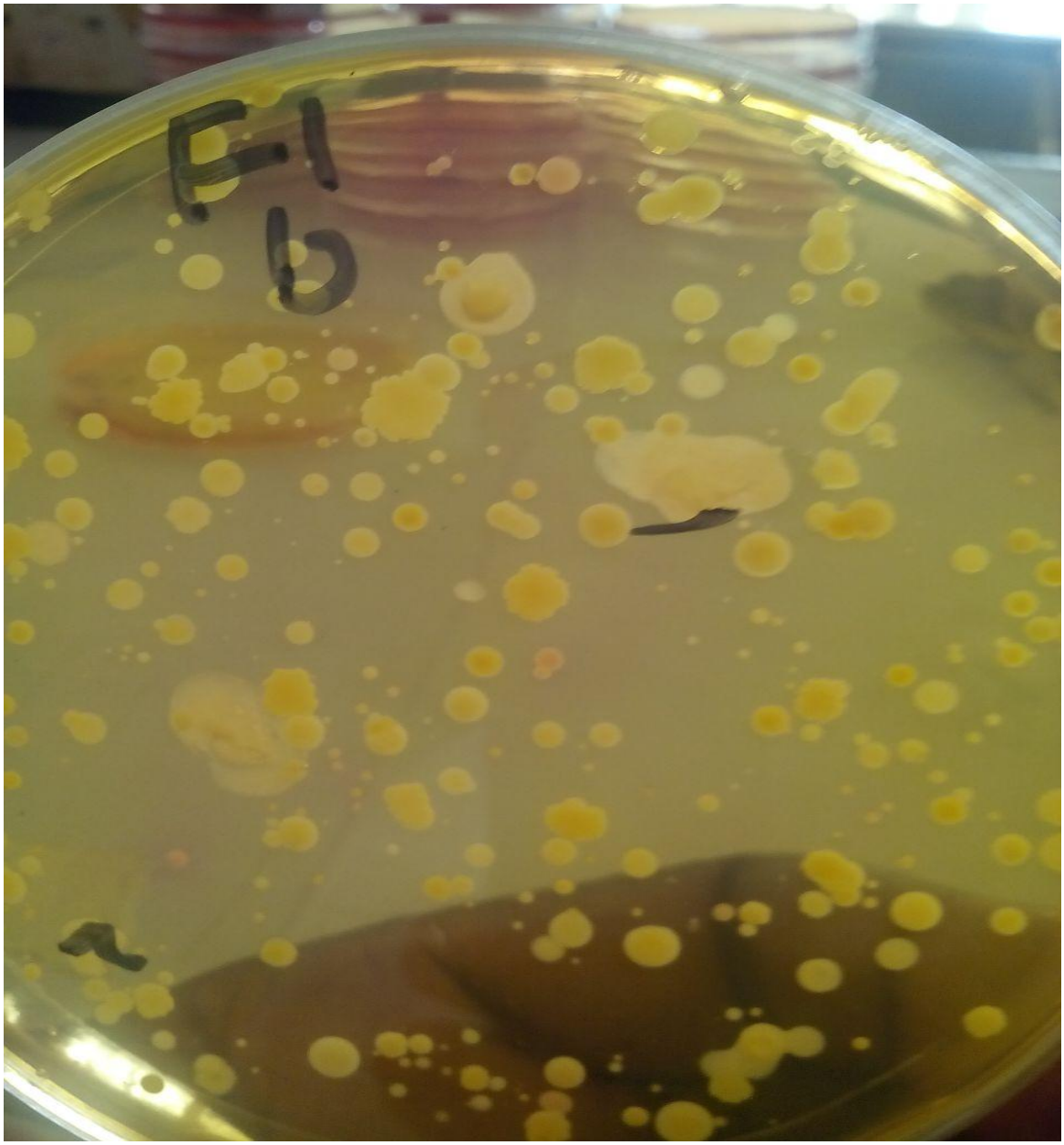

Plate VI: Pure culture of Staphylococcus isolate after incubation period cultivatedon Mannitol salt agar.

