PHYTOCHEMICAL AND MEDICINAL PROPERTIES OF EXTRACTS OF THE LEAVES OF *PEUCEDANUM WINKLERI* H. WOLFF

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AHMADU BELLO UNIVERSITY, ZARIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT FOR THE AWARD OF A MASTER DEGREE IN ORGANIC CHEMISTRY

DEPARTMENT OF CHEMISTRY,

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Decleration page

I declare that the work in this Thesis entitled Phytochemical and medicinal properties of extracts of the leaves of *Peucedanum winkleri* H. Wollf has been carried out by me in the Department of Chemistry, Ahmadu Bello University. 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.

Madumelu Mark

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Name of Student	Signature	Date

Certification page

This thesis entitled PHYTOCHEMICAL AND MEDICINAL PROPERTIES OF EXTRACTS OF THE LEAVES OF *PEUCEDANUM WINKLERI* H. WOLFF by MARK MADUMELU meets the regulations governing the award of the degree of Master of Organic Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. G.I. Ndukwe (Signature) .....

..... Date.....

Chairman Supervisory Committee

Dr (Mrs) R.G. Ayo (Signature) .....

..... Date.....

Member Supervisory Commitee

Prof. V.O. Ajibola (Signature) .....

..... Date.....

Head of Department

Prof. A.A. Joshua (Signature) .....

..... Date.....

Dean, School of Postgraduate Studies

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I give all thanks to God almighty for given me his grace and tremendous strength to carry out and effectively complete this study and research work.

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Dedication

I dedicate this research work to God Almighty who in his infinite mercy and love gave me the grace, wisdom, knowledge and strength to successfully complete this research work.

Abstract

Phytochemical investigation of the leaves of Peucedanum winkleri. H. Wolff, revealed the presence of carbohydrates, free reducing sugar, cardiac glycoside, saponins, steroids, flavanoids, alkaloids, tannins and triterpenes. The extracts were screened for their antimicrobial activity against Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Corynebacterium ulcerans, Klebsiella pneumoniae, Streptococcus pyogenes, Candida albicans, Candida tropicalis, Candida krusei and Proteus mirabilis. The result indicated that the extracts inhibited the growth of one or more test pathogens. The ethyl acetate extract showed a broad spectrum of antimicrobial activity.4-(4-Chlorophenyl)-3-cyclohexyl-1-methyl-6-(2-naphthoyl)-1,2,3,4-tetrahydro-1,2,4,5-Tetrazine was isolated from the ethyl acetate extract of the plant leaves by means of Vacuum liquid chromatography (VLC), Thin layer chromatography (TLC) and Preparative thin layer chromatography (PTLC) using a solvent mixture of 1:1 v/v petroleum-ether and ethyl acetate. The stucture was established mainly by MS, IR, ^{1}H and ^{13}C NMR including DEPT spectroscopy. Also the isolated compound was able to inhibit the growths of some of the microorganisms used for the studies. The result of the study justified the use of the plant against several infectious diseases.

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CHAPTER ONE

1.0 INTRODUCTION

1.0.1 BACKGROUND OF THE STUDY

Medicinal plants have always been associated with cultural behaviours and traditional knowledge. The renaissance of interest in plant products has been stimulated by the use of plant extracts in chronic conditions for which conventional drugs is perceived to offer very little specificity in its target (Rhiouani *et al*., 1999). This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Akindele and Adeyemi, 2007).

The blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Also, the development of adverse effects and high microbial resistance to the chemically synthesized drugs, has forced men into ethnopharmacognosy. More so, in our local situation, degree of ignorance and illiteracy had forced many to abandon or neglect pharmaceutically formulated drugs in favour of locally prepared herbal remedies coupled with the fact that pharmaceutical products are increasingly being faked. Thus, the herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment (Joy *et al.,* 2001). Herbs are staging a comeback, herbal 'renaissance' is happening all over the globe and people returning to the naturals with hope of safety and security. By and large, the public is gradually drifting towards acceptance and usage of herbal preparations.

In Africa, traditional healers and remedies made from plants play important role in the health of millions of people (Adotey *et al.,* 2012). The users of these remedies, found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effects. The Pharmacognosy Society of Nigeria supports the acceptance of herbal remedies or treatment of ethnomedicinal practice along with conventional orthodox health care system (Ndukwe, 2004). This is largely due to the fact that plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al.,* 2006).

Biological organisms particularly plants produce two distinctly different types of chemical products. The first type, primary metabolites, which consists of compounds such as sugars and proteins that are common to most organisms and are essential for functional metabolism. Secondary metabolites, on the other hand, are chemicals unique to a single species or related group of organisms. Not until the 1990s that scientists fully realize that these secondary metabolites are more than mere leftovers from an organisms metabolic processes. These chemicals can function as communications tools, defense mechanisms or sensory devices. The biological activity of these chemicals is beneficial to the organism that produces them, but it is often harmful to other species, including humans (Swerdlow, 2000). This toxicity can adversely affect the functions of the entire human body or only a specific biological process, such as the growth of cancer cells (Yoder, 2005). Also, many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity of plants have been reported. In this way, certain foreign, naturally produced chemicals can act as powerful drugs when administered at the proper concentration (Yoder, 2005).

Natural products are important in health care. They can be used as starting materials for semisynthetic drugs. The main examples are plant steroids, which led to the manufacture of oral contraceptives and other steroidal hormones. Today, almost every pharmacological class of drugs contains a natural product or natural product analog (Eba, 2005). Similarly, it represents an excellent resource for the identification of new lead structures (Newman and Cragg, 2007).

It is estimated that 25% of all prescriptions dispensed in the USA contained a plant extract or active ingredients derived from plants. It is also estimated that 74% of the 119 currently most important drugs contain active ingredients from plants used in traditional medicine for health care (Farnsworth *et al.,* 1985), these traditional medicines are primarily plant-based (De-Pascual-Teresa *et al.,* 1996). Another study of the most prescribed drugs in the USA indicated that a majority contained either a natural product or a natural product was used in the synthesis or design of the drug (Wakelin, 1986). Similarly, about 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources (Benowitz, 1996). Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics (Newman and Cragg, 2007).

Tropical and subtropical Africa contain between 40,000 – 45,000 species of plants with a potential for development and out of which 5,000 species are used medicinally (Van Wyk, 2008). Still there is a paradox that in spite of this huge potential and diversity, the African continent has only contributed 83 out of the 1100 classic drugs globally (Van Wyk, 2008).

It is a fact that traditional systems of medicine have become a topic of global importance. Although modern medicine may be available in many developed countries, people are still turning to alternative or complementary therapies including medicinal herbs. Yet, few plant species that provide medicinal herbs have been scientifically evaluated for their possible medical applications (Adotey *et al.,* 2012). Herbs had been priced for their medicinal and therapeutic effects, flavouring and aromatic qualities for centuries (Joy *et al*., 2001). Similarly, the herbal drugs contain many chemical compounds naturally. In many cases, traditional healers claim the good benefit of certain natural or herbal products. But, it is only a few herbs, their extracts and active ingredients and also, the preperation containing them that their safety and efficacy data are available (Adotey *et al.,* 2012). No dought, plants extracts either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.,* 2006). However, it is a common practice among chemists that the content of any unlabelled bottle in the laboratory should be discarded. In this way, a truelly practicing chemist should dissociate himself or herself from uncharacterized drug no matter how effective the drug may be (Ndukwe, 2004). As such, It is therefore essential to separate out those compounds which are responsible for therapeutic effect and characterise them. They are called active constituents or principles.

Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc (Akindele and Adeyemi, 2007). Also, Isolation is a part of natural product chemistry, through which it is possible to separate different components and biologically active ones which can be incorporated as

ingredients in the modern system of medicine. Modern medicine has largely confined itself to the isolation or synthesis of single active ingredient for the treatment of spercific disease (Shoge, 2010). Chromatographic techniques are widely used for the separation, isolation and purification of chemical constituents from natural drugs (Devi *et al.*, 2012).

Clinical trials is an essencial aspect of drug discovery. It is necessary to establish and demonstrate the effectiveness of bioactive compounds (Natural drugs) to verify their effeciency. Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate and long-term side effects and their outcomes which are measured before the drug is widely applied to patients (Sasidharan *et al*., 2011). Going by the slogan that every drug is a poison, the poisonous nature of every drug necessitates that its prescription must be followed. Even at that, reports exist where manunfacturing companies withdraw their products from circulation because of adverse reports recieved from dispensers of such drugs (Ndukwe, 2004).

Many plants are chemically very variable depending on the locality where they are found with some of the constituents occurring only at certain seasons of the year (Adelani, 2007). As such, plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs. Some of them include : Alkaloids, Phenolics.

1.1 Alkaloids

Alkaloids are the largest single class of secondary metabolites known. They are those basic substances which contain one or more nitrogen atoms as part of a ring system that shows marked physiological effect on man and animals (Harbone, 1973). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms.. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Norobi *et al.,* 1994). Large numbers of drugs are derived from plants containing alkaloids. Many of the earliest isolated compounds with biological activities were named alkaloids due to their ease of isolation. Many alkaloid have marked effects on the central nervous system and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Caffeine is an alkaloid that provides a mild lift whereas alkaloid in datura causes severe intoxication and even death. Other medicinal uses of alkaloids includes: as an analgesic, antimalarial, antihypertensive, remedy for gout and remedy for cough (Wikipedia).

1.1.1 Phenolic compounds

Phenolics are polyhydroxyl benzene derivatives. They include anthocyanins that give grapes their purple colour. Phytoestrogens from soy and the tannins that give tea its astringency. Natural phenolic compounds play an important role in cancer prevention and treatment. Their various bioactivities are responsible for their chemopreventive propertties (Huang *et al.,* 2010).

1.1.2 Steroids

Steroids are important drugs used as hypotensives, cardiac depressants, sedatives and anti-dysentric agents (Tijjani *et al.*, 2011). Okwu (2001) reported the relationship of steroidal compounds with various anabolic hormones including sex hormones. Steroids have equally been reported to have antimicrobial activity (Quinlan *et al.,* 2000) and comfirmed to have antiviral properties (Neumann *et al,* 2004).

1.1.3 Glycosides

This is a general word that embraces all the many and varied combinations of sugars and aglycones or genins. The usual link between the sugar and aglycone is an oxygen linkage between the reducing group of a sugar and alcoholic or phenolic hydroxyl group of the aglycone (Isah, 2007). The aglycone is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore (Shoge, 2010).

1.1.4 Terpenoids

They are built up from terpene building blocks. Each terpene consists of two paired isoprene units. Based on the number of isoprene units, the names monoterpenes, sesquiterpenes, diterpenes and triterpenes are derived. Monoterpenes are responsible for fragrance of rose.

Many triterpenes and their aglycone have been reported to have varied uses as antiulcerogenic, anti-inflammatory fibrinolytic, antipyretic, analgesic and anti-edmatous in action (Ndukwe *et al.*, 2005).

1.1.5 Tannins

This is an old term denoting substances present in plant extracts that were able to combine with protein of animal hides, prevent their putrefication, and convert them into leather. This definition excluded simpler phenolic substances present with tannins such as gallic acid , catechin and chlorogenic acid etc. i.e, They have tanning property. It has also been claim to have some protective values against toxins when taken orally. They are equally claimed to have anti-viral and anti-tumor properties (Harbone and Boxter, 1993). Tannins is an important plant metabolite because of its remarkable uses therapeutically to strengthen a weakened heart (Isah, 2007). Similarly, tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendarp, 2006; Trease and Evans, 2000).

Two groups of tannins are usually recognized. They are hydrolysable and non hydrolysable tannins. Hydrolysable tannins are of pharmacological importance because of their antiviral and antitoxic properties. Non hydrolysable tannins have been used in medicine to aid the healing of wounds and burns because of their antiseptic properties. Catechin is an example of non hydrolysable tannins.

1.1.6 Flavanoids

Flavonoids comprise a large group of plants secondary metabolites characterized by a diphenylpropane structure (C6-C3-C6). They are widely distributed throughout the plant kingdom and are commonly found in fruits, vegetables and certain beverages. Flavonoids are pigments that are largely responsible for colour of many fruits, flowers and vegetables. They provide many health–promoting benefit. They act as antihistamine, which is useful in reducing allergy sympton, and help to reduce inflammations associated with various forms of arthritis (Guardia *et al.*, 2001)

Numerous preclinical and some clinical studies suggest that flavonoids have potentials for the prevention and treatment of several diseases. Some epidemiological studies support a protective role of diets rich in foods with flavonoids and a reduced risk of developing cancer and cardiovascular diseases (Lopez-Lizaro, 2009). Preclinical *in vitro* and *in vivo* investigations have shown plausible mechanisms by which flavonoids may control cancer and cardiovascular protection (Middleton *et al.*, 2000). In addition to their preventive potential, certain flavonoids may be useful in the treatment of several diseases. Some evidence supporting the therapeutic potential of flavonoids comes from the study of plants used in traditional medicine to treat a wide range of diseases, which has shown that flavonoids are common bioactive constituents of these plants (Middleton *et al*., 2000; Ren *et al*., 2003; Wang, 2000; Lopez-Lizaro, 2002).

1.1.7 Saponins

Saponin has been reported to have anti microbial effects (Mahato *et al.*, 1988, 1992) and could serve as precusors of steroidal substances with a wide range of physiological

activities (Madusolomo *et al.*, 1999). Many saponins and their aglycone have been reported to have varied uses as antiulcerogenic, anti-inflammatory fibrinolytic, antipyretic, analgesic and anti-edmatous in action (Ndukwe *et al.*, 2005). Just *et al.*, (1998) revealed the inhibitory effect of saponins on inflammed cells.

People have been exploring nature, particularly plants, in search of new drugs with healing powers. The search for new drugs which are plant-derived has been receiving renewed interests among researchers globally in view of discovering new drugs that possess potency to combat the menace of drug resistant pathogenic microorganisms, antitumor and anticancer agents (Mirza, 2007; Pimenta *et al.*, 2003). Also, the increased incidence of diseases for which there is yet an effective remedy for. Diseases like tuberculosis, pneumonia, typhoid fever, rheumatic fever and meningitis (Greenwood *et al.*, 1992) still pose a major challenge to modern chemotherapeutic agents. In the constant effort to improve the efficacy of orthodox medicine, researchers are increasingly turning their attention to the chemistry of natural products which has its roots in the empirical knowledge of ancient medicine and finds its continuity in folk medicine today (Kan, 1995).

1.2 Historically Important Natural Products

Some well known and important drugs have originated from natural sources. A few examples are given to indicate what an important impact these drugs have had on medical treatment and disease control. Of note is the fact that these drugs were isolated from natural sources many of which have been used by various cultures throughout history.Some of them include: morphine, quinine, etc.

1.2.1 Morphine

Morphine was first isolated from the plant opium poppy (*Papaver somniferum*) in 1861 (Cragg and Newmann, 2001). In ancient Mesopotamia the oils of *P. somniferum* were used as an analgesic (Cragg and Newmann, 2001). The discovery and isolation of morphine led to an increased interest in alkaloid chemistry and resulted in the development of other analgesic agents (Cragg and Newmann, 2001). Morphine was the first commercially available pure natural product, marketed in 1826 (Newmann *et al*., 2000).

1.2.2 Quinine

Malaria has been and continues to be a problem in many areas around the world including Nigeria. The native Amerindians of the Amazon region used the bark of the *Cinchona* tree to treat malaria (Clark, 1996). Quinine, the active component of *Cinchona* bark, was isolated in 1820 from *C. officinalis* (Cragg, 2002). Quinine was the first effective anti-malarial drug to be isolated (Phillipson, 2001). Other anti-malarial drugs such as chloroquine and mefloquine were synthetically modified version of quinine (Guza, 2004).

1.2.3 Penicillin

Penicillin was first discovered in 1929 by Fleming from the fungus, *Penicillium notatum* (Cragg and Newmann, 2001). This discovery was important to the development of antibiotics and changed medicine forever (Clark, 1996). The discovery of this revolutionary drug from a natural source prompted the investigation of nature for other novel compounds (Cragg, 2002).

1.2.4 Vinblastine and Vincristine

The plant *Catharanthus roseus*, commonly known as the Madagascar periwinkle, was used in some cultures as a folk remedy to treat diabetes (Cragg and Newmann, 2001). However, when *C. roseus* was evaluated for hypoglycemic compounds but no such compounds were found (Clark, 1996). Instead, the results led to the hypothesis that the plant extract contained a compound potentially useful for the treatment of cancer (Clark, 1996). Upon further investigation of *C. roseus* for anti-cancer agents, vinblastine and vincristine were isolated in 1954 (Noble, 1990). These drugs were developed by Eli Lily and are important agents in the treatment of cancer (Guza, 2004).

1.2.5 Paclitaxel

Random collection of plants by the United States Department of Agriculture (USDA) for the National Cancer Institute (NCI) yielded an extract with anti-cancer activity (Cragg and Newmann, 2001). The extract was from the Pacific yew tree (*Taxus brevifolia*) and the active compound, paclitaxel, was isolated in 1969 (Cragg, 2002). Several Native American tribes use various parts of *Taxus* trees for the treatment of a wide range of non-cancerous ailments *notatum* (Cragg and Newmann, 2001). Paclitaxel is an important anti-cancer drug in use today (Guza, 2004).

