Sudan University of Science and Technology

College of Post Graduate Studies

Characterization of Constituents of Degen Albasha(*Albizia lebbeck*(L) Fixed Oil Seed using GC-MS and its Antimicrobial Activity

توصيف مكونات الزيت الثابت لبذوردقن الباشا باستخدام كرواتوغرافيا الغاز مطياف الكتلةونشاطه كمضاد للميكروبات

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By

Areeg Makki Ismail Makki

Supervisor

Dr. Mohammed Sulieman Ali Eltoum

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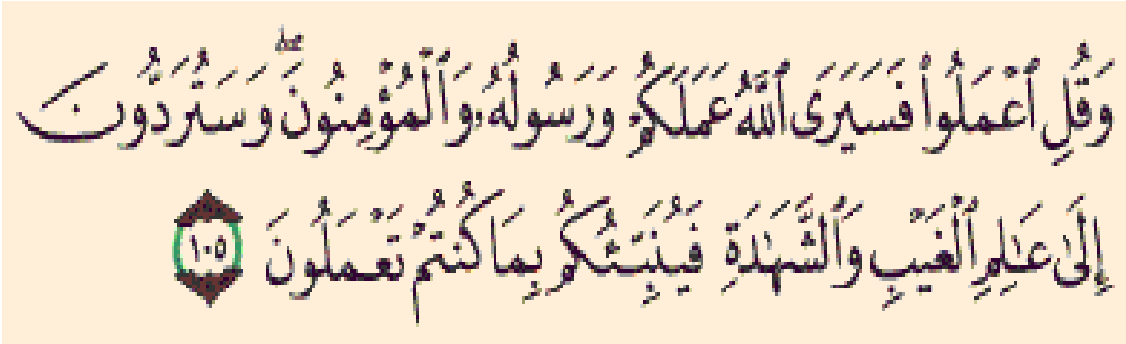
بسم الله الرحمن الرحيم


وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ
إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ١٠٥

(التوبة- 105)

صدق الله العظيم

Dedication

To

my parents

brothers , sisters and

friends

Acknowledgement

First of all I would like to thank **Almighty Allah** for giving me strength and patience to complete this research.

I would like to thank my supervisor **Dr. Mohammed Sulieman Ali Eltoum** suggestions, assistance patience and understanding throughout this research.

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Abstract

Albizia lebbeck(L) Benth is considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The therapeutic potential of plants has been well explored over a very long time period.

The present study was conducted to evaluate the phytochemical composition of the seeds extracts (Distilled Water and Petroleum ether) of *Albizia lebbeck* (L) Benth.

The phytochemical analysis showed that the aqueous extraction possess higher number of phytochemical constituents compared to other extract, Petroleum ether extract showed the absent of saponins and Tannis, compared to Aqueous extract absent only of terpens.

The *Albizia lebbeck* (L)Benth seeds was extracted by using soxhlet with solvent n- hexane gave amount of oil (3.5%v/w) and analyzed by GC-MS. The analysis revealed the presence of 16 components. The major components are: 9, 12-octadecadienoic acid (25.81%), 9-octadecenoic acid (15.27%),eicosanoic acid (27.80%) and Docosenoic acid (7.663%).

In disc diffusion bioassay *Albizia lebbeck* (L)Benth seeds oil showed activity against *Escherichia coli* (inhibition zone(15), *Staphylococcus aureus*(inhibition zone(15). It also exhibited significant partial activity against *Bacillus subtilis* (inhibition zone(12) *Pseudomonas aeruginosa* (inhibition zone(10) and *Candida albicans*(inhibition zone(10).

مستخلص البحث

يعتبر اللبخ (دقن الباشا) أحد النباتات الطبية التي تحتوي علي مركبات ذات فعاليه حيوية ولها خصائص علاجية عُرفت منذ زمن بعيد.أجريت هذه الدراسة لتحديد التركيب الكيميائي لمستخلص البذور(الماء المقطروالإيثر البترولي) وكشف التحليل الكيميائي النباتي الأولي للمستخلص المائي احتوائه على عدد اكبرمن المكونات مقارنه بمستخلص الإيثر البترولي واظهرت النتائج غياب الصابونين والتانين في مستخلص الإيثر البترولي بينما غياب التربينات فقط في المستخلص المائي.