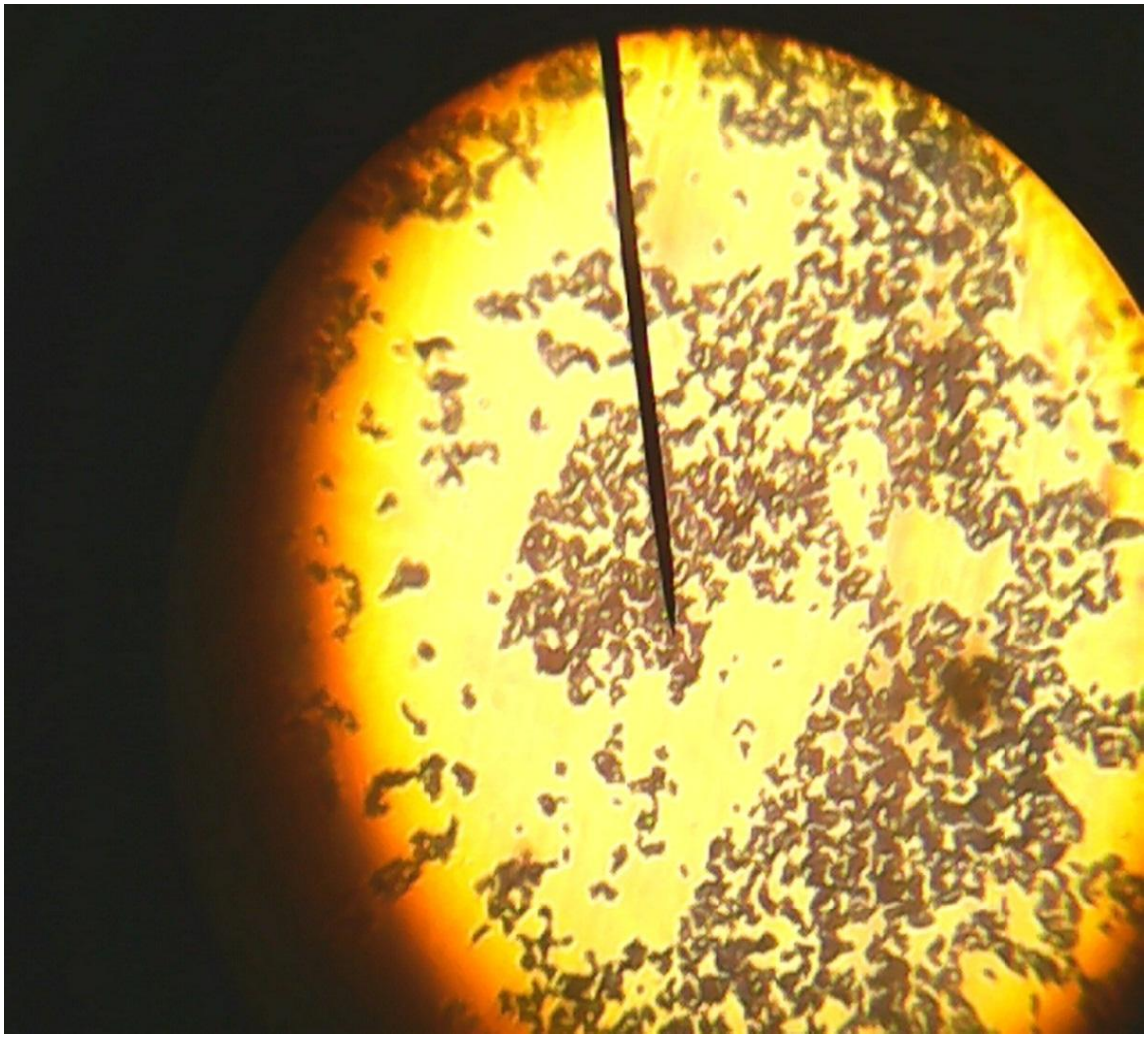

Plate VII: Photomicrograph of Staphylococcus isolate after Gram staining

Lugol's iodine, Acetone-alcohol decolourizer, and Safranin. Dried smear of the isolate obtained was fixed and stained with crystal violet solution (primary dye) for 60 seconds, rinsed with tap water and drained to avoid diluting with the mordant. It was further flooded with iodine solution (mordant) for 30 seconds and rinsed. Then the decolourizer was applied drop wise on the tilted slide until all free colours has been removed and subsequently rinsed with tap water. The slide was further flooded with safranin for 20-30 seconds, rinsed and air dried. Finally, all slides were examined under the microscope (Olympics)at ×100 oil immersion objective lens (Cheesbrough, 2006).

3.6 Conventional Biochemical Test

a) Catalase test**:** Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide into oxygen and water. This was done by addition of a drop of the bacterial suspension to a drop of hydrogen peroxide on a clean microscope slide. The appearance of effervescence and bubbling is an indication of a positive reaction. (Cheesbrough, 2006).

b) Coagulase test**:** Coagulase is an enzyme that causes the conversion of soluble fibrinogen into fibrin by a process different from normal clotting mechanisms which do not require $Ca^{2+,}$ hence citrated plasma is readily clotted in the presence of the enzyme coagulase. A drop of sheep plasma was placed on a grease free slide followed by the addition of a drop of the bacterial suspension in saline. The preparation was rocked and observed for agglutination after two minutes. Appearance of agglutination is indicative of positive reaction. This was done to distinguish *S. aureus* species from other members of the genus Staphylococcus (Cheesbrough, 2006).

c) Haemolysis on blood agar**:** Blood agar is a non-selective medium that was prepared by adding 25ml of sterile defribinated sheep blood to prepared sterile 500ml nutrient agar after cooling agar to 50°C and mixed gently to avoid forming air bubbles. The mixture was then dispensed in to Petri dishes, allowed to solidify and the test organism was streaked on the plates. The plates were incubated at 37°C for 24 hours.*S. aureus* species were observed to produce yellow to cream colony pigmentation of 1-2mm in diameter after incubation and some strains are beta- haemolytic as evidenced by clear zones due to haemolysis of red blood cells. (Cheesbrough, 2006).

d) Fermentation of mannitol**:***S. aureus* ferments mannitol and is able to grow well on mannitol salt agar, a selective medium containing peptone, *lab-lemco* powder, mannitol, 70-100 g/l sodium chloride phenol red, pH 7.3-7.7 at room temperature and agar. This was prepared according the manufacturer's instructions by suspending 11.1g in 100ml of distilled water, Sterilized at 121°C, and cooled to room temperature prior to dispensing in Petri dishes.

3.7 Microgen® Staph Latex Agglutination Test

Microgen® Staph latex Agglutination test (See Plate VIII) is a sensitive and specific test for the identification of *S. aureus* from primary plate culture which overcomes the problems associated with the traditional tests by detecting both coagulase and protein A offering a rapid and accurate identification of *S. aureus* in 2 minutes.Microgen® Staph contained all ancillary reagents needed to complete the test which includes positive and negative control, slide cards, and the plastic sticks for picking of culture.