1.3 Importance of natural products to drug discovery

For many decades, synthetic chemicals as drugs have been effective in the treatment of most diseases. The pharmaceutical industry has synthesized over 3 million new chemicals in their effort to produce new drugs (Eba, 2005). Despite their successes in

developing drugs to treat or cure many diseases, the treatment of certain diseases such as cancer, AIDS, heart disease and diabetes has not been a complete success due to the complexity of these diseases (Eba, 2005).

Over the centuries, people have been living in close association with the environment and relying on its flora and fauna as a source of food and medicine. As a result, many societies have their own rich plant pharmacopeias. In developing countries, due to economic factors, nearly 80% of the population still depends on the use of plant extracts as a source of medicine (Akindele and Adeyemi, 2007).

Natural products also play important roles in the health care system in developed countries. The isolation of the analgesic morphine from the opium poppy (*Papaver somniferum*) in 1816 led to the development of many highly effective pain relievers (Benyhe, 1994). The discovery of penicillin from the filamentous fungus *Penicillium notatum* by Fleming in 1929 had a great impact on the investigation of nature as a source of new bioactive agents (Bennett and Chung, 2001).

Natural products can also be used as starting materials for semisynthetic drugs. The main examples are plant steroids, which led to the manufacture of oral contraceptives and other steroidal hormones. Today, almost every pharmacological class of drugs contain a natural product or natural product analog (Eba, 2005). The investigation of higher plants has led to the discovery of many new drugs. So far only a small portion of higher plants has been investigated. In Nigeria, only a few plant species present in the country have been subjected to scientific evaluation for their potential chemoterapeutic values. As such, plants still remain a big reservoir of useful chemical compounds not only as drugs, but also as templates for synthetic analogues (Eba, 2005).

1.4 Aims and objective of the research

1.4.1 Aims

This research is aimed at establishing the medicinal potencials and claims of the plant *Peucedanum winkleri* H. Wollf and also isolate and characterize some compounds that may be responsible for the claimed ethnomedicinal values using organic analytical techniques.

1.4.2 Objectives

The objectives of this work includes:

i. Collection, proper boatanical identification, drying and grinding of plant material.

ii. Phytochemical screening of the plant material.

iii.Total extraction of ground plant material using methanol and then partitioning of dry methanol extract using petroleum ether, chloroform and ethyl acetate.

iv. Antimicrobial screening of the extracts.

v. Analytical separations and purification involving several chromatographic techniques.

vi. Verification of the purity of the isolated compound(s).

vii. Testing the potency of the isolated compound(s) on some microorganisms.

1.4.3 Justification of the research

The choice of *Peucedanum winkleri* as the plant of interest in this work is based on its claimed vast ethnomedicinal uses among traditional medicine practitioners in the tropics including Nigeria. It is therefore essential to scietifically study the plant in order to

ascertain its medicinal potentials and also isolate and characterize the medicinally active components of the plant with the help of analytical organic techniques.

1.4.4 Scope and limitation of the research

This research thesis is limited to phytochemical screening, antimicrobial screening, isolation, characterization and structural elucidation. But due to limited laboratory facilities, not all the active components may be completely elucidated. However, this challenge will not in anyway act as constraints towards progress and production of a sound work as relevant and updated materials will be used.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Apiaceae (alt. **Umbelliferae**),

Commonly known as carrot or parsley family, it is a group of mostly aromatic plants with hollow stems. The family is large, with more than 3,700 species spread across 434 genera, it is the sixteenth largest family of flowering plants (Stevens, 2001). Included in this family are the well known plants: angelica, anise, arracacha, asafoetida, caraway, carrot, celery, centella asiatica, chervil, cicely, coriander/cilantro, cumin, dill, fennel, hemlock, lovage, Queen Anne's Lace, parsley, parsnip, sea holly, and the now extinct silphium.

Most Apiaceae are annual, biennial or perennial herbs (frequently with the leaves aggregated toward the base), though a minority are shrubs or trees. Their leaves are of variable size and alternately arranged, or alternate with the upper leaves becoming nearly opposite. Most commonly crushing leaves emits a marked smell, aromatic to foetid, but absent in some members. The flowers are nearly always aggregated in terminal umbels, simple or compound, often umbelliform cymes, rarely in heads.

The defining characteristic of this family is the inflorescence: a simple or compound umbel. Flowers across the Apiaceae are fairly uniform and are usually perfect (hermaphroditic) and actinomorphic, but some are andromonoecious, polygamomonoecious, or even dioecious (as in *Acronema*), with a distinct calyx and corolla, but the calyx if often highly reduced, to the point of being undetectable in many

species, while the corolla can be white, yellow, pink or purple. Pollination of one flower by the pollen of a different flower of the same plant (geitonogamy) is common. The Umbels are unique in their floral uniformity. They encourage free pollination by unspecialised pollinators an interesting evolutionary adaptation.The effect of this promiscuous pollination allows it to increase distribution.

The fruits are non-fleshy schizocarp of two mericarps, each with a single seed; they separate at maturity and are dispersed by wind. The seeds have an oily endosperm (Watson and Dallwitz, 1992; She *et al.,* 2005) and generally contain large quantities of fatty oils, with the fatty acid petroselinic acid occurring universally throughout the family while rarely being found outside of the Apiaceae.

This family is generally well known for being distinguishable from each other but with reference to habitat, flowering time, leaf shape and aroma they can be easily identified.

The Apiaceae was first described by John Lindley in 1836 (Lidley, 1836). The name is derived from the type genus *Apium*, which was originally used by Pliny the Elder circa 50 AD for a celery-like plant (Michael, 2010). The alternative name for the family, Umbelliferae, derives from the inflorescence being generally in the form of a compound umbel. The family was one of the first to be recognized as a distinct group in Jacques Daleschamps' 1586 *Historia generalis plantarum*. With Robert Morison's 1672 *Plantarum umbelilliferarum distribution nova* it became the first group of plants for which a systematic study was published.

Many members of this family are cultivated for various purposes. The plant structure includes a tap root, which can be large enough to be useful in food, as with parsnips (*Pastinaca sativa*), carrots (*Daucus carota*), and Hamburg parsley (*Petroselinum crispum*). Many plants of this group are also adapted to conditions that encourage heavy concentrations of essential oils, and as a result some are flavourful aromatic herbs. Examples are parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*), and dill (*Anethum graveolens*). The plentiful seeds of the umbers, likewise, are sometimes used in cuisine, as with, coriander (*Coriandrum sativum*), fennel (*Foeniculum vulgare*), cumin (*Cuminum cyminum*), and caraway (*Carum carvi*).

Other notable cultivated Apiaceae include chervil (*Anthriscus cerefolium*), angelica (*Angelica* spp.), celery (*Apium graveolens*), arracacha (*Arracacia xanthorrhiza*), poison hemlock (*Conium maculatum*), sea holly (*Eryngium* spp.), asafoetida (*Ferula asafoetida*), galbanum (*Ferula gummosa*), cicely (*Myrrhis odorata*), anise (*Pimpinella anisum*), lovage (*Levisticum officinale*), and hacquetia (*Hacquetia epipactis*) (Watson and Dallwitz, 1992).

Apiaceae species contain compounds with several and different biological activities, such as antibacterial, anticancer, hepatoprotective, vasorelaxant, and cyclooxygenase inhibition (Vita *et al.,* 2011).

Many species in Umbelliferae family have been proved and used in homoeopathy. Similarly, members of this family have the medicinal tendancies to ameloriate: Alcoholism, Amenorrhoea, Anaemia, Anorexia, Anxiety, Appetite loss, Arthritis, Asthma, Black water fever, Bladder disorder, Breath refrehsner, Bronchitis, Bruises, Burns, Catarrh, Cholera, Colic, Conjuctivities, constipation, Coughs, Cramp, Diarrhoea, Digestive

complaints, Dizziness, Dropsy, Epilepsy, Fatigue, Intermitent fever, Flatulence, Fungal infection, Gingivitis, Gout, Haemorrhoids, Herpes simplex, Hiccoughs, Indigestion, Inflammation, Influenza, Intestine disorder, Jaundice, Kidney complaints, Lactation stimulant, Laryngitis, Menopausal problems, promoting menstral discharge, Menstral disorder, Migraine, Narcotics, Nausea, Obesity, Oedema, Abdominal pain, Premenstral tension, Prostraite complaint, Pneumonia, Rash, Rhrumatism, Sedative, Skin cleanser, Sore throat, Stomach disorder, Bladder stones, Kidney stones, Syphilis, Tumour, Ulcers, UTI, Vginal discharge, Vitiligo, Whooping cough and worms etc. Many plants in this family, such as wild carrot, have estrogenic properties and have been used as a folk medicine for birth control. Most notable for this use is the extinct giant fennel, silphium. *Conium maculatum* has been used as a sedative and in treatments for arthritis and asthma in addition to its most famous use: as a "humane" method of killing criminals and philosophers. *Daucus carota* has been used as a diuretic, as a stimulant, to aid in uterine ailments, and one time as a treatment for jaundice. According to the Greek physician and botanist Dioscorides, "[Parsley] —especially Bastard or Wild Parsley—provokes venery and bodily lust and erection of the parts." Fennel has been used to treat nausea; species of Ferula have been used to ease constipation and coughs; Cuminum cyminum has been used as a treatment for diarrhea; coriander for halitosis; *Eryngium* spp. to treat genitourinary problems; Centella asiatica to treat syphilis; and *Ferula assa-foetida* has been used as a carminative, antispasmodic, and stimulant (Wikipedia).

The poisonous members of the Apiaceae have been used for a variety of purposes globally. The poisonous *Oenanthe crocata* has been used to stupefy fish, *Cicuta douglasii* has been used as an aid in suicides, and arrow poisons have been made from various other

family species. *Daucus carota* has been used as colouring for butter and its roots used as a coffee substitute. *Dorema ammoniacum*, *Ferula galbaniflua*, and *Ferula sumbul* are sources of incense.(Wikipedia).

Polyacetylenes can be found in Apiaceae vegetables like carrot, celery, fennel, parsley and parsnip where they show cytotoxic activities (Zidorn *et al.*, 2005). Similarly, Lars and Kirsten (2006) reported that these bioactive aliphatic C17-polyacetylenes have shown to be highly toxic towards fungi, bacteria, and mammalian cells, and to display neurotoxic, anti-inflammatory and anti-platelet-aggregatory effects and to be responsible for allergic skin reactions. Similarly, the effect of these polyacetylenes towards human cancer cells, their human bioavailability and their ability to reduce tumour formation in a mammalian in vivo model indicates that they may also provide benefits for health (Lars and Kirsten, 2006). It is noteworthy that Apiaceae species contain compounds with several and different biological activities, such as antibacterial, anticancer, hepatoprotective, vasorelaxant, and cyclooxygenase inhibition (Vita *et al.*, 2011).

2.2.0 Some important members of the Apiaceae, their medicinal effects and compounds isolated from them

***Athamanta sicula* L.,**

Is a member of Apiaceae. Is an annual perennial herb and known in Sicilian popular medicine as “spaccapietre” (rock splitters), because fresh roots infusions are indicated as diuretic and used in the treatment of diseases of the urinary tract, and to dissolve kidney stones (Fiori, 1970; Lentini, 2000). Vita *et al.*, (2011) isolated two aromatic compounds

from the Acetone extracts of the plant namely: Apiol, a white powder having a molecular mass of $C_{12}H_{14}O_4$ and M.P of 56^0C from the flower and stem bark and Myristicin, a colourless oil with molecular mass of $C_{11}H_{12}O_3$ from the leaves of the plant. Also the Acetone extracts of leaves, flowers, and stems of *A. sicula* L., Apiol and Myristin was evaluated for its antibacterial and cytotoxic potencials as well as Antimicrobial activity against bacterial and fungal strains and antiproliferative activity against a group of human cancer cell lines (K-562, NCI-H460, and MCF-7). Their result indicated that all acetone extracts, Apiol and Myristicin was inactive as antimicrobial agents at the maximum tested concentration of 200 μg/mL, but induced significant antiproliferative activity on the tested cancer cell lines, suggesting that both apiol and myristicin could be tested as novel treatment in cancer chemotherapy (Vita *et al.,* 2011).

***Chaerophyllum aureum* L. (Apiaceae, tribe Scandiceae)**

Is a perennial herb growing in the mountainous to sub-alpine regions of Europe. The aromatic smell of this plant is similar to that of carrots. *Ch. aureum* has considerable nutritional values and is used as pasture herb by cattle (Hegi, 1975). (Gonnet, 1985, 1986) reported that flavonoid glycosides have been isolated from its leaves. Sub-aerial parts of the plant yielded two polyacetylenes, falcarinol, falcarindiol, three lignans, namely nemerosi , deoxypodorhizone, deoxypodo-phyllotoxin, two phenylpropanoids, 1-hydroxymyristicin and its angeloyl ester. 1-hydroxymyristicin and its angeloyl ester compounds were isolated for the first time from this plant material and their structures were elucidated by means of extensive 1- and 2-dimensional NMR spectroscopy and high

resolution mass spectrometry. In bioautographic tests on TLC plates the dichloromethane extract showed a significant antimicrobial activity. Falcarindiol was identified as the main active principle whereas the phenylpropanoids and lignans showed no activity. HR Mass spectrometric analysis of 1-hydroxymyristicin indicated a molecular formula of $C_{11}H_{12}O_4$ (Rollinger *et al.,* 2003).

***Heracleum persicum* Desf. ex Fischer**

Is an Iranian aromatic medicinal plant from Umbelliferae. Their fruits are used as flavouring agent in food products. Sedaghat *et al.,* (2011), reported that essential oils were separated from *Heracleum persicum*. The larvicidal activity was evaluated against laboratory-reared larvae of *An. stephensi* according to standard method of WHO. Result of this study showed that its oil was effective against *An. stephensi* with LC_{50} and LC_{90} values of 20.10 and 44.51 ppm, respectively. And concluded that the plant essential oil can serve as a natural larvicide against *An. stephensi.*

***Seseli sibiricum* Linn.**

Three new coumarins, seselinal, sesibiricol and sibirinol, and 12 known coumarins have been isolated from the umbels of *Seseli sibiricum*. The new coumarins have been characterized as 5, 7-dimethoxy-8-(2-methyl-2-formylpropyl)-2H-1-benzopyran-2-one, 5-(3-methylbut-2-enyloxy)-7-methoxy-8-(2-hydroxy-3-methylbut-3-enyl)-2H-1-benzopyran-2-one and 5,7-dimethoxy-8-(2-hydroxy-3-methylbut-3-enyl)-2H-1-benzopyran-2-one, respectively. The known ones were identified as sesibiricin, isosibiricin, osthol, coumurrayin, sesebrin, sesebrinol, sibiricin, imperatorin, bergapten, xanthotoxin,

isopimpinellin and mexoticin (Banergee *et al.,* 1980 umbel genus). Also, from the roots of of *Seseli sibiricum*, three new coumarins, sesebrin(7-methoxy-5-[3-methylbut-2-enyloxy]-8-[3-methyl-2,3-epoxybutyl] coumarin), sesebrinol(7-methoxy-5-[3-methylbut-2-enyloxy]-8-[3-methyl-2,3-dihydroxybutyl] coumarin) and sibiricol (7-methoxy-5-hydroxy-8-[3-methylbut-2-enyl] coumarin) were isolated, in addition to sitosterol and 7 known coumarins, isobergapten, bergapten, phellopterin, sibiricin, coumurrayin, osthenol and meranzin hydrate (Kumar *et al.*, 1978).

***Angelica pachycarpa* Lange.**

Mendez *et al.,* (1983) reported the isolation of novel furocoumarin from unripe fruits of *Angelica pachycarpa* and was characterized as (+)-tert- O-methylbyakangelicin. (+)-tert-O-Methyloxypeucedanin hydrate, neobyakangelicol, (+/-)-byakangelicol, (+)-byakangelicin, phellopterin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, pangeline and umbelliprenin were also identified along with (+/-)-byakangelicin monoacetate, which proved to be an artifact.

***Bupleurum wenchuanense* R.H.Shan & Yin L**

From the roots of *Bupleurum wenchuanense*, 14 derivatives of saikosaponin were isolated and identified as 2''-O-β-d-xylopyranosylsaikosaponin b2, 3'',6''-O,O-diacetylsaikosaponin b2, 2''-O-β-d-glucopyranosylsaikosaponin b2, saikosaponin b2, 6''-O-acetylsaikosaponin b2, saikosaponin d, 2''-O-acetylsaikosaponin d, 3''-O-acetylsaikosaponin d, 6''-O-acetylsaikosaponin d, 16-epichikusaikoside, prosaikogenin G, saikosaponin a, 2''-O-acetylsaikosaponin a and 3''-O-acetylsaikosaponin a. The first two

compounds are new derivatives of saikosaponin and this is the first isolation of prosaikogenin G from a plant. Some of the compounds showed cytotoxic activity (Si-Qi *et al.*, 1993).

***Ferulago macrocarpa* (Fenzl) Boiss.**

This is a perennial herb which grows in western Iran. Previous phytochemical studies of *Ferulago* have led to the isolation of various coumarins and volatile oils. Some of the isolated coumarins have shown antimicrobial, antioxidant, cytotoxic and acetylcholinesterase inhibitor activities. In addition, the essential oils of many other *Ferulago* species have exhibited antimicrobial activities. From the acetone extract of the fruits of this plant using repeated open column chromatography in normal phase and HPLC using petroleum ether, toluene, ethyl acetate and n-heptane as mobile phase resulted in isolation of these compounds: bornyl acetate and 1,10-di-epi-cubenol. The structures of these compounds were elucidated using NMR and MS spectra. The compounds are mainly used as antioxidant, antifungal, antibacterial and anti-inflammatory agents (Sajjadi *et al.*, 2012).

From the hexane extract of the aerial parts of *Ferulago Bernardii* (Apiaceae), Khalighi *et al.*, (2006) isolated four coumarins, namely prantschimgin **1**, oxypeucedanin **2**, psoralen **3** and umbelliferone **4**; β-sitosterol **5**; and nonacosane **6** by Column Chromatography (CC), Preparative Thin Layer Chromatography (PTLC) and crystallization. The structures were elucidated by means of melting point, UV, IR, MS, ^{1}H and ^{13}C-NMR spectra. They also reported that the presence of compounds **1**, **2**, **3** and **5** in

some others *Ferulago* species could be used as chemotaxonomic marker in genus *Ferulago*.

Dall'Acqua *et al*., (2010) isolated three coumarin derivatives (umbelliprenin 1, coladonin 2 and coladin 3), three daucane ester derivatives (siol anisate 4, ferutinin 5 and 1-acetyl-5-angeloyl lapiferol 6), two phenol derivatives (2-epilaserine 7 and epielmanticine 8) and one polyacetylene (9-epoxyfalcarindiol 9) by the bioassay-guided approach from the dichloromethane extract. Their structures were characterized on the basis of spectral methods (1D and 2D NMR, and MS spectroscopy). Also, they reported that all isolated compounds were able to inhibit the AChE (IC_{50} 1.2-0.1mM) although at higher doses if compared to galantamine (6.7 μM) measured in the same conditions. The most active compounds were the daucane derivative siol anisate 4 and the epielmanticine 8, with IC_{50} of 0.172 and 0.175 mM respectively.

***Lomatium macrocarpum* (Hook. & Arn.) C. & R**

Roots of *Lomatium macrocarpum* (Hook. & Arn.) C. & R. yielded osthol (7-methoxy-8-[3-methyl-2-butenyl]-coumarin) and a chromone, 2-methyl-5-hydroxy-6-[3-methyl-2-butenyl]-7-methoxychromone, identified spectroscopically and by synthesis. The aerial parts of the plant also contained this chromone along with sibiricin (5,7-dimethoxy-8-[3-methyl-2,3-epoxybutyl]-coumarin) and a new coumarin named macrocarpin. By spectroscopy and chemical degradation macrocarpin was shown to be 7-methoxy-8-(3-methyl-4-[2-methyl-cis-2-butenoyloxy]-cis-2-butenyl) coumarin (Steck, 1973).

***Foeniculum vulgare* Mill.**

It is commonly known as fennel. It is a well known and important medicinal and aromatic plant widely used as carminative, digestive, lactogogue and diuretic and in treating respiratory and gastrointestinal disorders. Its seeds are used as flavourings in baked goods, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures. Phenols, phenolic glycosides and volatile aroma compounds such as trans-anethole, estragole and fenchone have been reported as the major phytoconstituents of this species (Manzoor *et al*., 2012). An acylated kaempferol glycoside, namely kaempferol-3-O-α or β-L-(2.,3.-di-*E*-*p*coumaroyl)-rhamnoside (1) was isolated from the flowers of *Foeniculum vulgare* Mill. and *F. dulce* DC. It is thus isolated for the first time from family Apiaceae. In addition, the different organs of both plants afforded six flavonoid glycosides - namely afzelin (kaempferol-3-O-α or β-L-rhamnoside) (2), quercitrin (3), isorhamnetin-3-O-α or β-D-glucoside (4), isoquercitrin (5), rutin (6), and miquelianin (quercetin-3-O-α or β-D-glucuronide) (7). Structure elucidation of the above mentioned flavonoids was achieved by UV, ^{1}H- and ^{13}C-NMR, 1H-1H COSY, HMQC and EI-MS (Fathy *et al.*, 2002). Phytochemical study of the aerial parts of *Foeniculum vulgare* led to the isolation of seven compounds isolated for the first time from this species. Furthermore, Amar *et al*., (2011) reported antimicrobial activity of the plant crude extract especially against fungal strains.

Two new flavonoids, 8-hydroxyisoscoparin (1) and luteolin 7-O-glucoside 4"-sulfate (2), along with eight known flavonoids, including luteolin 7-O-glucoside 2"-sulfate, were isolated and identified from *Washingtonia filifera*. All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, CI-MS, FABMS and UV. The antioxidant activities of various *W. filifera* extracts were determined (EL Sayed *et al.*, 2006).

2.3 Some important members of the *Peucedanum* genus, their medicinal effect and compounds isolated from them.

Peucedanum ruthenicum M. Bieb.

This is a glabrous perennial plant that distributed in the north and central part of Iran (Salimian, 2003). Some species of this genus have been used traditionally in treatment of colds (Gan, 1965), coughs due to pathogenic wind-heat, accumulation of phlegm, and heat in the lung (Kong *et al.*, 1996), anti-tussive, and are used as anti-asthmatic and as a remedy for angina (Tang and Einsehbrand, 1992). From *P. ruthenicum*, a Bulgarian *Umbelliferae*, peucedanin (furanocoumarin) and a coumarin (peuruthenicin) in the roots and rutin (flavonol glycoside) in the flowers have been isolated from the methanolic extract of aerial parts (Soine *et al*., 1973).. Similarly, Alavi *et al.*, (2009) isolated four flavonoids namely Isorhamnetin 3-0-rutinoside, rutin, quercetin, morin and two phenolic acids namely caffeic acid and *p*-coumaric acid by Paper Chromatography (PC) and crystallization. Their structures were elucidated by MS, ^{1}H, ^{13}C NMR spectra.

Peucedanum ostruthium L. (Masterwort)

The rhizomes of *Peucedanum ostruthium* L.(Masterwort) are traditionally used in the alpine region as ingredient of liqueurs and bitters, and as a herbal drug. The medicinal plant is also used traditionally in Austria and has been found to have anti-inflammatory properties. The major components of the essential oils of herb and rhizome of *P. ostruthium* were sabinene (35.2%), 4-terpineol (26.6%), β-caryophyllene (16.1%) and α-humulene (15.8%) (Cisowskia *et al.*, 2001). It has been reported previously that coumarins

are the major bioactive constituents of extracts from *P. Ostrithium* rhizomes (Hiemann and Schantl, 1998; Hiemann *et al.*, 1996). Its main coumarins, oxypeucedanin hydrate, oxypeucedanin, ostruthol, imperatorin, osthole, isoimperatorin, and ostruthin, was simultaneously identified and quantified using a sensitive and specific high-performance liquid chromatography−diode-array detection−mass spectrometry (HPLC-DAD-MS) method (Sylvia *et al*., 2011). Khaled and Szendrei (1975) reported the presence of Coumarin glycosides in *Peucedanum ostruthium* plant. Reisch and Khaled (1975) reported the presence of 5-Alkoxy-furanocoumarins in *Peucedanum ostrithium* plant. Similarly, Reisch and Khaled (1975) reported the presence of new chromones in *Peucedanum ostrithium* plant. Hiermann *et al.,* (1996) isolated two new coumarins namely 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin and the 3′-acetate of oxypeucedanin hydrate from the roots of *Peucedanum ostruthium*, and their structures were established mainly by mass spectrometry and 2D NMR technque.

Peucedanum ***praeruptorum*** **Dunn (PPD)**

The root of *Peucedanum praeruptorum* Dunn (PPD) is a commonly used in traditional Chinese medicine for the treatment of asthma. Its major constituents, coumarins, were presumed to be responsible for its efficacy. This claim was ascertained by (Xiong *et al*., 2012) in their research using female mice their result concluded that PPD showed great therapeutic potential for the treatment of allergic asthma. Two novel angular-type furanocoumarin glycosides, peucedanoside A and peucedanoside B, along with a known compound apterin, were isolated from the roots of *Peucedanum praeruptorum* Dunn. Their chemical structures were determined by MS, NMR spectroscopy and chemical analysis.

Complete assignments of the ^{1}H and ^{13}C NMR spectroscopic data were achieved by 1D and 2D NMR experiments including DEPT, HSQC, HMBC and ROESY (Chang *et al*, 2007). (Zhang *et al*, 2010) reported a new compound, 9,10-dihydrophenanthrinic acid, 9,10-dione-3,4-methylenedioxy-8-methoxy isolated from the roots of *Peucedanum praeruptorum* Dunn and the characterization was on the basis of spectral evidences. A new angular dihydropyranocoumarin named qianhucoumarin H and four known coumarins, peucedanocoumarin, 5,8-dimethoxypsoralen, isoscopoletin, umbelliferone along with anchoic acid were isolated from the root of *Peucedanum praeruptorum*. By spectral analysis and chemical evidence, the structure of qianhucoumarin H was elucidated as 3′(*S*)-angeloyloxy-4′(*R*)-isovaleryloxy-3′,4′-dihydroseselin and the absolute configurations were established by chemical correlations with known compound (Ling yi *et al.*, 1996). A new angular dihydropyranocoumarin, named qianhucoumarin was isolated from the root of *Peucedanum praeruptorum* . By spectral analysis and chemical evidence, the structure was elucidated as 3′(*S*)-acetoxy-4′(*S*)-tigloyloxy-3′,4′-dihydroseselin, and the absolute configurations were established by chemical correlation with known compounds (Ling yi *et al*., 1996). Liu *et al.*, (2004) isolated coumarins using a preparative high-speed counter-current chromatography (HSCCC) method for isolation and purification of coumarins from *Peucedanum praeruptorum* Dunn. This was successfully established by using light petroleum–ethyl acetate–methanol–water as the two-phase solvent system in gradient elution mode. Four kinds of coumarins and another unknown compound were obtained and yielded 5.3 mg of qianhucoumarin D, 7.7 mg of Pd–Ib, 35.8 mg of (+)-praeruptorin A, 31.9 mg of (+)-praeruptorin and 6.4 mg of unknown compound with the purity of 98.6%, 92.8%, 99.5%, 99.4% and 99.8% in one-step separation, respectively. The structures of the

coumarins were identified by ^{1}H NMR and ^{13}C NMR. Two minor coumarins, including a new compound qianhucoumarin J, were isolated from *Peucedanum praeruptorum* by a multi-step separation procedure using high-speed counter-current chromatography (HSCCC) and preparative high performance liquid chromatography (prep-HPLC) monitored by high performance liquid chromatography coupled with mass spectrometry (HPLC/MS) analysis. The structure of the new compound was elucidated mainly by analysis of its 1D and 2D NMR spectral data. Its absolute configuration was determined by chemical degradation followed by chiral HPLC analysis (Huo *et al.*, 2010).

According to Zhang *et al.,* (2003) differentiation therapy for myeloid leukemia offers great potential as a supplement to the current treatment modalities. They investigated if the pyranocoumarins, (±)-4′-*O*-acetyl-3′-*O*-angeloyl-*cis*-khellactone (or angular pyranocoumarin, APC) isolated from the medicinal plant *Peucedanum praeruptorum* Dunn, could induce human acute myeloid leukemic HL-60 cells to differentiate and elucidated the molecular mechanism(s) involved. Their research concluded and suggested that APC are potent inducers of HL-60 cell differentiation along both the myelocytic and monocytic lineages and are potential agents for differentiation-treatment of leukemia.

***Peucedanum verticilare* Koch**

Alavi *et al.,* (2009) reported that the major constituents of *P. verticillare* leaf and branch oil were sabinene and trans-anethole. β-Caryophyllene, α-Phellandrene, cis-β-farnesene and β-bisabolene were components of *P. verticillare* dried fruit oil and sabinene

was the constituent of *P. verticillare* fresh fruit oil (Daniele *et al.,* 1978). Similarly, analysis by GC and GC/MS of the essential oil obtained from aerial parts and fruit of *Peucedanum verticillare* revealed 32 components. The major constituents were found to be sabinene and (*E*)-anethole in the leaf and branch oil, sabinene in the fresh fruit oil and *β*-caryophyllene, *α*-phellandrene, (*Z*)-*β*-farnesene and *β*-bisabolene in the dried fruit oil (Daniele *et al.,* 2000).

***Peucedanum zenkeri* Engl. ex H. Wolff**

The methanol extract of *Peucedanum zenkeri* L. seeds showed antimicrobial activity which is concentrated in the n-hexane fraction. Bioactivity-guided chromatographic fractionation of the seeds of *P. zenkeri* led to the isolation and characterization of five major coumarins, umbelliprenin, imperatorin, bergapten, isopimpinellin and byakangelicin, as well as two minor coumarins, 7-methoxy coumarin and 5-hydroxy-8-methoxy psoralen. Amongst the isolated compounds only imperatorin, bergapten and isopimpinellin were found to possess anti-microbial activities (Ngwendson *et al.,* 2003).

***Peucedanum tauricum* Bieb.**

From the essential oil of fruits of Peucedanum *tauricum* Bieb., two guaiane type sesquiterpene hydrocarbons guaia-1(10),11-diene and guaia-9,11-diene were identified. Their structures were assigned using 1D and 2D NMR analysis. The relative configurations of the compounds were established by 2D-NOESY experiments while the absolute

configurations were deduced through chemical correlations with (+)-γ-gurjunene and capillary GC analysis using modified cyclodextrins as the stationary phases. From the dichloromethane extract of the less volatile fraction of the fruits, coumarins, viz. peucedanin, oxypeucedanin hydrate and officinalin isobutyrate were isolated. Officinalin isobutyrate was confirmed to be 6-carbomethoxy-7-isobutyroxycoumarin by its 1D and 2D NMR data as well as by conversion into officinalin by alkaline hydrolysis. Peuruthenicin, a positional isomer of officinalin, is assigned structure on spectral basis. Bergapten was identified by its mass spectrum. This is the first report on the isolation of oxypeucedanin hydrate and officinalin isobutyrate compounds from *P. Tauricu*. (Tesso *et al.,* 2005).

***Peucedanum japonicum* Thunb**

P. japonicum is a traditional herb in Ryukyu Island with an anti-oxidant activity (Okabe *et al.,* 2011). The antidiabetic activity-guided fractionation and isolation of the 80% EtOH extracts from *Peucedani Radix (Peucedanum japonicum, Umbelliferae)* led to the isolation and characterization of a coumarin and a cyclitol as active principles, that is, peucedanol 7-O-beta-D-glucopyranoside and myo-inositol. Their structures were identified by spectroscopic methods. Peucedanol 7-O-beta-D-glucopyranoside showed 39% inhibition of postprandial hyperglycemia at 5.8 mg/kg dose, and myo-inositol also significantly inhibited postprandial hyperglycemia by 34% (Lee *et al*, 2004). According to Chang-yhi *et al.,* (1991), bioactivity-guided fractionation of a chloroform extract of the aerial parts of *Peucedanum japonicum* afforded two new cytotoxic pyranocoumarins, (+)-trans-khellactone and (+)-trans-4'-acetyl-3'-tigloylkhellactone, as well as a known cytotoxic

pyranocoumarin, (+)-cis-4'-acetyl-3'-angeloylkhellactone. Two new angular-type dihydropyranocoumarins, peujaponisin and (-)-visnadin, were isolated from the roots of *Pencedanum japonicum* and their structures determined as 3'(S)-senecioyloxy-4'(S)-isovaleryloxy-3',4'-dihydroseselin and 3'(S)-2-methylbutyryloxy-4'(S)-acetoxy-3',4'-dihydroseselin, respectively, by spectroscopic analysis and chemical reactions (Ikeshiro *et al.,* 1992). Four new khellactone esters, (−)-*trans*-3′-acetyl-4′-senecioylkhellactone, (±)-*cis*-3′-acetyl-4′-tigloylkhellactone, (±)-*cis*-4-tigloylkhellactone, (+)-*trans*-4′-tigloylkhellactone, together with 14 known coumarins, isoimperatorin, psoralen, bergapten, xanthotoxol, cnidilin, (−)-selinidin, (−)-deltoin, (+)-pteryxin, (+)-peucedanocoumarin III, xanthotoxin, imperatorin, (+)-marmesin, (+)-oxypeucedanin hydrate, (+)-peucedanol and three chromones, eugenin, (−)-hamaudol, (+)-visamminol, have been isolated from the root of Formosan *Peucedanum japonicum*. The structures of the new compounds were elucidated by spectral data. Among the isolates, seven compound, eugenin, (−)-selinidin, (+)-pteryxin, imperatorin, bergapten, cnidilin and (+)-visamminol, show strong antiplatelet aggregation activity *in vitro* (Chen *et al.,* 1996). Bioactivity-guided fractionation of a chloroform extract of the roots of *Peucedanum japonicum* afforded a new cytotoxic pyranocoumarin, (-)-cis-khellactone, and a known cytotoxic pyranocoumarin, (+)-cis-4'-acetyl-3'-angeloylkhellactone (Chang yih *et al*., 1992). Two new angular-type dihydropyranocoumarins, peujaponisinol A and B, were isolated from the roots of *Peucedanum japonicum* and the structures determined as 3'(S)-senecioyloxy-4'(S)-hydroxy-3', 4'-dihydroseselin and 3'(S)-hydroxy-4'(S)-senecioyloxy-3', 4'-dihydroseselin, respectively, by spectroscopic and chemical methods (Ikeshiro *et al.,* 1993). Four coumarins were isolated from chloroform extract of the root of *Peucedanum japonicum* and

identified as praeruptorin A (1), xanthotoxin (2), psoralen (3) and bergapten (4) on the basis of spectroscopic methods. The inhibitory activities of these coumarins on monoamine oxidase prepared by mouse brain were tested. The IC_{50} values of them were shown to be 27.4 μM (1), 40.7 μM (2), 35.8 μM (3), and 13.8 μM (4),in vitro (Huong *et al,* 1999). A new coumarin glycoside, peujaponiside [(*R*)-peucedanol 7-*O*-*β*-d-apiofuranosyl-(1→6)-*β*-d-glucopyranoside], was isolated from the roots of *Peucedanum japonicum*, and its structure was elucidated on the basis of spectroscopic and chemical methods (Ikeshiro *et al.*,1994).