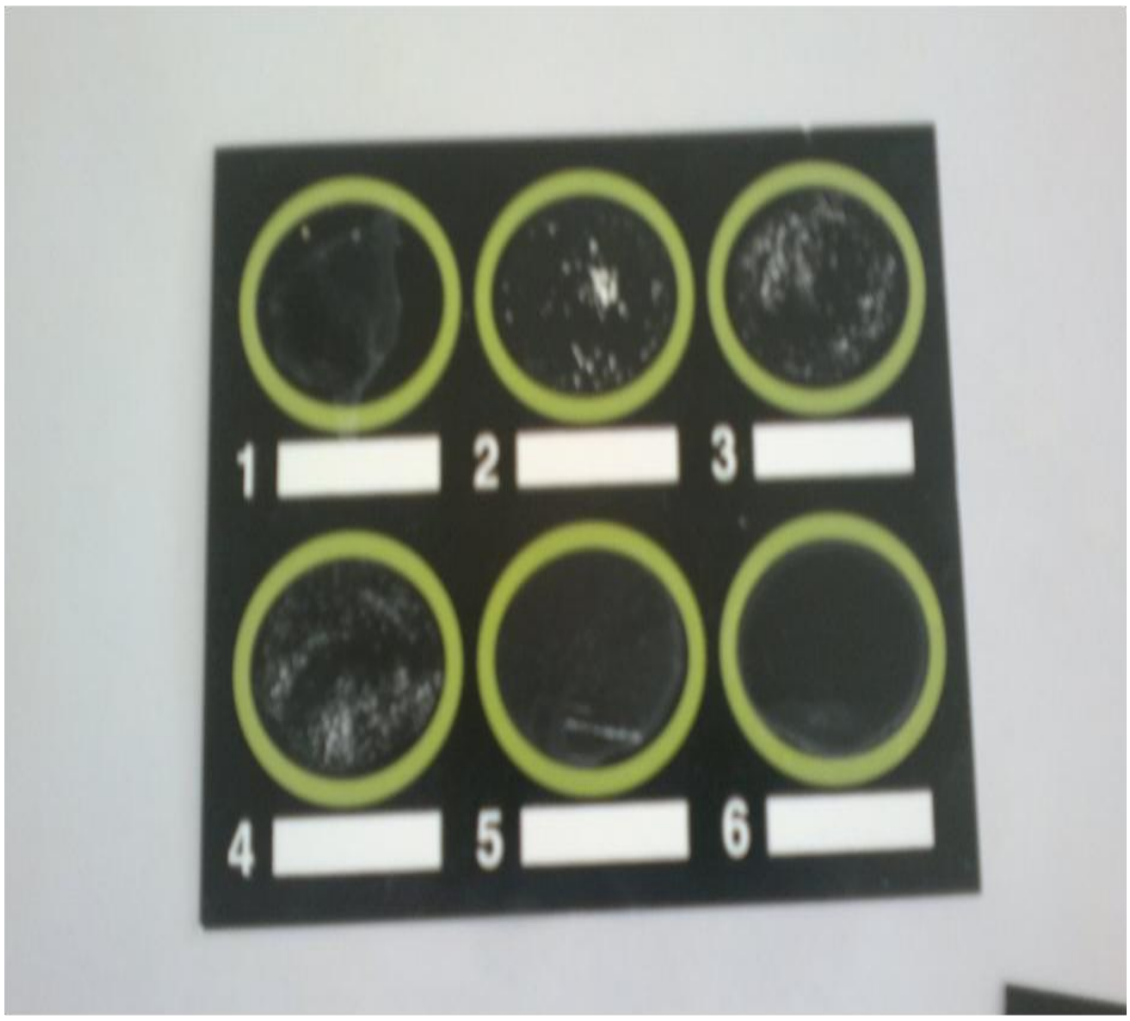

Plate VIII: Staph latex agglutination test

3.8 Identification of *S. aureus*

Identification of *S. aureus* was done using Microgen® kit (See plate IX) after obtaining a pure culture.

3.8.1 Microgen® *S. aureus* identification kit

3.8.1.1 Principle of the test

The Microgen® Staph-ID system comprises a single micro well test strip containing 12 standardized biochemical substrates which have been selected on the basis of extensive computer analysis of published databases for the identification of the genus Staphylococcus. The dehydrated substrates in each well are reconstituted with a suspension of the organism to be identified prepared in the suspending medium provided. If the individual substrates are metabolized by the organism, a colour change occurs during incubation or after addition of specific reagents. The permutation of metabolized substrates can be interpreted using the Microgen® Identification System Software (MID-60) to identify the test organism. Microgen® Staph-ID has been developed for the identification of commonly encountered *Staphylococcus* species.

3.8.1.2 Kit biochemical tests description

The Microgen® Staph-ID system has been designed to perform the following biochemical tests:

Fermentation of Sugars: includes fermentation of sucrose, Trehalose, mannitol, N-Acetyl Glucosamine, mannose and turanose in which Phenol red changes from red to yellow as a result of acid produced from the carbohydrate fermentation. A positive reaction is indicated by yellow or orange colour and a negative reaction is indicated by a red colour in the microwell.

PlateIX: Microgen® *S. aureus* identification kit

Alkaline Phosphatase: involves the hydrolysis of p-nitrophenyl phosphate by alkaline phosphatase that results in the production of yellow p-nitrophenol as a positive reaction while negative reaction is colourless.

Glucosidase: involves the hydrolysis of p-nitrophenyl β D glucopyranoside by β Glucosidase that results in the production of yellow o-nitrophenolas positive reaction and colourless indicating negative reaction.

Glucuronidase: involves the hydrolysis of o-nitrophenyl β D glucuronide by β Glucuronidase resulting in the production of yellow p-nitrophenol as positive reaction while colourless indicates negative reaction.

Nitrate: Nitrate is reduced to nitrite which forms a deep red complex after the addition of α-Naphthylamine and Sulphanilic Acid indicating positive reaction while yellow colour indicates a negative reaction.

Urease: involves the hydrolysis of urea resulting in the formation of ammonia leading to an increase in pH which turns phenol red from yellow to pink / red as positive reaction while straw to pale salmon pink colour indicates a negative reaction.

Arginine: Arginine is converted to ornithine, ammonia and CO_2 by arginine dihydrolase resulting in an increase in pH and a change in colour of the bromothymol blue from green to blue colour as positive reaction. At 48 hours green reactions are negative.

PYR:involves the hydrolysis of L-pyrrolidonyl-α-naphthylamide by the enzyme pyrrolidonylarylamidase resulting in a red to deep pink colour as positive reaction and a colourless to pale pink colour as negative reaction.

3.8.1.3 Identification criteria

On the Microgen® Staph-ID Report Form, the substrates were organized into triplets (sets of 3 reactions) with each substrate assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet formed a single digit of the Octal Code that was used to determine the identity of the isolate. The Octal Code was entered into the Microgen® Identification System Software (MID-60), which generates a report of the five most likely organisms in the selected database.

3.8.1.4 Test procedure

A single colony from 18-24 hour culture of the bacterial isolate to be identified was emulsified in the suspending medium supplied in the kit and mixed thoroughly. Carefully, the adhesive tape sealing the microwell test strip(s) was peeled to the edge but not removed completely and a sterile Pasteur pipette was used to add 3-4 drops (approximately 100µl) of the bacterial suspension to each well of the strip(s). Wells 10 and 11 were overlaid with 3-4 drops of mineral oil and the microwell test strips were resealed with the adhesive tape `removed earlier and incubated at 35-37°C for 18-24 hours. After incubation, the adhesive tape was removed and all positive reactions were recorded with the aid of the colour chart and substrate reference table (included in the booklet). After recording the results on the forms provided, reagents were added to appropriate microwells in which 1 drop of PYR reagent was added to well 12 and read after 10 minutes for the formation of a very deep pink/red colour indicating a positive result. The nitrate reduction test was also performed on well 9 after reading and recording the β Glucuronidase reaction by adding 1 drop of Nitrate A reagent and 1 drop of Nitrate B reagent to the well and read after 60 seconds for the development of a red colour indicating that nitrate has been reduced to nitrite. All these additional results were also recorded on the forms provided.