***Peucedanum decursivum* (Miq.)**

Maxim (Zihuaqianhu in Chinese) is a Chinese medicinal plant. Coumarin compounds from the plant was isolated and purified using preparative high-speed counter-current chromatography (HSCCC) method. This was successfully achieved by using light petroleum–ethyl acetate–methanol–water (5:5:7:4, v/v) as the two-phase solvent system. Nodakenetin (2.8mg), 6.1mg of Pd-C-IV, 7.3mg of Pd-D-V, 4.7mg of ostruthin, 7.8mg of decursidin and 11.2mg of decursitin C with the purity of 88.3%, 98.0%, 94.2%, 97.1%, 97.8% and 98.4%, respectively, were separated in one-step from 150mg of crude sample of the plant. After purified by HSCCC again with light petroleum–ethyl acetate–methanol–water (5:5:4:5, v/v) as the two-phase solvent system, the purity of Nodakenetin reached 99.4%. The structures of all the compounds were identified by ^{1}H NMR and ^{13}C NMR (Liu *et al*., 2005).

Peucedanum ***arenarium var. arenarium***

Zheleva *et al*., (1976) reported that the roots of *Peucedanum arenarium var. arenarium* revealed the presence of 4 new natural coumarins. By spectral data their structures were elucidated as follows: peuarin, 3'-angeloyl-4'-methylisokhellactone; peuchlorin, 3'-angeloyl-4'-(2"-hydroxy-2"-methyl-3"-chloro)-butyroylisokhellactone; peuchlorinin, 3'-(2"-methylepoxy)-butyroyl-4'-(2"-hydroxy-2"-methyl-3"-chloro)-butyroylisokhellactone; peucloridin 3',4'-di-(2"-hydroxy-2"-methyl-3"-chloro)-butyroylisokhellactone. (Kuznetsova *et al.,* 1978) reported the presence of (-)-3'(R)-hydroxy-4'(S)-methoxy-3'4'-dihydroxanthyletin from the roots of *Peucedanum arenarium.*

Peucedanum ***oreoselinum*** **(L.)Moench**

A new diterpenoid has been isolated from roots of *Peucedanum oreoselinum*. Mainly by spectroscopic methods, its structure was shown to be (+)-(E)-7-hydroxymethyl-2,6,10,14-tetramethyl-2,9,13-pentadecatrien-6-ol, yet without specification of stereochemistry at C-6 and C-7. Furthermore the roots afforded a high yield of falcarindiol, (+)-(Z)-heptadeca-1,9-dien-4,6-diyn-3,8-diol (Lemmich, 1979). (Lemmich and Gylle, 1988) reported that a new dihydrofuranocoumarin has been isolated from the roots of *Peucedanum oreoselinum,* and the structure established as (8S,9R)-2'-angeloyloxy-9-isovaleryloxy-8,9-dihydrooroselol mainly on the basis of spectroscopic evidence.

Peucedanum ***terebinthaceum*** **Fischer et Turcz.**

Ganbaatar *et al*., (2008) submitted that a potentially medically valuable pyranocoumarin (+)-pteryxin was isolated for the first time from *Peucedanum*

terebinthaceum Fischer et Turcz. And the structure, rigorously proved to be (+)-pteryxin was achieved using mass spectrometry, NMR, IR, and UV spectroscopy.

***Peucedanum luxurians* Tamamsch**

From *Peucedanum luxurians*, the rare coumarins stenocarpin, stenocarpin isobutyrate, officinalin, officinalin isobutyrate, 8-methoxypeucedanin and the known xanthotoxin, isoimperatorin, bergaptene, peucedanin and cnidilin were isolated and identified by means of spectral data (Chinou *et al.*, 2007).

***Peucedanum paniculatum* L.,**

Is an endemic species to Corsica, Eight cyclolavandulyl esters (β-cyclolavandulyl and β-isocyclolavandulyl acetate, propionate, isobutyrate, isovalerate) were identified in the leaf and root oils of *Peucedanum paniculatum* L., by comparison of their spectroscopic data with those of synthetic materials. Their structures were elucidated by spectroscopic methods (Vellutuni *et al.*, 2005). Vellutini *et al.*, (2007) reported that from the dichloromethane extract of the roots of *Peucedanum paniculatum* L., two new coumarins, were isolated. Their structural elucidationed were acertained on the basis of spectroscopic studies, particularly 1D and 2D NMR spectroscopy, as 6-(7′-β-cyclolavandulyl)-7-hydroxycoumarin and a spirodihydrofurano-coumarin.

***Peucedanum nebrodense* (Guss.) Strohl.**

Acetone extract of *Peucedanum nebrodense* (Guss.) Strohl., a rare endemic species from the Madonie mountains (Sicily), was tested in vitro for its antimicrobial activity against bacterial reference strains and antiproliferative activity against K562 (human chronic myelogenous leukemia), HL-60 (human leukemia) and L1210 (murine leukemia) cell lines. The acetone extract showed antiproliferative IC_{50} values in the range of 14–0.27μg/ml (Schillaci *et al.*, 2003).

Peucedanum ***ledebourielloides* K.F.Fu**

Two cancer cell growth inhibitory esters, 1,2-dipalmitoyl-3-glucosyl glycerol and 1,6-dihydroxy-hexane-bis-palmitoyl ester, together with arachidic acid-2-hydroxy-glycerol ester, daucosterol, and oleanolic acid, were isolated from the roots of *Peucedanum ledebourielloides*. The structures were determined by spectroscopic analysis. The esters displayed significant activity against the SGC-7901, HT-29, and HL-60 cancer cell lines (Zheng *et al.*, 2010).

***Peucedanum alsaticum* L.**

The hexane of the fruits extracts of *Peucedanum cervaria* and *P. alsaticum* were examined for antimicrobial activity and analyzed for their fatty acid contents against twelve reference bacterial and yeast strains. The result showed that fourteen fatty acids were

identified among which Oleic and linoleic acids were found to be dominant. The extracts from both plants examined exhibited inhibitory effects against *Gram*-positive strains tested with different *MIC* values (0.25–2 mg/ml); however, extract from *P. alsaticum* possessed stronger antibacterial properties and a broader spectrum. The growth of *Gram*-negative bacteria and *Candida* spp. strains was not inhibited even at the highest extract concentration used (*MIC*>4 mg/ml). Standard fatty acids exhibited inhibitory effects towards all bacterial and yeast strains used in the study; however, the majority of bacteria were more sensitive to linoleic than to oleic acid. The research concluded that the results revealed, for the first time, that hexane extracts obtained from fruits of *P. alsaticum* and *P. Cervaria,* possess moderate *in vitro* antibacterial activity against *Gram*-positive bacteria including staphylococci. Linoleic and oleic acids appear to be the compounds responsible for this effect, and a synergistic antimicrobial effect between these two fatty acids was indicated (Skalicka-Woźniak *et al,* 2010).

From the fruits *Peucedanum alasticum*, by means of preparative high-speed counter-current chromatography (HSCCC) method using a two phase solvent system composed of n-hexane-ethyl acetate-methanol-water in the ratio (1:1:1:1), (Skalicka-Woźniak *et al*., 2009) reported that its dichloromethane extract afforded two new compounds – alsaticol and alsaticocoumarin A. The identification of the compounds were achieved by means of NMR and MS methods. They concluded that HSCCC offers a rapid method of obtaining new natural compounds. Similarly, (Hadacek *et al.*, 1987) reported that the lipophilic root extract of *Peucedanum alsaticum* afforded four highly unstable butenolides which could be separated by HPLC. Whereas the stereochemistry of three olefinic derivatives, previously isolated only as their methyl ethers, were now established

by ^{1}H NMR, the structure of a new acetylenic lactone was additionally confirmed by ^{13}C NMR. They concluded that the compounds were probably formed by condensation of unsaturated C_{18}-acids with pyruvate. (Skalicka-Woźniak *et al.,* 2012) reported that a high-performance counter-current chromatography (HPCCC) method was applied for the first time for the preparative separation and purification of three rare compounds which occur as minor constituents in the fruits of *Peucedanum alsaticum* L. Using 1g of the crude extract in a single run: 5-substituted coumarin notoptol and two dihydropyranochromones: divaricatol and ledebouriellol using a two-phase solvent system composed of *n*-hexane/ethyl acetate/methanol/water (1:1:1:1) was developed. The components purified and collected were analyzed by high-performance liquid chromatography. The result showed that the method yielded 0.7 mg of notoptol, 1.46 mg of ledebouriellol at purity of 99.5%, and 10 mg of mixtures of divaricatol, alsaticol and alsaticocoumarin, where divaricatol present 22% by peak area. They concluded that it was the first time that minor notoptol, ledebouriellol, and divaricatol were isolated in a single run using HPCCC method and the first time the compounds were identified in *Peucedanum* genus.

Yan *et al.*, (2008) isolated fifteen compounds and identified as Umbelliferone, Coumurayin, Mexoticin, Marmesin, Ammijin, Delton, Selinidin, Anomalin, Isopteryxin, Ferulic acid, Falcarindiol, Stearic acid, beta-sitosterol, Daucosterol and d-Mannitol *Peucedanum delavayi* by manifold chromato-graphy methods, and their structures were determined by spectral analysis. All these compounds were isolated from the plant for the first time.

***Peucedanum officinale* L**

The volatile constituents of *Peucedanum officinale* L were isolated by hydrodistillation and the essential oil were analyzed by GC and GC/MS. Thirty-eight compounds representing 98.7% of the oil in leaf were identified, of which fenchone (27.7%), (E)-β-ocimene (18.7%) and β-pinene (8.1%) were major. Twenty-two compounds representing 99.7% of the oil in the seeds were identified, of which fenchone (32%), (E)-β-ocimene (17.8%), and (Z)-β-ocimene (9.4%) were major (Jaimand *et al.*, 2006).

Peucedanum arenarium Waldst. & Kit., *P. austriacum* (Jacq.) Koch, *P. coriaceum Reichenb*., *P. longifolium* Waldst. & Kit, *P. officinale* L., *P. oreoselinum* (L.) Moench, *P. ostruthium* L., and *P. palustre* (L.) Moench accumulate different structural types of coumarins including simple coumarins, linear furanocoumarins, linear dihydropyranocoumarins, angular dihydrofuranocoumarins and angular dihydropyranocoumarins. (Hadacek *et al,* 1994) conducted a bioasay screening on the diverse coumarins from *Peucedanum* for insecticidal activity using Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) as test organism. Result indicated the majority of the linear furanocoumarins and the angular dihydrofuranocoumarin athamantin as active compounds. Oxygenation of the prenyl residue of linear furanocoumarins decreased activity. Further formation of an ester with angelic acid even resulted in complete inactivity. Similarly, (Hadacek *et al.*, 1994) compared five active linear furanocoumarins, bergapten, isopimpinellin, xanthotoxin, isoimperatorin, and imperatorin, and two linear furanocoumarins with a substituted furan ring, peucedanin and 8-methoxypeucedanin, in a dietary utilization bioassay. Relative growth rate (RGR) and relative consumption rate

(RCR) divided the tested coumarins in three groups of similar activity. Isopimpinellin and peucedanin slightly decreased RGR and RCR of the treated larvae, and xanthotoxin, isoimperatorin, and 8-methoxypeucedanin heavily decreased RGR and RCR. Bergapten and imperatorin differed by the lowest RGR values and rather high RCR values. The effects caused by these two coumarins indicate specific postingestive toxicity.They concluded that the results obtained from their study had added to the reputation of coumarins to be an effective chemical defense, postulating that chemical diversity is a necessary trait for well-defended plants.

2.4 *Peucedanum winkleri* H. Wolff species and its medicinal effects

Peucedanum winkleri, is one of the plants that are extremely used as a herbal preparation in some parts of Nigeria, Cameroun, East Africa (Gabon, Rwanda, Tanzania, Kenya, Uganda), Asia (China) and Europe. The plant is found to be of medicinal importance among traditional medicine practitioners in these localities. The plant is called (Ochochoyo) in Idoma language and (Reken giwa) in Hausa language of Nigeria. It is found useful in the treatment of respiratory diseases, pneumonia, meningitis, ostoemyelitis, arthritis, high fever, typhoid fever, unirary tract infection, thrush, abdominal pain and diarrhoea and mastitis in diary cows. It is equally used as an analgesic.

On a wider dimension, the organisms that causes these problems to the patients are the target of this research. In this circumstances, they are: *Staphylococus aureus, Methicillin Resistance Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae,Ccandida albicans, Candida tropicalis* and *Proteus mirabilis.* These are organisms that causes huge damage to body tissues. Researches are continously

going on how to handle these microbes effectively due to their devastating and persistent nature.

To the best of our knowledge and literature available, their is no phytochemical, antimicrobial screeninig report or compound isolated and characterized from this species.

2.5 Bacterial agents

Salmonella (Gram-negative)

Salmonella belongs to the the Enterobacteriaceae. It is an anaerobic gram-negative bacillus which is able to grow on wide range of relatively simple media. Their normal habitat is the animal intestine. They can be differenciated from other members by their biochemical characteristics and antigenic structure (Greenwood *et al.,* 1992). *Salmonella* are widely distributed in nature. All vertebrates appear capable of harboring *Salmonella* in their gut. It has also been isolated from a wide range of arthropods such as flies, cockroaches, fleas and ticks. *Salmonella typhi* and *Salmonella paratyphi* A, B and C are primarily human pathogens which are rarely isolated from animals except human beings. *Salmonella paratyphi*, which is essencially a human pathogen, is occationally isolated from cattle, pigs and other poultry animals. The cycle of transmission in this host has not been fully demostrated (Christie, 1987). Humans infected by these organisms are characterized by a long incubation period of 10-14 days, followed by a septicaemic illness and an enteric fever. The ability of Salmonella entering and surviving within macrophages and other cells particularly in the liver and kidney occasionally lead to persistent infection and the chronic carear state where sufferers from *Salmonellae* infection continues to excrete the organism in their stool for days or weeks after complete clinical recovery.

In the treatment and clinical management of enteric fever due to *Salmonellae,* the problem of bone marrow toxicity and widespread emergence of chloramphenicol resistance in *Salmonella typhi* in many parts of the world prompted the search for alternative drugs and among these are ampicillin and cotrimoxazole and they may have shown comparability in efficacy to chloramphenicol (Greenwood *et al.*, 1992).

***Escherichia coli* (Gram-negative)**

The specie *Escherichia coli* encompasses a great variety of strains that include purely commensal organisms as well as those possessing combinations of virulence determinant which enable them to act as specific pathogens of the gut and of extra intestinal sites especially the urinary tract.

Escherichia coli are a widespread intestinal parasite of mammals and birds. It is always present whenever there is fecal contamination but it does not lead an independent existence outside the animal body. Some strains are pathogens in man and animals and cause some septic infection and diarrhoea. It is the most common type of acute urinary tract infection. *Esteerichia coli* also cause neonatal meningitis and septicemia as wll as sepsis in operation wounds and abscesses in a variety of organs (Greenwood *et al.,* 1992).

Escherichia coli that causes urinary tract infection often originates in the gut of the patient. It is naturally sensitive to many antibiotics, although moderately resistant to benzyl penicillin. It is sensitive to ampicillin, tetracycline, chloramphenicol, streptomycin, gentimicine, sulphonamides etc. Many strains however have acquired plasmids conferring resistance to one or more of these drugs. Extra intestinal *E. Coli* infections are treated with

spercific antimicrobial therapy which preferably requires the results of laboratory test for sensitivity guide.

***Klebsiella* (Gram-negative)**

The genus *Klebsiella* tend to be slightly shorter and thicker than other enterobacteria. It is a straight rod of about 1-2micro m long and 0.5-0.8 micro m wide. The capsular material is produced in greater amounts in media rich in carbohydrates. A useful characteristic of all strains of *klebsiella pneumonia* species, aerogenes and pneumoniae is the ability to form gas within four (4) days from starch, which practically no other member of the Enterobacteriaceae is capable of doing.

Clinical isolates of *Klebsiella* are generally resistant to a wider range of antibiotics than most *E. coli* strains. They are nearly always naturally resistant to ampicillin and amoxicillin but usually sensitive to gentamicin and cephalosporins. Resistance to chloramphenicol and tetracycline varies from strain to strain. *Klebsiella* are fairly common cause of urinary tract infections and occationally give rise to bronchopneumonia. *Klebsiella* infection of the urine often responds to trimethoprinn, nitrofurantoin, co-amoxiclav or oral cephalosporins. Pneumonia and other serious infections require vigorous treatment with aminoglycoside or a cephalosporin as cefotaxime (Greenwood *et al*., 1992).

***Staphylococci* (Gram-positive)**

There are four types of *Staphyloccoci;*

Penicillin-sensitive strains

These are almost sensitive to all anti *Staphyloccoci* antibiotics like penincillin, erythromycin, lincosamides and fusidic acid.

B-Lactamase (penicillinase) producing strains

These are resistant to all methicillin, flucloxacllin and nafcillin. Most strains of *Staphyloccocus aureus* isolated from patients belongs to this group (Eastman and Adams, 1983).

Antibiotic resistant strain

All these strains produce B-lactamase which are additonally resistant to one or more of the anti-staphylococcal antibiotics, most frequently to tetracycline and erythromycin, less commonly to lincosamides, fusidic acid and amino glycosides. Resistant strains of this kind are in a minority and are almost always isolated from hospital contracted infections.

Methicillin-resistant strains

Although these strains are usually detected in test of methicillin, they are also resistant to flucloxacillin and virtually all β-lactam agents and are believed to have epidemiological significance as a group. They require treatment with vancomycin or teicoplanin (Greenwood *et al.,* 1992).

2.6 Phytochemical screening

This involves the grouping of various constituents of the plants based on the various functional groups present. Because of the heterogeneous chemical nature of plants constituents, most of them cannot be identified in plant extract using a single method. A standard method described by Sofowora (1993) is a general phytochemical screening method to detect the presence or absence of various classes of natural produts e.g.

saponins, flavanoids, alkaloids, glycosides, tannins etc. Equally, other spercific test may be carried out to detect the presence or absence of a suspected group of compounds.

2.7 Isolation of Constituents

The separation and purification of plant constituents is mainly carried out using one or a combination of chromatographic techniques i.e, Thin layer chromatography (TLC), column chromatography (CC), high performance liquid chromatography (HPLC), vacuum liquid chromatography (VLC) and gas chromatography (GC). The choice of a particular technique depends greatly on the solubility properties and volatility of the compounds to be saperated (Harborne, 1973).

Vacuum liquid chromatography is a recently improved technique that is extensively used to separate the constituents of plant into its various components. This provides rapid method of separation and purification of plant extracts for further investigation of the isolated compound.

2.8 Identification and Charaterization of Isolated Constituent

Once a plant constituent has been isolated and purified, it is then important to first determine the class of compound and then find out which particular substance it is within thst class.

Complete identification within the class depends on measuring other properties and comparing with those in literature, i.e, melting point, boiling point, optical rotation (for optically active compounds) and the use of spectroscopic techniques such as ultraviolet, infrared, nuclear magnetic resonance, mass spectroscopy and x-ray diffraction make

characterization of organic molecules from plant extracts a lot easier (Amako, 1999). A known plant extract can usually be identified on the above basis. Again, direct comparison with authentic material (if available), should be carried out as final confirmation. If an authentic material is not available, careful comparison with literature data may be good enough for its identification. If the compound is a new one, all the above data should be sufficient to characterize it. Although, it is always preferable to confirm the identification of such new compounds through chemical degradation and laboratory synthesis (Isah, 2007).

2.9 Biological Screening

An isolated and charecterized compound, which has no benefit to mankind, results in waste of time and resources. A new trend has resulted where bioassay-guided screening and purification of plant extracts ensures that the isolated compound will have biological activity and therefore stand a good drug discevery through subsequent structure-related-activity studies (Sofowora, 1993). Such bioassay test, includes: pharmacological screening, toxicity test and antimicrobial screening test.

2.9.1 Antimicrobial Screening

In determining the potency of plant extracts against microorganisms, this method ranked the highest and the best among the biological tests for bioactivity. Antimicrobial screening can be said to be a measure of the response of growing population of microorganism to antimicrobial agent (Hugo and Rusell, 1993). One of these techniques is the agar diffusion assay method in wich the samples under test are applied into some form

of well (reservoir) in a thin-layer of agar seeded with indicator organism. The drug is allowed to diffuse into the the medium and after incubation, a zone of growth inhibition forms as a circle around the reservoir i.e, in the case where the drug contains active principle, which inhibits the growth of indicator organism. The diameter of zone of inhibition is measured and is related to the concentration and activity of the antimicrobial agent in the reservoir.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials used for extraction and phytochemical test

Materials:

Soxhlet extractor

Round bottom flask (1000 cm^3)

Rotavapor

Vacuum pump

Heating mantles

Steam bath

3.1.1 Reagents used for extraction

Petroleum-ether 60-80^{0}C

Chloroform

Ethyl acetate

Methanol.

All solvents used are analar gtade by Sigma Aldrich and were all distlled before use.

3.1.2 Reagents used for phytochemical test

90% Ethanol.

10% NaOH.

10% Hydrochloric acid.

Concentrated Hydrochloric acid.

Chloroform.

Dilute Sulphuric acid.

Concentrated Sulphuric acid.

5% H_2SO_4 in 5% Ethanol.

5% Acetone.

28% Ammonia solution.

Acetic anhydride ($C_4H_6O_3$).

Lead sub-acetate solution.

Gelatine.

Sodium picrite paper.

Magnesium metal.

Molisch's reagent (solution of alpha-Naphthol 10% in alcohol).

Mayer's reagent.

Fehling's solution.

Ammonical Silver Nitrate Solution: Silver nitrate (3.0 g) was dissolved in 50ml of distilled water and ammonia solution was added drop wise until the initial precipitate of silver oxide dissolved. This resulting solution was made up to 100ml in a volumetric flask.

Molisch Reagent: 10% of alpha-naphthol solution in alcohol.

Dragendorff's Reagent: Bismuth nitrate (0.85 g) was dissolved in 10ml of acetic acid and 40ml distilled water was added to give a stock solution (A). Potasium

iodide (8.0 g) was equally dissolved in 20 ml of distilled water to give a stock solution (B). 5 ml of each of the stock solution (A and B) were mixed with 20 ml of 33% acetic acid followed by 100ml distilled water to give the Dragendorff's reagent.

Mayer's Reagent: Mercuric chloride (1.36 g) was dissolved in 60ml of distilled water to give solution (A). Potasium iodide (5.0 g) was dissolved in to give solution B. Both solutions A and B were mixed together and made up to 100ml mark.

Fehling's Solution: Solution A was made by dissolving copper sulphate (7.0 g) in 0.1ml of sulphuric acid with sufficient distilled water and made up to 100ml mark. Solution B was made by dissolving of sodium potasium tartrate (35.2 g) and 15.4 g of sodium hydroxide in sufficient distilled water and made up to 100ml mark. Equal volumes of solutions A and B were mixed before use.

Iodine Solution: Iodine crystals (2.5 g) were added to 5.0 g of potasium iodide and 25 ml of distilled water was added to the mixture to give iodine solution.

3.2 Materials used for Anti-microbial test

Petri-dishes (150 mm by 15 mm)

Cotton wool

Bunsen burner

Distilled water

Incubator

Syringe (2 cm^3 and 5 cm^3) and needles

Test tubes

Pipettes

Autoclave

Sterile pipettes

Disinfectant

DMSO (10%)

3.2.1 Micro organisms for Anti-microbial test

Staphylococcus aureus

Methicillin Resistant S. aureus

Streptococcus pyogenes

Corynebacterium ulcerans

Escherichia coli

Salmonella typhi

Proteus mirabilis

Pseudomonas aeruginosa

Klebsiella pneumoniae

Candida albicans

Candida krusei

Candida tropicalis

3.3 Materials for Vacuum liquid chromatography (VLC)

Vacuum pump

Pressure flask

Cintered funnel of porousity number 3

Silica gel

Salite

Beakers

Measuring cylinder

Micro pipette

3.3.1 Materials for Thin layer chromatography (TLC)

Chromatographic Tank

(20 by 20 cm) transparent glass plates

Gallenklamp Spreader

Ultraviolet lamp (366nm)

Silica gel as stationary phase

3.3.2 Instrumentation and general techniques

Gas Chromatography-Mass Spectroscopy (GC-MS)

Agilent Gas Chromatogram (6890N model) coupled to 5973N Mass Selective Detector was used.

Fourier transform Infra-Red Spectroscopy

Spectra were obtained on a SHIMADZU FTIR spectrophotometer 8400S series.

Thin-Layer Chromatography (TLC)

This was done using silica gel for TLC supplied by BDH and also by the use of commercially prepared one.

Nuclear magnetic resonance (NMR)

Bruker Avance 400 MHz tunned to 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR was used.

3.4 Sample collection, Identification and preparation

The leaves of the plant were collected fresh from Shika village Zaria, Kaduna State, Nigeria in November 2011. It was identified by Mallam Musa Galah of the Herbarium unit of Biological Sciences Department, Faculty of Science, Ahmadu Bello University Zaria Nigeria. A voucher specimen with number 900062 was deposited in the Herbarium. The plant material was air dried, pulverized using a wooden morter and pestle and stored in polythene bags until needed for use.

3.5 Extraction of plant material

The pulverized leaves of the plant (300 g) was weighed and packed into a soxhlet extractor. Methanol was used to extract the plant material exhaustively. Concentration of the extract was done *in vacuo* using rotary evaporator to give fairly dried crude extract. Extract was further allowed to dry in air until a constant weight was achieved. The dried extract was then partitioned using: petroleum ether 60-80^{0}C, chloroform and ethylacetate exhaustively and respectively. Their respective extracts were concentrated *in vacou* using rotary evaporator and then dried in air until a constant masses were achieved and also

residual mass for the methanol. The crude exracts were then subjected to antimicrobial screening.

3.6 Phytochemical Screening of plant material

This was carried out on the leaves of the plant using the standard technique described by Trease and Evans, 1989; Sofowora, 1993.

3.6.1 Test for Carbohydrates

Molsch's test

Few drops of Molisch reagent reagent was added to a little quantity of extract in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the test tube. A lower purple to violet colour was formed at the interface.

Fehling's test

To 2 ml of extract, 5 ml of a mixture of fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for few minutes. A brick red precipitate was observed.

3.6.2 Test for Cardiac Glycosides

Kella-Killiani test

Extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degree. 1 ml of concentrated sulphuric acid was added down the side. A purple ring colour was observed at the interface.

Kedde's test

1ml of 2% 3,5-dinitrobenzoic acid in 95% alcohol was added to extract. The solution was made alkaline with 5% sodium hydroxide. This gave a brownish colour.

Salkwoski's test

The extract (0.5 g) was dissolved in 2 ml of chloroform and few drops of concentrated H_2SO_4 were added to form a lower layer. A reddish brown colour was observed at the interface.

3.6.3 Test for Anthraquinones Derivatives

Tesst for free Anthraquinones (Borntrager's test)

Small portion of the extract was shook with 10 ml of benzene and filtered. 5ml of 10% of ammonia solution was added to the filtrate and stirred. A yellow colour was observed.

Test for combined Anthracene (modified Borntrager's test)

Sample was boiled with 5 ml of 10% hydrochloric acid for 3 mins. This was hydrolysed by the glycosides to yield aglycone which are soluble in hot water only. The solution was filtered hot, the filtrate was cooled and extracted with 5ml benzene. The benzene layer was filtered off and shaken gently with half its volume of 10% ammonia solution. A yellow colour was observed.

3.6.4 Test for Saponins

Frothing test

Small quantity of the extract was dissolved in 10 ml of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb which endured for a while was observed.

3.6.5 Test for steroids and tritenpenes (Lieberman-Burchards test)

Equal volume of acetic anhydride was added to the extract. 1ml of concentrated sulphuric acid was added the down side the test tube. Red colour was observed immediately and later.blue-green colour was formed.

3.6.6 Test for Flavanoids

Shinoda test

About 0.5 g of extract was dissolved in 1-2 ml of 50% methanol in the heat. Methalic magnesium and four or five drops of conc HCl were added. A red colour was observed.

Sodium hydroxide test

Few drops of aqueous NaOH was added to 5ml of extract, a yellow colour was observed.

3.6.7 Test for Tannins

Lead sub-acetate solution was added to a solution of the extract. A yellow coloured precipitate was observed.

Ferric chloride test

About 0.5 ml of extract was dissolved in 10 ml of distilled water, then filtered. Few drops of ferric chloride solution was added to the filtrate. Green precipitate was observed.

3.6.8 Test for Alkaloids

Mayer's test

Few drops of Mayer's reagent was added to sample of the extract in a test tube. Cream colour precipitate was observed.

Dragendorff's test

Few drops of this reagent was added to the extract. A red precipitate was observed.

3.7 Anti-microbial screening of crude methanol extract

The antimicrobial activities of this extract was determined using some human pathogens which were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. All the isolates were checked for purity and maintained in slants of nutrient agar for the bacteria and in slants of Sabouraud dextrose agar for the fungi.

The extract (0.5 g) was weighed and dissolved in 10 ml of DMSO to obtain a concentration of 50 mg/ml of the extract. This was the initial concentration of the extract used to check the antimicrobial activites of the plant.

Mueller Hinton agar was the medium used as the growth medium for the test microbes. The medium was prepared according to the manufacturer's instruction, sterillized at 121^0 C for 15 mins, poured into sterile petri dishes and was allowed to cool

and solidify. Agar-well diffusion method was used for the antimicrobial screening of the extract.

The sterilized medium was then seeded with 0.1 ml of the standard inocullum of the test microbes, the inocullum was spread evenly over the surface of the medium by the use of sterile swab. By the use of a standard cork borer of 6mm in diameter, a well was cut at the centre of each inoculated medium.

The solution of the extract (0.1 ml) was then introduced into each well on the inoculated medium. The inoculated medium was incubated at 37^0C for 24 hours after which, each plate was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result was recorded in millimetres. The process was repeated for each of the crude extracts.

3.7.1 Minimun inhibition concentration of crude methanol extract

The minimun inhibition concentration of the crude methanol extract was carried out using the broth diffusion method. Mueller Hinton broth was prepared according to the manunfacturare's instructions. 10mls was dispensed into test tube and was sterillized at 121^0C for 15 mins. The broth was allowed to cool.

MC-Farland's standard turbidity scale number 0.5 was prepared to give turbid solution.

Normal saline was prepared, 10mls was dispensed into sterile test tube and the test tube microbe was inoculated and incubated at 37^0C for 6 hours.

Dilution of the test microbe in the normal saline was done until the turbidity marched that of MC-Farland's scale by visual comparison, at that point the test microbe had a concentration of about 1.5×10^8 cfu/ml.

Two fold serial dilution of the extract in the broth was made to obtain the respective concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml for the crude extract of methanol.

Having obtained different concentrations of the extracts in the sterile broth, 0.1ml of the standard inoculum of the test microbe in the normal saline was then inoculated into the different concentrations of the extract in the broth. Incubation was made at 37^0C and left for 24 hours after which, each test tube was observed for turbidity or growth. The lowest concentration of the extract in the broth, which showed no turbidity was recorded as the MIC. The process was repeated for each crude extract of pet-ether, chloroform and ethyl acetate respectively.

3.7.2 MBC/MFC of crude methanol extract

MBC/MFC were carried out to check whether the test microbes could be killed or only their growth were inhibited.

Mueller Hinton ager was prepared and sterillized at 121^0C for 15mins. The medium was poured into sterile petridishes and was allowed to cool and solidify.

The contents of the MIC in the serile dilutions were then subcultured onto the prepared medium. Incubation was made at 37^0C for 24 hours after which each plate was observed for colony growth, the MBC/MFC was the plate with lowest concentration of the extract without a colony growth. The process was repeated for each crude extract.

3.8 Vacuum liquid chromatography (VLC)

This was performed on the ethyl acetate extract of the leaves which showed the highest antimicrobial values of the plant to separate the components in the extract according to their similarity in polarity and rf values using first, 20ml combinations of petroleum ether and ethyl acetate solvents in gradient ratio of 100% : 0% intially to 0% : 100% in order of increasing polarity. Then, 20 ml combination of chloroform and methanol solvents in gradient ratio of 100% : 0% intially to 80% : 20% also, in order of increasing polarity. For each mixture of solvent, fractions corresponding to that was collected in a flask and labelled.