3.9 Preservation of Confirmed Isolates

S. aureus confirmed isolates were maintained on nutrient agar (Deben Diagnostics Ltd U.K) slants and subcultured on to fresh slants after every three weeks (twenty isolates). It was prepared according to manufacturer's instructions by suspending 2.3 grams of the medium in 100ml of distilled water, mixed and allowed to stand until the mixture is uniform. The mixture was heated with gentle agitation and boiled for one or two minutes until completely dissolved. It was further dispensed in to Bijou bottles, sterilized at 121°C and allowed to solidify on a slanting position. The isolates were then inoculated on to the slants and incubated at 37°C for 24hrs.

3.10 Antibacterial Susceptibility Test

Mueller Hinton Agar (Titan Biotech Ltd. Bhiwadi- 301 019, Rajasthan, India.) was used for the antibacterial susceptibility test.It was prepared according to manufacturer's instructions by suspending 3.8 grams of the medium in 100ml distilled water, Sterilized at 121°C, and cooled to room temperature prior to dispensing in Petri dishes.

3.10.1 Preparation of extract concentration

Preparation of extract concentration was carried out as described by Srinivasan*et al*., (2009). Stock solution of the plant extracts were prepared by adding 1gram of each plant extract in 10ml of 50% dimethylsulphuroxide (DMSO) as reconstituting solvent to make 100mg/ml stock solution. From the stock solution, 50mg/ml, 25mg/ml, and 12.5mg/ml concentrations were prepared using Two-fold serial dilution method. These concentrations were labeled and kept in Bijou bottles for subsequent use.

3.10.2 Preparation of turbidity standard

McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that number of bacteria will be within a given range as described by Cheesbrough, (2006). Firstly, $BaCl_2$(1% w/v) and H_2SO_4 (1% v/v) were prepared by dissolving 1g of $BaCl_2$ in 100ml of sterile distilled water and 1ml of concentrated H_2SO_4 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5 McFarland scale was prepared by adding 9.95ml of H_2SO_4(1% v/v) to 0.05ml of $BaCl_2$(1% w/v) with constant stirring to maintain a suspension of $BaSO_4$ (1% w/v) whose density is equivalent to 1.5×10^8 CFU /ml or 150million/ml approximate cell density of bacteria. The barium sulphate suspension in 4- to 6-ml aliquots were transferred in to screw-cap tubes, tightly sealed, and stored in the dark at room temperature to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial inoculum.

3.10.3 Standardization of bacterial inoculum

For inoculum standardization, density of isolated cultures was adjusted equal to that of 0.5 McFarland standards (1.5×10^8CFU/ml) by suspending some quantity of the bacterial culture in to 2ml of sterile physiological saline as suspending medium. The physiological saline was prepared by dissolving 8.5g of $NaCl_2$ in 1litre of distilled water and sterilised. To aid comparison, the test organisms and standard were compared against a white background with contrasting black lines.

3.10. 4 Susceptibility of *S. aureus* isolates to plant extracts

The antibacterial activity of *Daucuscarota*L.root and leaf extracts (Methanol) against the test organism was evaluated by using agar well diffusion method of sensitivity test described by Srinivasan*et al*., (2009). Mueller hinton agar plates were inoculated with

100μl of standardized inoculum of each isolates (in triplicates) using a micropipette of 100μl size and spread uniformly with sterile swab sticks. Wells of 5 mm size were made with sterile cork borer into the agar plates containing the bacterial inoculum. Using the micropipette, 100μl volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml each of the extracts were poured into wells of inoculated plates. The plates thus prepared were left at room temperature for 1h allowing the diffusion of the extracts into the agar and then incubated for 18 to 24 hrs at 37°C. The diameter of inhibition zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated to the nearest whole number (Srinivasan*et al.*, 2009). To check the activity of the extracts, the reconstituting solvent (DMSO) was used as negative control. Commercially available standard antibiotic Ciprofloxacin disk was used as positive control parallel with the extracts. For this antibiotic, inhibition zones were interpreted in accordance with the CLSI (Clinical Laboratory Standards Institute) interpretation guideline (2012).

3.11 Determination of Minimum Inhibitory Concentration (MIC)

Extracts which exhibited activity against the test organism were further assayed for their minimum inhibitory concentrations (MIC). The Broth dilution method was employed using Mueller hinton broth as described by Andrews (2001). Two- fold serial dilutions of the reconstituted extracts were made to obtain the following concentrations; 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. From the Mueller hinton broth prepared, 2ml broth was added to each of the test tubes labeled as containing 2ml of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentrations of each extract resulting in two sets of five different test tubes for the organism that were then inoculated with 100μl inoculum size of the test

organisms. Mueller hilton broth samples with 100μl of active inoculum of standardized bacterial isolates and tubes were incubated for 24hrs at 37°C. The MIC determined as the lowest concentration of the extract which inhibited the organism and results were observed in the form of turbidity (Andrews, 2001). Negative controls were set up to contain Mueller Hinton broth only and Mueller Hinton broth with extract only. Positive control was set up to contain Mueller Hinton broth and the test organism only (Andrews, 2001).

3.12 Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) was determined from the MIC tube and the other tubes following the MIC tube by subculturing an aliquot from the tubes on to nutrient agar plates and incubated at 37°C for 24hrs. The lowest concentration of the extracts that yielded no growth was recorded as the MBC (Andrews, 2001).Negative controls were set up as nutrient agar only and nutrient agar with extract only. Positive control was set up to contain nutrient agar and the test organism only (Andrews, 2001).

3.13 Statistical Analysis

The data obtained were presented in tables and figures and were analysed statistically using the S.A.S (Statistical Analysis System) package- SAS 9.1. Means were compared using student's *t*-test analysis of means to determine the level of significance. ANOVA was used to compare means of the plant extracts at different concentrationsand the positive control antibiotic if there is any statistically significant difference in the diameter of zones of inhibition and P values < 0.05 is considered significant. Subsequently, these were further ranked by the Duncan's multiple range tests (DMRT).

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents ofMethanolic Extracts of Carrot Root and Leaf

Based on the phytochemical screening conducted on the methanolic extracts of carrot root and leaf, the results were presented in Table 1. Table 1 shows the presence of Carbohydrate, cardiac glycoside, tannins, triterpenes, flavonoids and alkaloids in the methanolic extract of carrot root (MECR). Cardiac glycoside, triterpenes, saponins, dragendoff's and Wagner's alkaloids were present in the methanolicextracts of the leaf (MECL). MECR contained 8 fractions while MECL contained 5 fractions of phytoconstituents. MECR and MECR were similar for the presence of Cardiac glycoside, triterpenes and alkaloids, and absence of Anthracene derivatives and unsaturated steroids. They vary however for the presence of Carbohydrate, Tannins, Flavonoids and Meyer's alkaloids fractions which were present in MECR and absent in MECL.

4.2 Identification and Characterization of Test Organisms

The stagnant water isolates of Staphylococcusobtained from four different stagnant water bodies are presented in Table 2. Table 2 shows that a total of 120 samples were obtained from the water bodies in which 53 isolates were positive (rods and cocci). Out of the total positive isolates, 28 were obtained as presumptive isolates of Staphylococcus.

Table 1: Phytochemical constituents ofmethanolic extracts of carrot root and leaf

TestType	Phytoconstituents	MECR	MECL

Molish	Carbohydrate	+	-

Gram reaction	**N %**	**Cell morphology**	**N %**	**Cell arrangement**	**N %**	**Inference**
Liebermann-Buchard		Unsaturated Steroids		-	-	
Liebermann-Buchard		Triterpenes		+	+	
Keller-Kiliani		Cardiac Glycoside		+	+	
Frothing		Saponin Glycoside		-	+	
Ferric chloride		Tannins		+	-	
Sodium hydroxide		Flavonoids		+	-	
Mayer’s		Alkaloids		+	-	
Dragendorff’s				+	+	
Wagner’s				+	+	
Bontrager’s		FreeAnthraceneDerivatives		-	-	

Key:
+ = Positive
- = Negative
MECR= Methanolic Extract Carrot Root
MECL= Methanolic Extract Carrot Leaf

Table 2: Gram Reaction and Microscopic Examination of Staphylococcusisolates

Positive	53	44.17	Cocci	28	52.83	Clustered	25	47.17	Staphylococcus
Negative	67	55.83							
Total	120								

The results of Gram reaction and Microscopy of stagnant water isolatesof Staphylococcusobtained from four different water bodies are presented in Table 2. Table 2 shows that all the 53(44.17%) isolates were gram positive in which

Table 3: Cultural and physiological properties of stagnant water isolates of Staphylococcus

28(52.83%) were cocci, clustered and Staphylococcus in terms of cell morphology, cell arrangement and inference respectively. 20(71.43%) were selected as the best for biochemical test.

4.3 Cultural and Physiologic Properties of stagnantwaterIsolatesof Staphylococcus

The cultural and physiologic properties of stagnant water isolates ofStaphylococcusare presented in Table 3. Table 3 shows that all the 20 Staphylococcus isolates were gram positive, most golden yellow slightly raised colonial morphology. They all had cocci cell morphology, net clustered cell arrangement. However, one tested negative to catalase reaction, 4 to Coagulase test and 5 to both Mannitol and Latex agglutination tests. Only 9 isolates showed positive to all tests, the rest showed mixed results.

4.4BiochemicalCharacterizationandIdentificationofStagnantwaterIsolatesof *S.aureus*usingMicrogen®*S.aureus*IdentificationKit

The result of Microgen® *S. aureus* identification kit containing 20 test strips conducted on twenty(20) isolates is presented in Table 4 where fifteen (15) isolates were identified as*Staphylococcus aureus* and four (04) were identified as *Staphylococcus hominis* and one (01) as *S. haemolyticus.*

Sample	Colonial Morphology	Catalase	Coagulase	Mannitol	Latex agglutination	Inference
AA1	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
AA4	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
KG1	Golden yellow, slightly raised	+	-	-	-	Staphylococcus
KG5	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
KW3	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
KW5	Golden yellow, slightly raised	+	-	-	+	Staphylococcus
MG1	Golden yellow, slightly raised	+	+	-	-	Staphylococcus
MG2	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
MG3	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
AA1a	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
AA2b	Golden yellow, slightly raised	-	+	+	+	Staphylococcus
AA3c	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
AA4d	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
KG2a	Golden yellow, slightly raised	+	+	+	-	Staphylococcus
KG4d	Golden yellow, slightly raised	+	+	-	-	Staphylococcus
KW1a	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
KW2b	Golden yellow, slightly raised	+	-	-	-	Staphylococcus
KW3c	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
MG1a	Golden yellow, slightly raised	+	+	+	+	Staphylococcus
MG3c	Golden yellow, slightly raised	+	-	+	+	Staphylococcus
-=Negative		1	4	5	5	
+= Positive		19	16	15	15	

Key AA1- MG3c = Sample code

Table 4: Biochemical characterization and identification of stagnant water isolates of *S. aureus* using microgen® *S. aureus* identification kit