3.9 Thin layer chromatography (TLC)

This was done on the VLC fractions collected to ascertain the number of components present in each fraction and also to combine VLC fractions that has similar components in them based on their similarity in rf value and colour they show under the UV-lamp. TLC was done using commercially pre-coated silica gel plate prepared by MERCK, Germany. The VLC fractions were respectively dissolved in minimal amount of ethyl acetate and they were spotted at the base of the plate cut to 5 by 5 cm and developed using combinations of petroleum ether and ethyl acetate solvents in the ratio of 1 : 1 in a chromatographic tank for 4 minutes. The plate was then observed under the UV-lamp at 366nm for the components thus separated. These samples were further purified using PTLC and 10 mg of a brown solid was obtained. It melting point was determined to be 140 – 145 ^{0}C.

3.10 Spectroscopic measurements

The FT-IR spectra of the isolated component was obtained using SHIMADZU FTIR – 8400S in chloroform and recorded in cm^{-1}. The carbon-13 (^{13}C) and proton-NMR (^{1}H) spectra analysis of the pure component were recorded on a MHz spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as internal reference. All chemical shifts (δ) were reported in ppm from internal TMS. GC-MS was carried out on the isolated compound.

CHAPTER FOUR

4.0 RESULTS/DATA PRESENTATION AND ANALYSIS

Table 4.1. Phytochemical screening on the leaves of *Peucedanum winkleri.* H. Wolff

Property Tested	Leaves of *P. winkleri*
Carbohydrates	+
Free Reducing Sugar	+
Cardiac glycosides	+
Saponins	+
Steroids	+
Flavanoids	+
Alkaloids	+
Anthracene	-
Tannins	+
Triterpenes	+

Key : + = Present - = Absent

Table 4.2. Amount of extracts from 300 g leaves of *Peucedanum winkleri.* H. Wolff by total extraction with methanol followed by partitioning with solvents in increasing order of polarity.

Solvent Medium	Weight of Extract (g)	Percentage recovery (%)
Petroleum-ether (60 - 80^0C)	4.30	10.29
Chloroform	6.50	15.55
Ethyl acetate	4.20	10.05
Methanol	26.80	64.11

Table 4.3. Antimicrobial activity sensitivity test of petroleum ether (EB), chloroform (AT), ethyl acetate (CN) and methanol (SZ) extract

Test organism	EB	AT	CN	SZ
Staphyloccocus aureus	S	S	S	S
Methicillin resistance S. aureus	S	S	S	S
Streptococcus pyogenes	R	R	R	R
Corynebacterium ulcerans	R	R	R	R
Escherichia coli	S	S	S	S
Salmonella typhi	S	S	S	S
Proteus mirabilis	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R
Klebsiella pneumoniae	S	S	S	S
Candida albicans	S	S	S	S
Candida krusei	R	R	R	R
C. tropicalis	S	S	S	S

Key : S = Sensitive, R = Resistant

Table 4.4. Zone of inhibition of the extract against the test micoorganism (mm)

Test organism	EB (0.5mg/mol)	AT (0.5mg/mol)	CN (0.5mg/mol)	SZ (0.5mg/mol)
Staphyloccocus aureus	21	20	24	21
MRSA	21	20	27	21
Streptococcus pyogenes	0	0	0	0
Corynebacterium ulcerans	0	0	0	0
Escherichia coli	26	25	29	25
Salmonella typhi	24	23	26	25
Proteus mirabilis	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0
Klebsiella pneumoniae	25	25	29	27
Candida albicans	22	20	24	24
Candida krusei	0	0	0	0
Candida tropicalis	21	20	23	21

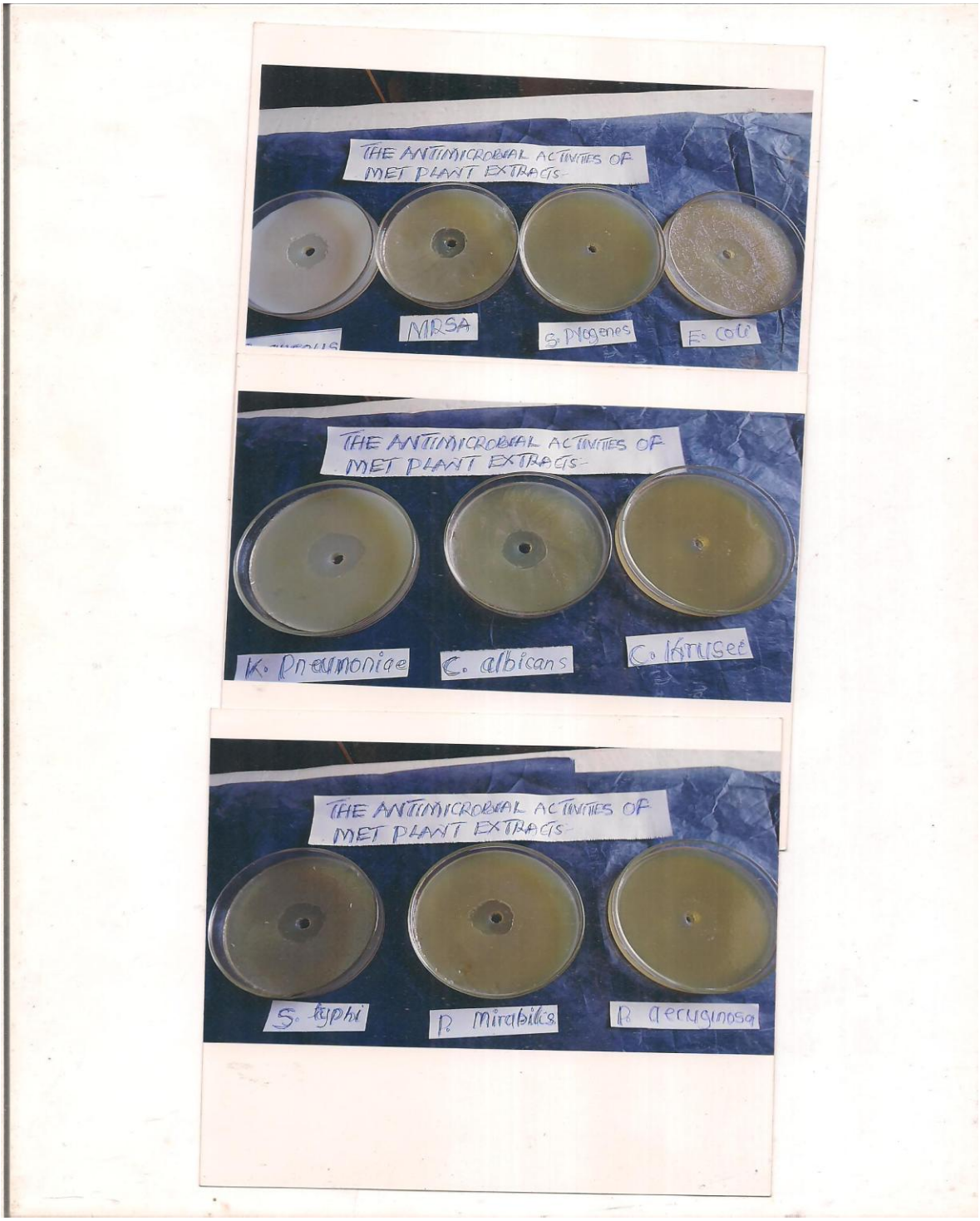

THE ANTIMICROBIAL ACTIVITIES OF MET PLANT EXTRACTS
MRSA
S. Pyogenes
E. coli
THE ANTIMICROBIAL ACTIVITIES OF MET PLANT EXTRACTS
K. Pneumoniae
C. albicans
C. Krusei
THE ANTIMICROBIAL ACTIVITIES OF MET PLANT EXTRACTS
S. typhi
P. Mirabilis
P. aeruginosa

Plate I

Table 4.5. Minimum inhibition conc (MIC) the EB extract

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Staphyloccocus aureus	-	-	0*	+	++
Methicillin resistance S. aureus	-	-	0*	+	++
Escherichia coli	-	-	-	0*	+
Salmmonella typhi	-	-	0*	+	++
Klebsiella pneumoniae	-	-	-	0*	+
Candida albicans	-	-	0*	+	++
Candida tropicalis	-	-	0*	+	++

Key : - No tubidity (No growth), 0* MIC, + Turbidity (Light growth), ++ Moderate turbidity

Table 4.6. Minimum inhibition conc (MIC) the AT extract

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Staphyloccocus aureus	-	-	0*	+	++
Methicillin resistance S. aureus	-	-	0*	+	++
Escherichia coli	-	-	-	0*	+
Salmmonella typhi	-	-	0*	+	++
Klebsiella pneumoniae	-	-	-	0*	+
Candida albicans	-	-	0*	+	++
Candida tropicalis	-	-	0*	+	++

Key : - No tubidity (No growth), 0* MIC, + Turbidity (Light growth), ++ Moderate turbidity.

Table 4.7. Minimum inhibition conc (MIC) the CN extract

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Staphyloccocus aureus	-	-	0*	+	++
Methicillin resistance S. aureus	-	-	-	0*	+
Escherichia coli	-	-	-	0*	+
Salmmonella typhi	-	-	-	0*	+
Klebsiella pneumoniae	-	-	-	0*	+
Candida albicans	-	-	0*	+	++
Candida tropicalis	-	-	0*	+	++

Key : - No tubidity (No growth), 0* MIC, + Turbidity (Light growth), ++ Moderate turbidity.

Table 4.8. Minimum inhibition conc (MIC) of the SZ extract

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
Staphyloccocus aureus	-	-	0*	+	++
Methicillin resistance S. aureus	-	-	0*	+	++
Escherichia coli	-	-	0*	+	++
Salmmonella typhi	-	-	-	0*	+
Klebsiella pneumoniae	-	-	-	0*	+
Candida albicans	-	-	0*	+	++
Candida tropicalis	-	-	0*	+	++

Key : - No tubidity (No growth), 0* MIC, + Turbidity (Light growth), ++ Moderate turbidity.

Table 4.9. MBC and MFC for EB extract

Test organism	MBC/MFC in mg/ml				
	50	25	12.5	6.25	3.125
Staphyloccocus aureus	0*	+	++	+++	++++
Methicillin resistance S. aureus	0*	+	++	+++	++++
Escherichia coli	-	0*	+	++	+++
Salmonella typhi	-	0*	+	++	++++
Klebsiella pneumoniae	-	0*	+	++	+++
Candida albicans	0*	+	++	+++	++++
Candida tropicalis	0*	+	++	+++	++++

Key : - = No colony growth, 0* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

Table 4.10. MBC and MFC for AT extract

Test organism	MBC/MFC in mg/ml				
	50	25	12.5	6.25	3.125
Staphyloccocus aureus	0*	+	++	+++	++++
Methicillin resistance S. aureus	-	0*	+	++	+++
Escherichia coli	-	0*	+	++	+++
Salmonella typhi	0*	+	++	+++	++++
Klebsiella pneumoniae	-	-	0*	+	++
Candida albicans	0*	+	++	+++	++++
Candida tropicalis	0*	+	++	+++	++++

Key : - = No colony growth, 0* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

Table 4.11. MBC and MFC for CN extract

Test organism	MBC/MFC in mg/ml				
	50	25	12.5	6.25	3.125
Staphyloccocus aureus	-	0*	+	++	++++
Methicillin resistance S. aureus	-	0*	+	++	+++
Escherichia coli	-	-	0*	+	++
Salmonella typhi	-	0*	+	++	+++
Klebsiella pneumoniae	-	-	0*	+	++
Candida albicans	-	0*	+	++	+++
Candida tropicalis	-	0*	+	++	+++

Key : - = No colony growth, 0* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

Table 4.12. MBC and MFC for SZ extract

Test organism	MBC/MFC in mg/ml				
	50	25	12.5	6.25	3.125
Staphyloccocus aureus	0*	+	++	+++	++++
Methicillin resistance S. aureus	0*	+	++	+++	++++
Escherichia coli	-	0*	+	++	+++
Salmonella typhi	-	0*	+	++	+++
Klebsiella pneumoniae	-	0*	+	++	+++
Candida albicans	0*	+	++	+++	++++
Candida tropicalis	0*	+	++	+++	++++

Key : - = No colony growth, 0* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

Table 4.13. Antimicrobial activity sensitivity test of standard drugs and pure component isolaletd on the test microbes

Test organism	Cefuroxine	Sparfloxacin	Erythromycin	Fluconazole	MK2
Staphyloccocus aureus	S	S	R	R	S
Methicillin resistance S. aureus	S	S	R	R	S
Streptococcus pyogenes	S	S	S	R	R
Escherichia coli	S	S	R	R	S
Salmonella typhi	R	S	R	R	S
Pseudomonas aeruginosa	R	S	S	R	S
Proteus mirabilis	R	S	S	R	R
Klebsiella pneumoniae	S	S	S	R	S
Candida albicans	R	R	R	S	S
Candida krusei	R	R	R	S	R

Key : S = Sensitive, R = Resistant, MK2 = pure component from ethyl acetate extract

Table 4.14. Zone of inhibition of the standard drugs against the test micro organism

Test organism	Cefuroxine	Sparfloxacin	Erythromycin	Fluconazole	MK2
Staphyloccocus aureus	22	36	0	0	22
Methicillin resistance S. aureus	30	34	0	0	27
Streptococcus pyogenes	30	30	24	0	0
Escherichia coli	30	35	0	0	25
Salmonella typhi	0	30	0	0	25
Pseudomonas aeruginosa	0	39	22	0	27
Proteus mirabilis	0	27	29	0	0
Klebsiella pneumoniae	40	47	32	0	24
Candida albicans	0	0	0	32	20
Candida krusei	0	0	0	34	0

Concentration of standard drugs : Cefuroxine = 40µg/ml, Sparfloxacin = 40µg/ml, Erythromycin = 50µg/ml and Fluconazole = 50µg/ml.

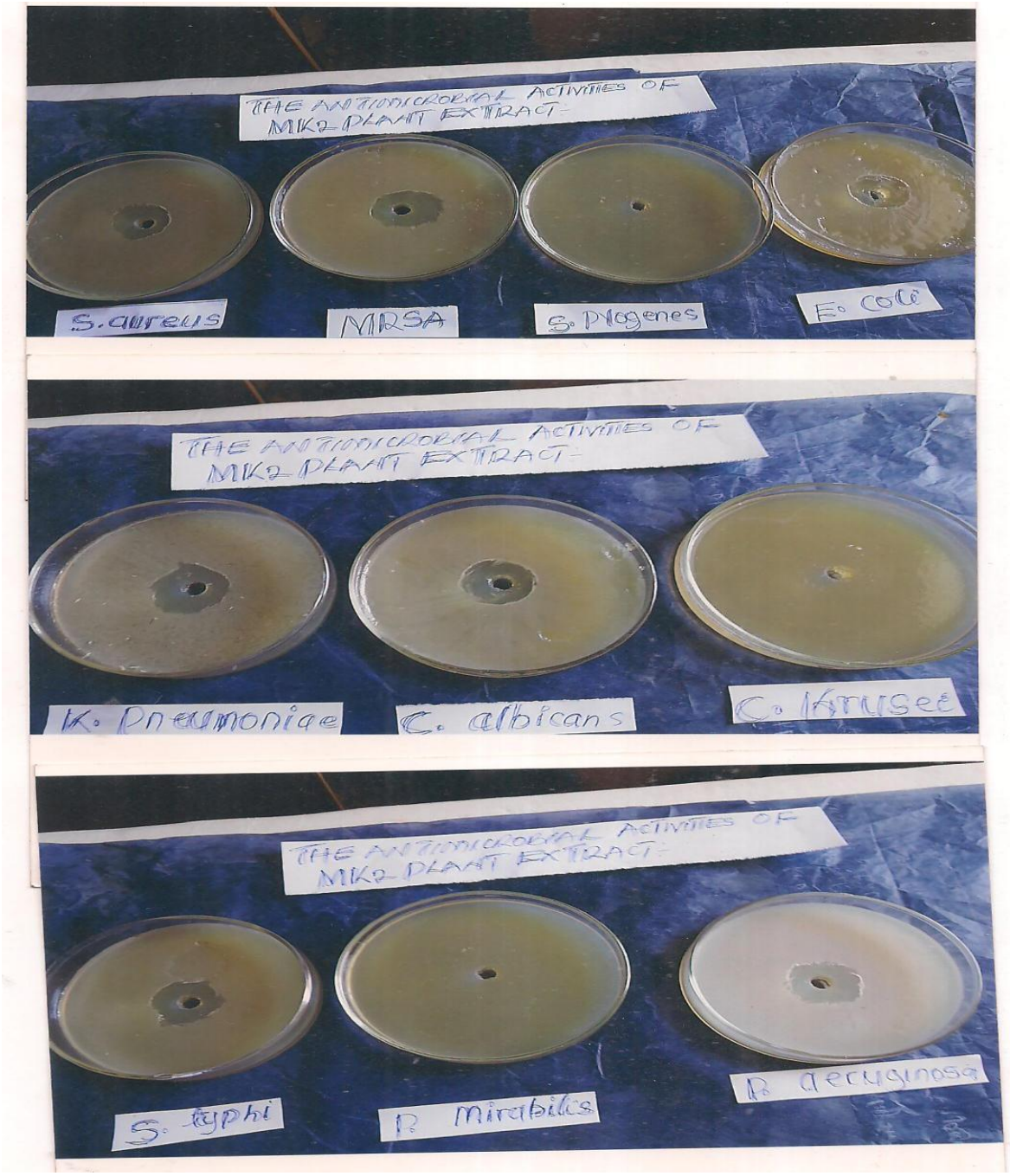

THE ANTIMICROBIAL ACTIVITIES OF MK2-PLANT EXTRACT-
S.aureus
MRSA
S. Pyogenes
E. coli
THE ANTIMICROBIAL ACTIVITIES OF MK2-PLANT EXTRACT-
K. Pneumoniae
C. albicans
THE ANTIMICROBIAL ACTIVITIES OF MK2-PLANT EXTRACT-
S. typhi
P. Mirabilis
P. aeruginosa

Plate II

Table 4.15.Minimum inhibition conc (MIC) of the pure component from ethyl acetate extract

Test organism	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml
Staphyloccocus aureus	-	-	0*	+	++
Methicillin resistance S. aureus	-	-	-	0*	+
Escherichia coli	-	-	0*	+	++
Salmonella typhi	-	-	0*	+	++
Pseudomonas aeruginosa	-	-	0*	+	++
Klebsiella pneumoniae	-	-	0*	+	++
Candida albicans	-	-	0*	+	++

Key : - = No growth, 0* = MIC, + = Turbidit (Light growth), ++ = Moderate turbidity.