SC	L	C	N	S	T	M	N	M	T	P	B	B	U	A	PYR	% probability	Profile No	Final identification
AA1			+	+	+	-	-	-	+	+	-	+	+	-	-	80.22%	16164	*S. aureus subsp aureus*
AA4			+	+	+	-	-	-	-	+	+	-	+	+	-	99.98%	16066	*S. aureus subsp aureus*
KG1			-	+	+	-	-	-	-	+	+	+	+	-	-	84.5%	06074	*S. hominis*
KG5			-	+	+	-	+	-	+	+	+	+	+	-	-	98.32%	06574	*S. aureussubsp aureus*
KW3			+	+	+	-	+	-	-	+	+	+	+	-	-	85.28%	16474	*S. aureus subsp aureus*
KW5			+	-	-	-	-	-	-	+	+	+	-	-	-	89.24%	10070	*S. hominis*
MG1			+	-	+	-	+	+	+	+	+	+	+	-	-	76.85%	12774	*S. hominis*
MG2			+	+	+	-	-	-	-	+	+	+	+	+	-	99.99%	16076	*S. aureus subsp aureus*
MG3			+	+	+	-	+	+	+	+	+	+	+	-	-	99.97%	16774	*S. aureus subsp aureus*
AA1a			+	+	+	-	+	+	+	+	+	+	+	-	-	99.97%	16774	*S. aureus subsp aureus*
AA2b			-	-	+	-	+	+	+	+	+	+	+	+	-	92.33%	02776	*S. aureus subsp aureus*
AA3c			-	+	+	-	+	+	+	+	+	+	+	-	-	99.97%	06774	*S. aureus subsp aureus*
AA4d			+	+	+	-	+	+	+	+	+	+	+	+	-	100%	16776	*S. aureus subsp aureus*
KG2a			+	+	+	-	+	+	+	+	+	+	+	-	-	99.97%	16774	*S. aureus subsp aureus*
KG4d			-	-	+	-	-	-	+	+	+	+	+	-	-	99.18%	02174	*S. hominis*
KW1a			+	+	+	-	+	+	+	+	+	+	+	-	-	99.97%	16774	*S. aureus subsp aureus*
KW2b			+	-	+	-	+	+	+	+	+	+	+	-	-	54.74%	12774	*S. haemolyticus*
KW3c			+	-	+	-	+	+	+	+	+	+	+	+	-	52.49%	12776	*S. aureus subsp aureus*
MG1a			+	+	+	-	+	+	+	+	+	+	+	-	-	99.94%	16774	*S. aureus subsp aureus*
MG3c			+	+	+	-	+	+	+	+	+	+	+	-	-	96.47%	16774	*S. aureus subsp aureus*

KEY

S=Sample code.M=Mannose AA=Anguwan Alkali

L= Latex agglutination test T=Turanose

C=Colony pigmentation **P=**AlkalinePhosphataseKG=Kofan Gayan

N=Nitrate β= β- Glucosidase

S=Sucrose β= β- Glucuronidase KW=Kwarbai

T=Trehalose U=Urease

M=Mannitol A=Arginine MG=Magajiya

N=N-Acetyl Glucosamine PYR=Pyrrolidonylarylamidase

+= Positive - = Negative

4.5 Antibacterial Susceptibility Test of Carrot Leaf

The result of antibacterial susceptibility test of various isolates of *S. aureus* to different concentrations of methanolic extract of carrot leaf was presented in Table 5.Table 5 shows the resistance of various isolates of *S. aureus* to different concentrations of methanolic extract of carrot leaf. This indicates that the methanolic extract of carrot leaf showed no antibacterial effect.

4.6 Antibacterial Susceptibility Test of Carrot Root

The results of antibacterial susceptibility test of various isolates of *S. aureus* to different concentrations of methanolic extract of carrot root as well as the antibiotic used and their mean inhibition zone diameter are presented in Table 6 and Figure 2. Table 6 shows the susceptibility of various isolates of *S. aureus* to different concentrations of methanolic extract of carrot root where means with different superscripts alphabets along rows are significantly different at $P< 0.05$. There is no difference in the susceptibility of isolates KG5, MG2, AA2b,AA3c, KG2a, KW1a, KW3c,MG1a and MG3c to 100mg/ml and 50mg/ml 25mg/ml and 12.5mg/ml concentrations of methanolic extract of carrot root. Isolate KW3 has the highest activity at 100mg/ml. 50mg/ml and 25mg/ml with mean inhibition zone diameter of 31.00±1.14mm, 20.50±3.54mm, 14.00±2.83mm while isolate AA4d has the highest activity at 12.5mg/ml with diameter inhibition zone of 6.00±6.49mm along the rows. On the other hand, there was also no difference in the susceptibility of isolate AA1, AA4, and AA4d to 100mg/ml concentration and also MG3c and AA1a to100mg/ml of methanolic extract of carrot root. There was also no difference in the susceptibility of isolate AA1 and MG3 to 50mg/ml concentration and isolate AA1 and AA4d to 25mg/ml concentration.

Table 5: Susceptibility of various isolates of *Staphylococcus aureus* to different concentrations of leaf extracts of *Daucus carota* L.

Sample	Mean Inhibition Zone Diameters (mm) *Daucuscarota* leaf extracts (mg/ml)				
	100.0(mg/ml)	**50.0(mg/ml)**	**25.0(mg/ml)**	**12.5(mg/ml)**	**Ciprofloxacin (control)**
AA1	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
AA4	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
KG5	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
KW3	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
MG2	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
MG3	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
AA1a	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
AA2b	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
AA3c	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
AA4d	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
KG2a	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
KW1a	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
KW2b	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
MG1a	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
MG3c	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

AA1-MG3c=Sample-code AA=Anguwan Alkali

KG=Kofan Gayan KW=Kwarbai MG=Magajiya

00=No-inhibition

Table 6: Susceptibility of various isolates of *Staphylococcus aureus*to different concentrations of root extracts of *Daucuscarota*L.

SC	Mean Inhibition Zone Diameters (mm)				
	***Daucuscarota* L. root extracts (mg/ml)**				
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5 mg/ml**	**Ciprofloxacin(control)**
AA1	17.00±0.00^{c}	15.00±0.00^{bc}	12.00±0.00^{ab}	0.00±0.00^{b}	35.00±0.00
AA4	14.00±0.00^{c}	12.00±0.00^{d}	10.00±0.00^{b}	0.00±0.00^{b}	34.00±0.00
KG5	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	37.00±0.00
KW3	31.00±1.41^{a,a1}	20.50±3.54^{a,b1}	14.00±2.83^{a,c1}	0.00±0.00^{b,d1}	34.00±0.00
MG2	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	40.00±0.00
MG3	23.00±7.07^{b,ab1}	15.50±0.71^{bc,bc1}	13.00±1.41^{a,c2}	0.00±0.00^{b,d2}	28.00±0.00
AA1a	26.50±2.12^{b,b2}	16.50±0.71^{bc3}	13.50±0.71^{a,a2}	0.00±0.00^{bd3}	37.00±0.00
AA2b	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	30.00±0.00
AA3c	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	35.00±0.00
AA4d	16.50±2.12^{c,b4}	13.50±2.12^{cd,b4}	12.00±2.83^{ab,b4}	6.00±8.49^{a,b4}	34.00±0.00
KG2a	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	30.00±0.00
KW1a	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	28.00±0.00
KW3c	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	30.00±0.00
MG2a	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	32.00±0.00
MG3c	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{b}	37.00±0.00

NOTE: Values are expressed as means ± SD (standard deviation). Means with the different superscripts along columns and rows are significantly different at $P < 0.05$

AA=Anguwan Alkali KG=Kofan Gayan

KW=Kwarbai MG=Magajiya

a-d= across rowsSC=Sample code

a1-d3=across columns

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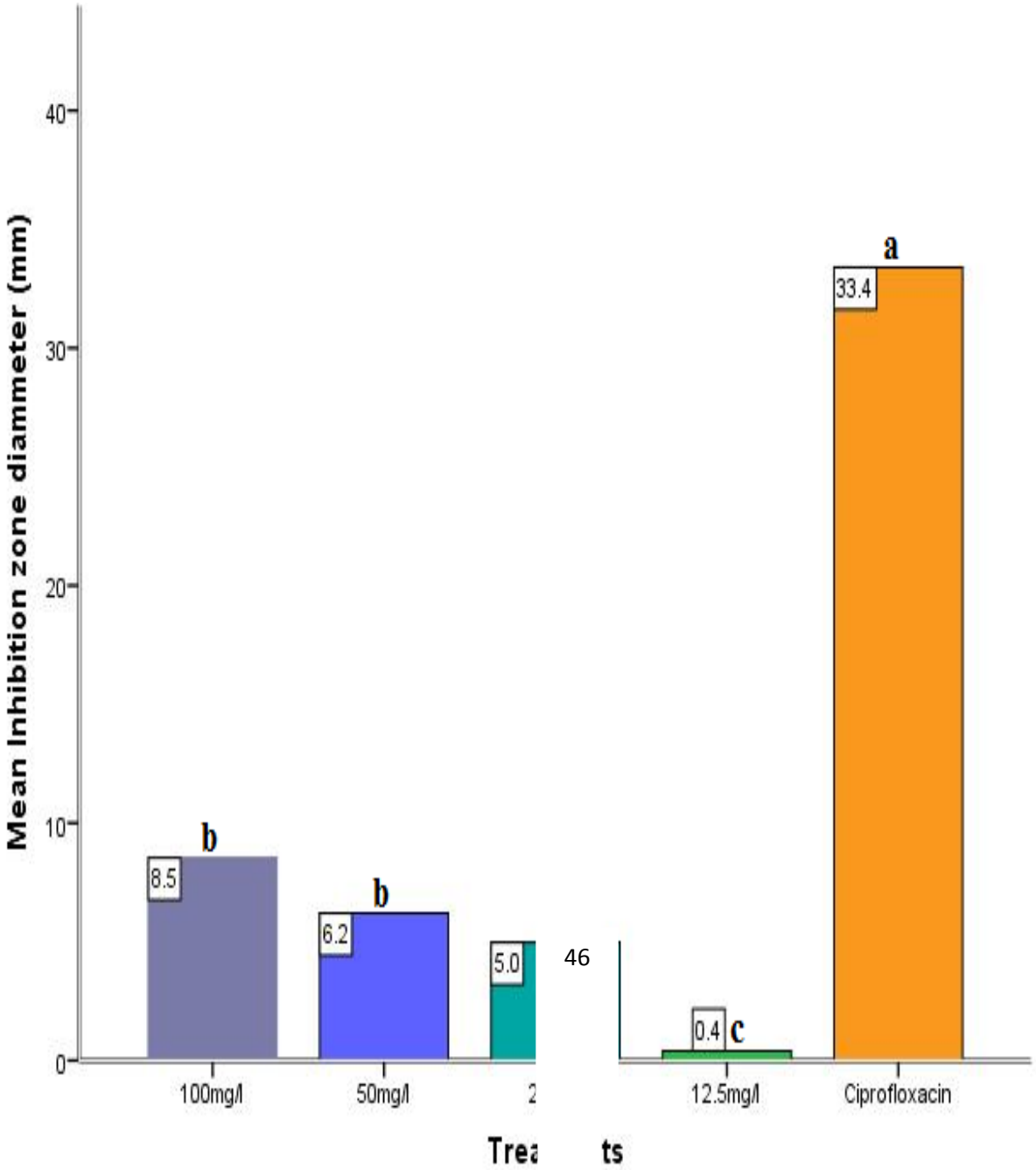

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The result in Table 6 also shows the susceptibility of various isolates of *S. aureus* to different concentrations of methanolic extracts of carrot root where means with different superscripts alphabets along columns are significantly different at $P < 0.05$. Isolate AA4dhas the least in all the concentrations across the columns. The control Ciprofloxacin shows no significant difference between all susceptible samples across the columns and rows.

4.7 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extracts of carrot root on susceptible *S. aureus* isolates are presented in Table 7. Table 7 shows the MIC and MBC of methanolic extracts of carrot root on susceptible *S. aureus* isolates where the root extract has the same MIC and MBC on isolate AA1 and KW3 as 6.25mg/ml MIC and 12.5mg/ml MBC; it also has the same MIC and MBC on isolate AA4 and AA1a as 25.0mg/ml MIC and 50.0mg/ml MBC; it has MIC and MBC of 12.5mg/ml and 25.0mg/ml on isolate AA4d and MIC and MBC of 3.125mg/ml and 6.25mg/ml on isolate MG3 respectively.

Table7:Determinationofminimuminhibitoryconcentration(MIC)andminimum bactericidal concentration (MBC)ofmethanolicextractsofcarrot root toselected*S. aureus*

SAMPLE	MIC	MBC
AA1	6.25	12.5
AA4	25.0	50.0
KW3	6.25	12.5
MG3	3.125	6.25
AA1a	25.0	50.0
AA4d	12.5	25.0

Key: MIC=Minimun Inhibitory Concentration. MBC= Minimum Bactericidal Concentration.

AA=Anguwan Alkali KG=Kofan Gayan KW=Kwarbai

MG=Magajiya (Sample Code)

CHAPTER FIVE

5.0 DISCUSSION

The results of the current study reveal the efficacy of methanolic extracts of carrot root and leaf based on evaluation of antibacterial activity on stagnant water isolates of *Staphylococcus aureus.* Methanolic extracts of carrot root recorded higher antibacterial activity than the leaf extracts though*Staphylococcus aureus*tends to be resistant to the leaf extracts (See Table 5).