Table 4.16. Minimum bactericidal / Fungicidal conc. (MBC/MFC) of the pure component from ethyl acetate extract

Test organism	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml
Staphyloccocus aureus	0*	+	++	+++	++++
Methicillin resistance S. aureus	-	0*	+	++	+++
Escherichia coli	-	0*	+	++	+++
Salmonella typhi	0*	+	++	+++	++++
Pseudomonas aeruginosa	-	0*	+	++	+++
Klebsiella pneumoniae	-	0*	+	++	+++
Candida albicans	0*	+	++	+++	++++

Key : - = No colony growth, 0* = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

GC-MS Analysis

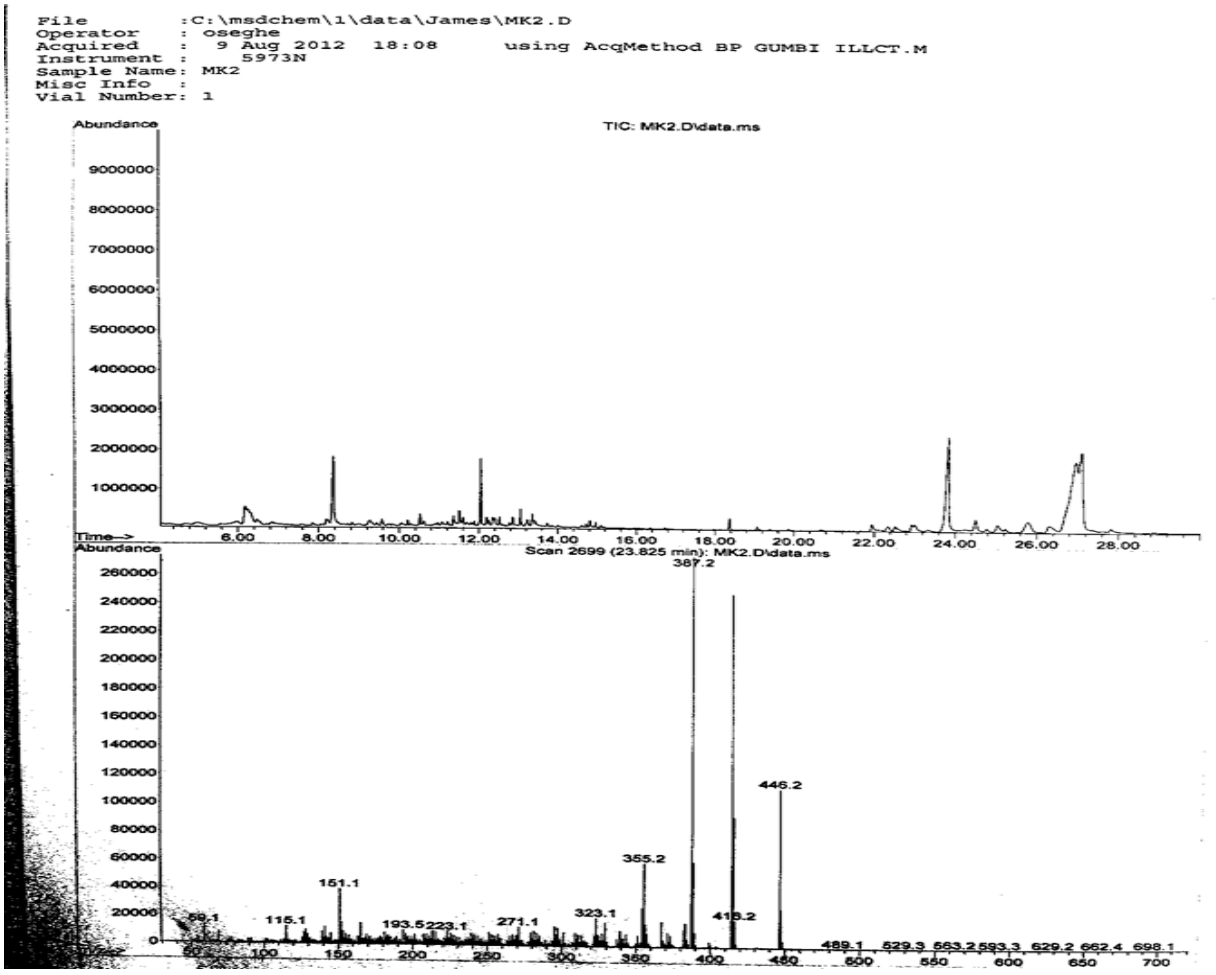
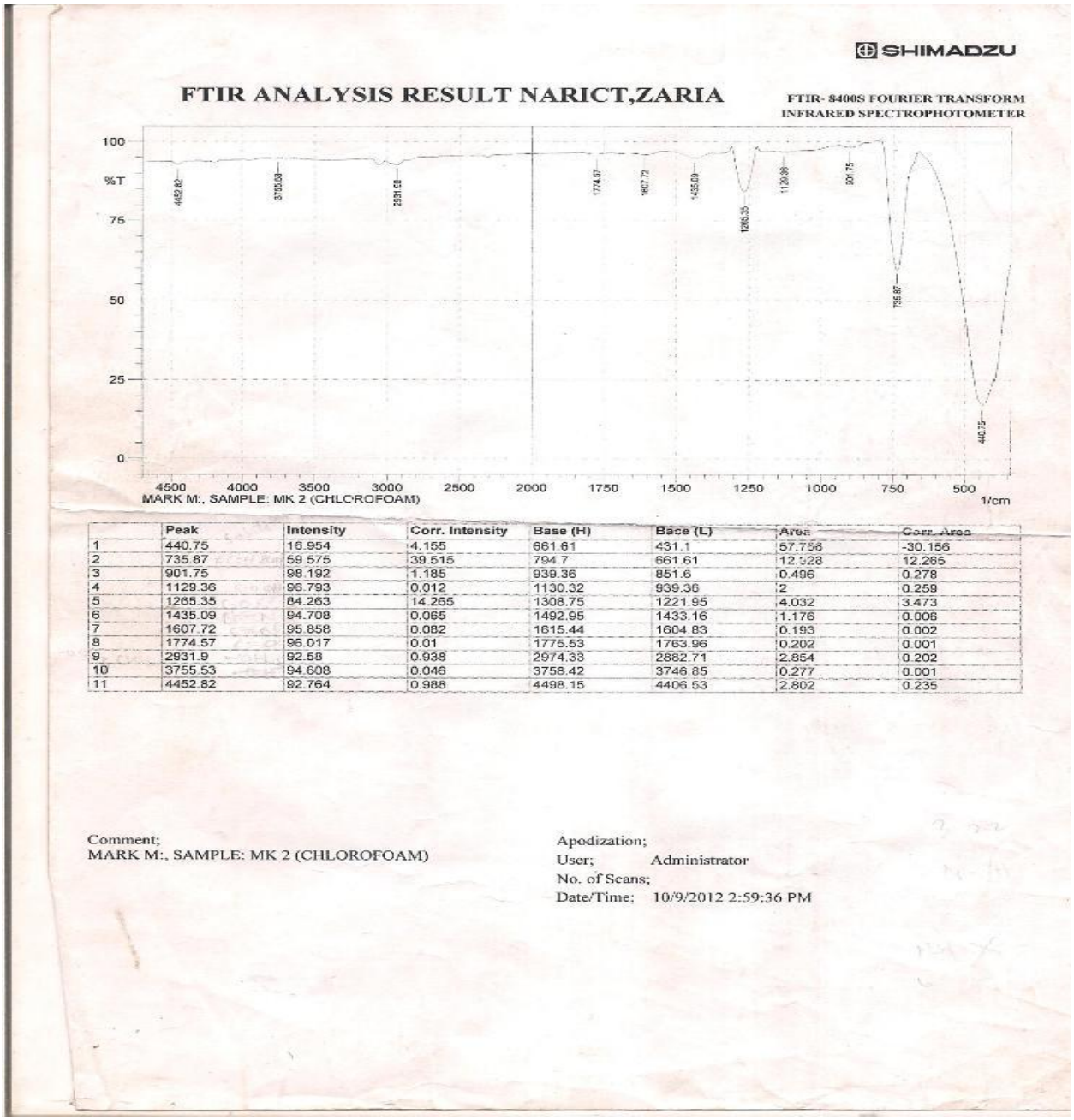


Figure 4.1

FT-IR Analysis



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	440.75	16.954	4.155	661.61	431.1	57.756	-30.156
2	735.87	59.575	39.515	794.7	661.61	12.528	12.265
3	901.75	98.192	1.185	939.36	851.6	0.496	0.278
4	1129.36	96.793	0.012	1130.32	939.36	2	0.259
5	1265.35	84.263	14.265	1308.75	1221.95	4.032	3.473
6	1435.09	94.708	0.065	1492.95	1433.16	1.176	0.006
7	1607.72	95.858	0.082	1615.44	1604.83	0.193	0.002
8	1774.57	96.017	0.01	1775.53	1763.96	0.202	0.001
9	2931.9	92.58	0.938	2974.33	2882.71	2.854	0.202
10	3755.53	94.608	0.046	3758.42	3746.85	0.277	0.001
11	4452.82	92.764	0.988	4498.15	4406.53	2.802	0.235

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Figure 4.2

^{1}H NMR Spectroscopy

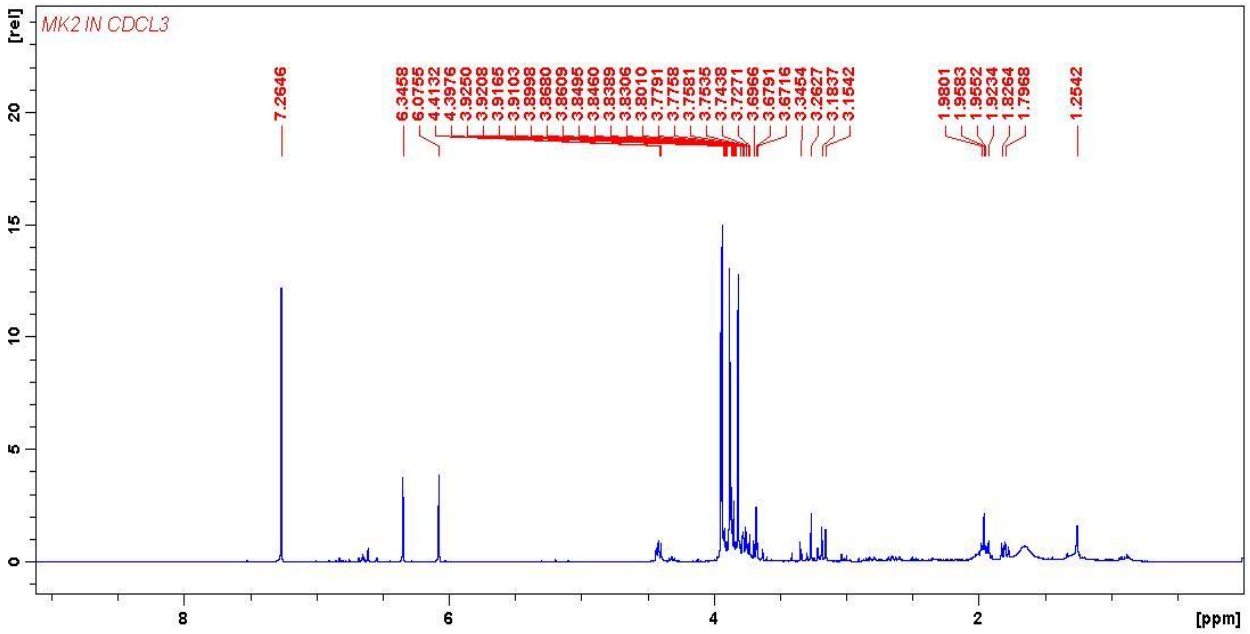

Figure 4.3

^{13}C NMR spectroscopy

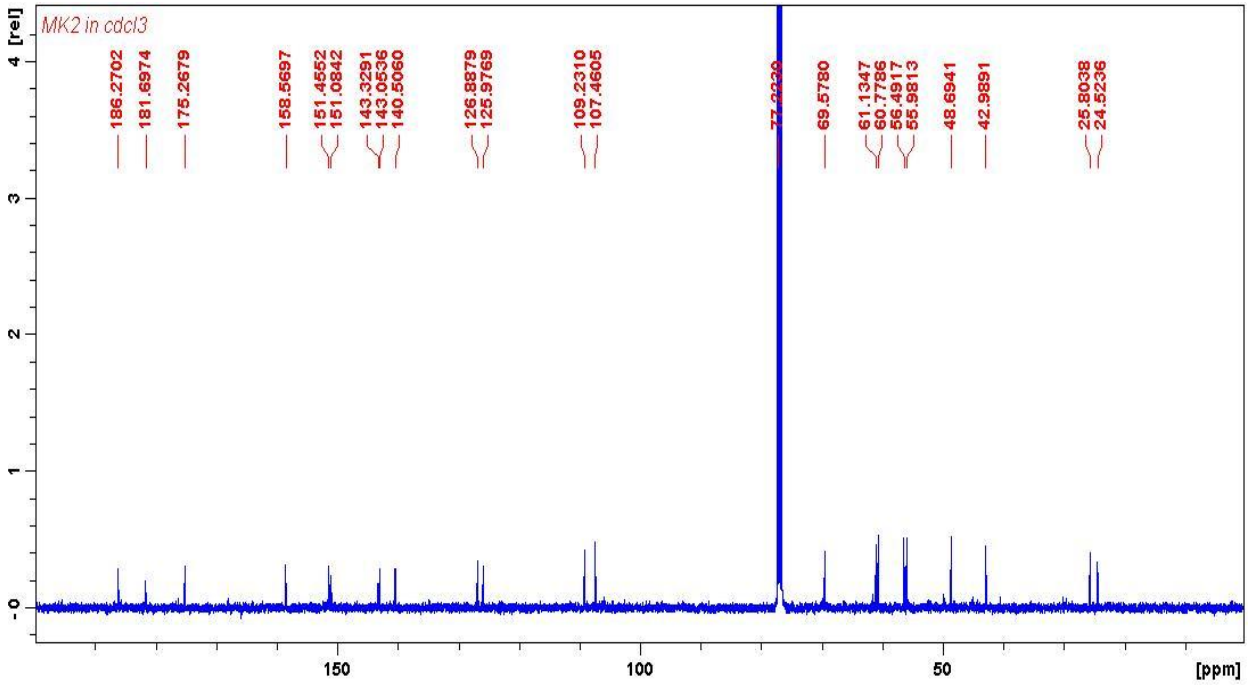

Figure 4.4

Dept spectroscopy

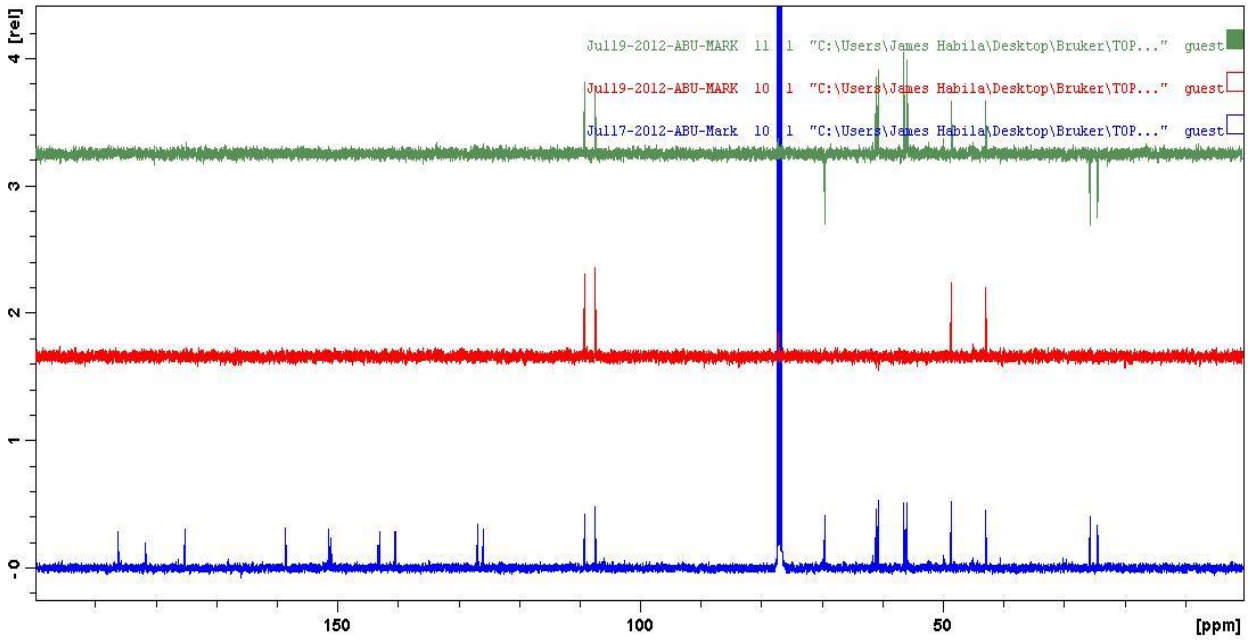

Figure 4.5

Table 4.17. Pure component spectra / Literature figure

^{1}H NMR from Lit.	^{1}H NMR of MK2	^{13}C NMR from Lit.	^{13}C NMR of MK2
8.46 – 7.12	7.26	184.20	186.27
4.67	-	141 – 115.5	140.51 – 107.46
4.30	4.41	70.40	69.58
3.20	3.18	42.90	42.99
1.90 – 1.20	1.98 – 1.25	40.20	-
		27.60	-
		25.30	25.80
		24.10	24.52

Key: MK2 = pure component

CHAPTER FIVE

5.0 DISCUSSION

Phytochemical Screening

From Table 4.1, it can be seen that the phytochemical screening of the leaves of the plant revealed the presence of carbohydrates, free reducing sugar, cardiac glycosides, saponins, steroids, flavanoids, alkaloids, tannins, triterpenes and absence of anthracene.