The study demonstrated that *S. aureus* is present along with *S.hominis* and *S. heamolyticus* in stagnant Waters of Zaria city, Nigeria. The study showed that *Daucus carota* root methanolic extract at 100mg/ml demonstrated comparable potency as Ciprofloxacin ($P<0.05$) to *S. aureus* growth. Among the methanolic extracts of carrot root, highest inhibitory activity was recorded with sample KW3 at 100mg/ml, 50mg/ml and 25mg/ml concentrations with mean inhibiting zone diameter ranging from 31.00±1.41, 20.50± 3.54, 14.00±2.83mm while sample AA4d has the highest inhibitory activity at 12.5mg/ml concentration with mean inhibition zone diameter of 6.00± 8.49mm. Iwu*et al*.,(1999) and Cowan, (1999) reported that the in vitro antibacterial activities of root, leaf and stem unsaponifiable, methanolic and aqueous extracts of *D.crinitus*against the employed bacteria were qualitatively assessed by the presence or absence of inhibition zones.

The antibacterial effects of carrot root were previously studied as reported by Dib*et al.,* **(**2010) on the evaluation of Partialchemicalcompositionandantimicrobialactivityof*Daucuscrinitus*extractsand concluded that the antimicrobial properties of the unsaponifiable, methanolic and aqueous extracts (leaves, stems and roots) tested on four microorganism species were active against *Bacillus cereus*, *Staphylococcus aureus*, *Eschericia coli* and *Candida albicans* which are deemed very dangerous

and very difficult to eliminate. Shaalan*et al*., (2006) investigated the activity of *D. carota*acetone, ethanol, hexane, and methanol extracts and found that carrot exhibited significant activity against *C*ulex*annulirostris*.

Similarly, it was also reported by Kumarasamy*et al*., (2005) on the assessment of biological activities associated with the major constituents of the methanol extract of 'wild carrot' (*Daucuscarota*)L.and concluded thatthe major constituents isolated from the methanol extract of *D. carota* seeds by reversed-phase preparative high performance chromatography were luteolin, luteolin 3'-O-beta-D-glucopyranoside and luteolin 4'-O-beta-D-glucopyranoside, three flavones. The constituents were assessed for their antibacterial and free radical scavenging activities, as well as toxicity towards brine shrimp. Among these three flavones, luteolin showed the highest degree of free radical scavenging activity (RC50 = 4.3 x 10(-4) mg/mL) in the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Both luteolin and its 4'-O-glucoside demonstrated bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* (Minimum Inhibitory Concentration (MIC) = 5.0 x 10(-2) - 1.0 x 10(-1) mg/mL). Nadia, (2009) on the effects of carrot extracts on *Pseudomonasaeruginosa* results revealed that the treatments with carrot extracts especially the alcoholic extract was more efficient to inhibit the growth of *Pseudomonas aeruginosain vitro*. It was also found that the alcoholic extract has more effect on the growth of bacteria which explain the efficiency of carrot to reduce the contamination by *Pseudomonas aeruginosa*(Ahmed *et al*., 2005). This gave an indication that the carrot extracts have inhibitory effect on the growth of *Pseudomonasaeruginosa in vitro.* In this study, sampleKW3 had the highest activity at 100mg/ml concentration of methanolic extract of carrot root with mean inhibition zone diameter of 31.0± 1.4mm along the rows respectively.

The MIC and MBC assay procedures are frequently used to evaluate some diverse agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Andrews, 2001). Antimicrobial agents with low activity against an organism usually gives a high MIC and MBC values, while those that are highly effective give low MIC and MBC values. In this study, the MIC and MBC values for sample AA1 and KW3 are the same with 6.25mg/ml and 12.5mg/ml respectively, so also that of sample AA4 and AA1a are the same with 25.0 and 50.0 and that of MG3 differs with 3.125 and 6.25 as MBC, and that of sample AA4dwith 12.5 and 25.0 as MBC respectively. This implies that the efficacy of methanolic extracts of the root could be as low as the MIC and MBC obtained in this study but this also confirms the strong efficacy of *Daucuscrinitus*extracts as was revealed by Dib*et al.,* **(**2010) on the evaluation of Partial chemicalcompositionandantimicrobialactivityof*Daucuscrinitus*extracts where the unsaponifiable extract of leaves, stems and roots shows a strong antimicrobial effect against *E.coli* with an MIC of 111.5, 111.1 and 126 µg/ml respectively and *S. aureus* with a MIC of 122.1, 122.1 and 136 µg/ml respectively and average activity on the *B. cereus* with a MIC of 255, 255 and 250 µg/ml respectively. The root extracts showed more inhibition than the leaf extract. This indicates that the antimicrobial properties are more concentrated in the root than in the leaves. This observation confirmed the common use of the roots of this plant in traditional healing process than the stem and leaves.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i. In conclusion, the result obtained in this study has clearly demonstrated that*S. aureus*is present significantly in stagnant waters of Zaria area with Kwarbai (KW3) having the highest value of 31.00±1.14mm, 20.50±3.54mm, 14.00±2.83mm and Anguwan Alkali (AA4) having the least value of 14.00±00mm, 12.00±00mm and 10.00±0.00mm along with insignificantly presence of *S. hominis*and *S. heamolyticus*.

ii. The study has also demonstrated the presence of some phytochemical constituents inmethanolic extracts in carrot root and leaf.

iii. The study showed that *Daucus carota* methanolic extractof root at 100mg/ml demonstrated comparable potency as ciprofloxacin ($P<0.05$) to *S. aureus*growth.

iv. The study demonstrated MIC values for Carrot root potency on *S. aureus* isolates ranging from 3.125-25.00mg/ml and MBC ranging from 6.25-50.00mg/ml.

6.2 Recommendation

- Further studies should be carried out on the antibacterial activity of carrot leaf extracts using otherbacteria stainssince *S. aureus*is resistant tothe leaf.
- Further research should be done on polymerase chain reaction (PCR) on S. *aureus* to assess the quantitative antibacterial activity.
- Other solvents like acetone and ethanol should be used to determine the susceptibility of carrot root and leaf at different concentrations on other types of bacteria.

- Standard drainage systems should be provided by the government to enable rain water and domestic waste products to move freely so as to eliminate the possibility of stagnant waters to be in place.

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