Extraction

Total and exhaustive extraction carried out on 300 g of the pulverized leaves of the plant material at a time using methanol after concentration *in vacuo* using rotary evaporator gave fairly dried crude extract which was further allowed to dry in air until a constant weight of 45.8 g was achieved. The dried crude extract was then partitioned using: petroleum-ether 60-80^{0}C, chloroform and ethyl acetate respectively. Their various extracts were concentrated *in vacou* using rotary evaporator and then dried in air until constant masses of 4.3, 6.5 and 4.2 g respectively were achieved and also, 26.8 g as residual methanol extract.

From Table 4.2, methanol extract gave the highest percentage recovery, followed by chloroform, then petroleum-ether and lastly ethyl acetate.

Antimicrobial

This was carried out on the four extracts generated after partitioning of crude methanol extract as well as the compound isolated from the ethyl acetate fraction using the agar well diffusion method. They were tested against twelve microorganisms namely *Staphyloccocus aureus, Methicillin Resistant S. aureus, Streptoccocus pyogenes, Corynebacterium ulcerans, Escherichia coli, Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Candida krusei* and *Candida tropicalis.* Also, their antimicrobial activities were compared with those of some standard drugs namely: Cefuroxine, Sparfloxacin, Erythromycin and Fluconazole. The results obtained were shown in Table 4.3.

All the four extracts of *Peucedanum winkleri* from Table 4.3, showed sensitivity to *Staphyloccocus aureus, Methicillin Resistant S. aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Candida albicans* and *Candida tropicalis* microorganism. Their zones of inhibition were measured in (mm) and ranges from 20-29 mm as shown in Table 4.4.

From Table 4.4, the ethyl acetate extract inhibited the growth of the test organisms more compared to the other extracts. The plant material contains phenolic compounds such as flavanoids and tannins, glycosides that have long been known to be toxic to microorganisms (Harbone, 1964, 1967). The possession of anti-mirobial activity by the extracts of *Peucedanum winkleri,* tends to support the local use of the plant to treat typhoid fever, pneumonia and as analgesic etc.

Based on the results, the minimum inhibitory concentrations (MIC) for the extracts, was carried out.

From Table 4.5 and 4.6, the petroleum ether and chloroform extracts could inhibit the growths of the test organisms *Staphyloccocus aureus, Methicllin Resistance S. aureus, Salmonella typhi, Candida albicans* and *Candida tropicalis* at the concentration of 12.5 mg/ml while *E. coli* and *Klebsiella pneumoniae* were inhibited at the concentration of 6.25 mg/ml.

Similarly, from Table 4.7 the ethyl acetate extract was able to inhibit *Staphyloccocus aureus, Candida albicans* and *Candida tropicalis* at the concentration of 12.5 mg/ml while *Methicllin Resistance S. aureus, E. coli, Salmonella typhi* and *Klebsiella pneumoniae* were inhibited at the concentration of 6.25 mg/ml.

Lastly, from Table 4.8, methanol extract was able to inhibit *Staphyloccocus aureus, Methicllin Resistance S. aureus, E. coli, Candida albicans* and *Candida tropicalis* at the concentration of 12.5 mg/ml while *Salmonella typhi* and *Klebsiella pneumoniae* were inhibited at the concentration of 6.25 mg/ml.

When the various inhibiting concentration were determined, the lowest concentrarion at which the organisms will be inhibited from further growth (MIC), this was then followed by the determination of the minimum concentration at which the organism will completely be exterminated known as the minimum bactericidal concentration (MBC) for bacteria and minimum Fungicidal concentration (MFC) for fungi.

From Table 4.9 of petroleum ether extract, the MBC/MFC for *Staphyloccocus aureus, Methicillin Resistance S. aureus, Candida albicans* and *Candida tropicalis* were 50 mg/ml while *E. coli, Salmonella typhi* and *Klebsiella pneumoniae* were 25 mg/ml. This indicates that *E. coli, Salmonella typhi* and *Klebsiella pneumoniae* can be completely exterminated at a low concentration compared to other organisms for this crude extract.

Also from Table 4.10 of chloroform extract, the MBC/MFC for *Staphyloccocus aureus, Salmonella typhi, Candida albicans* and *Candida tropicalis* were 50 mg/ml while that for *Methicillin Resistance S. aureus* and *E. coli* were 25 mg/ml and that for *Klebsiella pneumoniae* was 12.5 mg/ml. This indicates that *Klebsiella pneumoniae* can be completely extraminated at a low concentration compared to *Methicillin Resistance S. aureus* and *E. coli* which can also be completely exterminated at a low concentration compared to *Staphyloccocus aureus, Salmonella typhi, Candida albicans* and *Candida tropicalis* organisms for this crude extract.

Similarly, from Table 4.11 of ethyl acetate extract, the MBC/MFC for *Staphyloccocus aureus, Methicillin Resistance S. aureus, Salmonella typhi, Candida albicans* and *Candida tropicalis* were 25 mg/ml while *E. coli* and *Klebsiella pneumoniae* were 12.5 mg/ml. This indicates that *E. coli* and *Klebsiella pneumoniae* can be completely exterminated at a low concentration compared to other organisms for this extract.

Furthermore, from table 4.12 of methanol extract, the MBC/MFC for *Staphyloccocus aureus, Methicillin Resistance S. aureus, Candida albicans* and *Candida tropicalis* were 50 mg/ml while that for *E. coli, Salmonella typhi* and *Klebsiella pneumoniae* were 12.5 mg/ml. This indicates that *E. coli, Salmonella typhi* and *Klebsiella pneumoniae* can be completely exterminated at a low concentration compared to other organisms for this crude extract.

Lastly from Tables 4.9 and 4.12, the petroleum- ether and methanol crude extracts both have the same MBC/MFC values meaning they exterminate the test organisms at the same concentration. This shows that the components in the two extracts may be the same. Also the MBC/MFC of the plant leave extracts showed a higher concentration than that of

their MIC. The MBC/MFC was lowest in ethyl acetate crude extract compare to others which is an indication that the extract contains the highest concentration of the active compound.

From Table 4.13 above, it is observed that the isolated pure component, was sensitive to *Staphylococcus aureus, Methicillin Resistance Staphylococcus aureus, E. coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida albican*. Their zones of inhibition were measured as follows.

From Table 4.14, the isolated pure component, Cefroxine, Sparfloxacin, Erythromycin and Fluconazole showed relevant sensitivities to almost thesame sets of microorganisms.

From Table 4.15 of isolated pure component (MK2), the MIC for *Staphyloccocus aureus, E. coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida albicans were* 25 µg/ml while the *Methicillin Resistance S. aureus was* 12.5 µg/ml indicating that the isolated pure component inhibited the growths of *Methicillin Resistance S. aureus* at a low cocentration compared to other test organisms.

From Table 4.16 of isolated pure component (MK2), the MBC/MFC for *Staphyloccocus aureus, Salmonella typhi* and *Candida albicans* were 100 µg/ml while those of *Methicillin Resistance S. aureus, E. coli, Pseudomonos aeruginosa* and *Klebsiella pneumoniae* were 50 µg/ml. This indicates that *Methicillin Resistance S. aureus, E. coli, Pseudomonos aeruginosa* and *Klebsiella pneumoniae* can be completely exterminated at a lower concentration compared to other organisms for the isolated pure component.

All the four extracts and compound isolated from the ethyl acetate extract of the leaves of the plant and some of the standard drugs used showed relevant bioactivities on

the test microorganisms. Also, their inhibition of *Staphyloccocus aureus, Methicillin Resistance S. aureus, E. coli, Salmonella typhi, Klebsiella pneumoniae, Candida albicans, Candida tropicalis* and *Pseudomonas aeruginosa* organisms tend to support the local use of the plant in ethnomedicine for the treatment of high fever, typhoid fever, artrities, urinary tract infections and also, as an analgesic.

Gas Chromatography–Mass Spectrograph (GC-MS) of the pure component

From Figure 4.1, the GC analysis gave four components. The component with retention time of 23.825 minutes has a mass spectrograph with a m/z of 387.2 as the base peak and 446.2 as probably the molecular ion peak. Other prominent peaks were 115.1, 151.1, 355.2 and 416.2.

FT-IR spectrum of the pure component

From Figure 4.2, the FT-IR spectrum of the isolated pure component showed the following charateristic absorbances in cm^{-1}. 2931.9: typical of sp^3 C-H stretching vibration, 1774.57: typical of a C=O stretching vibration, 1607.72, 1500 - 1435: typical of C=C Ar stretching, 1265.35:: typical of a substituted or unsymmetrical benzene, 1120.36: typical of a C-N stretching vibration, 901 and 735.87: characteristic absorbance of N-H rocking mode.

^{1}H NMR spectra of the pure component

From Figure 4.3, the ^{1}H NMR ($CDCl_3$) (ð/ppm) spectra showed the following characteristics signals. 7.26: typical of Ar-H, 6.40 – 6.10: typical of NH proton, 4.41 –

4.40: typical of CH proton, 3.90 – 3.15: characteristics signals for the N – CH_3 protons and lastly, 1.98 – 1.25: typical of cyclohexyl protons.

^{13}C NMR spectra of the pure component

Similarly from Figure 4.4, the ^{13}C NMR ($CDCl_3$) (ð/ppm) spectra showed the following characteristics signals. 186.72 – 175.28: typical of C=O. 158.58 – 108.00: typical of C=C Ar. 140.51: typical of C=N. 69.58: typical of C-3. 61.00 – 49.00: typical of CH. 42.99: typical of NCH_3 and lastly 25.80, 24.52: typical of cyclohexyl carbons.

Dept spectroscopy of the pure component

From Figure 4.5, the dept 90 spectra, revealed the presence of four methine carbons (CH). Dept 135 revealed the presence of three methelene carbons (CH_2) as well as two methyl carbons (CH_3) and nine quatenary carbons in the pure compound isolated.

Based on these spectra informations and the GC-MS analysis, the isolated compound is proposed to be 4-(4-Chlorophenyl)-3-cyclohexyl-1-methyl-6-(2-naphthoyl)-1,2,3,4-tetrahydro-1,2,4,5-Tetrazine**.** The structure is given in figure 4.6

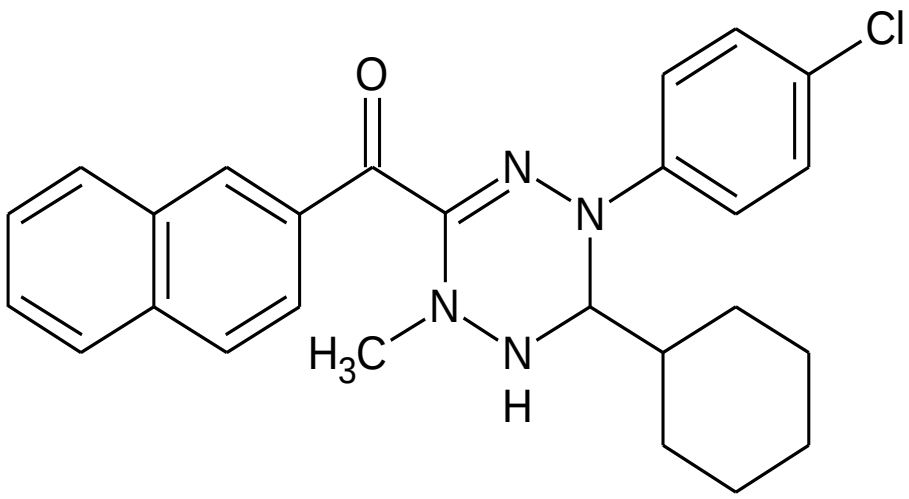

Figure 4.6. 4-(4-Chlorophenyl)-3-cyclohexyl-1-methyl-6-(2-naphthoyl)-1,2,3,4-tetrahydro-1,2,4,5-Tetrazine**.**

Figure 4.6 has a molecular weight of 446.98 and a molecular formular of $C_{26}H_{27}ClN_4O$. The structure was confirmed by its spectral data. Its FT-IR spectra showed absorption band of the aroyl carbonyl group at 1774.57 cm-1 region. In the ^{1}H NMR spectra, the signal of N-H proton of tetrazine ring recorded between 6.40 - 6.10 ppm and the N-methyl protons appeared as singlet in the range of 3.35 - 3.15 ppm. The ^{13}C NMR spectra show the expected resonance signals of the different carbons, especially the signal of C-3 of tetrazine ring around 69.58 ppm. This assignments are in good agreement with (El-Abadelah *et al.*,1988; Dalloul and Boyle, 2007; Dalloul and Abu-Shawish, 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

Peucedanum winkleri H. Wolff is a plant used in traditional medicine in Nigeria, the leaves of the plant were extracted by total extraction using the soxhlet extractor followed by partioning of the dried extract with petroleum ether, chloroform and ethyl acetate solvents in order of their increasing polarity. Phytochemical screening of the leaves of the plant, showed the presence of carbohydrate, free reducing sugar, cardiac glycosides, saponins, tannins, terpenes, steroids, flavanoids and alkaloids. The antimicrobial test of the leaves extracts had broad spectrum activity against the following pathogens: *Saphyloccocus aureus, Methicillin Resistance S. aureus, E. coli, Salmonella typhi, Klebsiella pneumoniae, Candida albicans* and *Candida tropicalis.* Similarly, antimicrobial test of the isolated pure component had broad spectrum activity against the following pathogens: *Saphyloccocus aureus, Methicillin resistance S. aureus, E. coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida albicans*. These activities could be due to the presence of secondary metabolites in them, thereby justifying the usage of the plant in folk medicine for the treatment of typhoid fever, high fever, pneumonia, rheumatic fever, arthritis, thrush, as an analgesic and so others.

Bioactive compound was isolated from the ethyl acetate fraction of the plant and based on the spectra data available, the compound was proposed to be 4-(4-Chlorophenyl)-3-cyclohexyl-1-methyl-6-(2-naphthoyl)-1,2,3,4-tetrahydro-1,2,4,5-Tetrazine. Which has high potential for biological activity, possessing a wide spectrum of antiviral and antitumor properties (Xu and Hu, 2008). They have been widely used in preparations of pesticides

and herbicides (Xu and Hu, 2008; Xu *et al.*, 2010). Tetrazine derivatives have high activity in chemical reactions (Trifonov *et al.,* 1998; Sun *et al.,* 2012).. Lang *et al.,* (1976) evaluated tetrazine derivative as active anti–inflammatory agents. This is in agreement with Sharma *et al,* (2008). Who proposed that a tetrazine ring can be an effective pharmacophore in various types of medicinal preparations.

In view of the vast medicinal potentials demostrated by the plant (*Peucedanum winkleri* H. Wolff), it is recommended that further work be carried out on this plant with the aim of isolating more useful active components from it and the pharmacological studies on the isolated components well carried out for their possible uses by humans.

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