وُجد ان كمية زيت البذور لنبات اللبخ(دقن الباشا) المتحصل عليه بالسكسوليت والهيكسان كمذيب (3.5٪ حجم / وزن) ، المكونات الكيميائيه لزيت بذور نبات اللبخ تم تحليلها بواسطه جهاز كروماتوغرافيا الغاز-طيف الكتله حيث وضح التحليل وجود (16) مركب اهمها:

حمض اللينوليك (25.81%) –حمض الاوليك (15.27%)- حمض الإيكوسينويك (27.80%) حمض الدوكوسينويك(7.663%)

الدراسه الحيوية لمضادات الميكروبات لزيت اللبخ (دقن الباشا) باستخدام طريقة الانتشار ابدي الزيت فعاليه جيدة ضد:البكتريا الإشريكية القولونية(منطقة التثبيط(15))-البكتريا العنقودية الذهبية(منطقة التثبيط(15))كما ابدي فعالية متوسطة ضد البكتريا العصوية الرقيقة (منطقة التثبيط(12))-البكتريا الزائفة الزنجارية(منطقة التثبيط(10)) والفطريات المبيضة (منطقة التثبيط(10)) .

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CHAPTER ONE

Introduction and Literature Review

1. General introduction

Plants serve as source of food and medicine because of the ability to convert the solar energy from the sun to metabolites, which are later converted to food and medicine for animal and human consumption. They also contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have preventive and curative properties. Resistance to synthetic drugs by many diseases in recent years has made health practices to change from curative to preventive medicine. Alkaloids, flavonoids, polyphenols, saponins, lignoids and vitamins are common phytochemicals popular in preventive medicine. Many common plants have been discovered by traditional healers to have medicinal properties for example bitter leaves used for diabetes; lemon grass used as stomachic and diuretic. Some essential oils bearing plants are used in a wide variety of consumer goods such as cosmetics, perfumes, detergents, soaps, toilet products, pharmaceuticals (antiviral, antibacterial, antiseptic , insecticides and antioxidant) confectionery food products, soft drinks and distilled alcoholic beverages (Trease, 1987; Oloyede, 2010b).

Albizia lebbeck(L)Benth (locally named, Degen Albasha) from Family: Fabaceae,it is ragne include southern and eastern Africa , from south Africa to sudan and Ethiopia , it is also found in India and Sri lanka. is a great interest due to its commercially valuable essential oils and widely used in traditional medicine, and thus the potentiality of *Albizia lebeck* (L)Benth *seeds* essential oil which could be the alternative approach Seeds are use to cure piles, diarrhea, scrofulous swelling, aphrodisiac and tonic to the brain and it oil is applied topically inleucoderma as antibacterial and anti-fungal (Sharma and Dubey (2015)).

Albizia lebbeck(L)Benth is also used traditionally in sudan as anti-asthmatic, anti-inflammatory, anti-fertility and anti-diarrhoeal, antiseptic, anti-dysenteric, anti-tubercular, leprosy, paralysis, helmenth infection (Folk Medicine at the H.E.J. (2013), Allergic rhinitis (Chulet et al., (2010). Astringent, to treat the eye, psychoactive, flu, lung problems, pectoral problems, cough, gingivitis, abdominal tumors (Sesoltani and Paulsen (2011). It is also used in the treatment of ringworms and wounds by washing the affected areas, gonorrhea, leucorrhoea and other genital diseases (Hassan et al., (2007).

The Main objectives of this study are to test a certain Sudanese plant namely *Albizia lebbeck*(L)Benth for their anti microbial activity and their ability to treatment of skin disease. The test of microbial was performed of an extract by using disk-diffusion method.

1.4 LITREATURE REVIEW

1.4.1 Natural product:

Natural products have been playing a significant role in human disease therapy and prevention. More than 60% and 75% of the chemotherapeutic drugs for cancer and infectious disease respectively are of natural origin, Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Almost 60% of the world population and about 80% of the population in developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs. Hence natural products play an important role in drug development programs in the pharmaceutical industry (Khalid et al., 1986).

The medicinal value of plants lies in some chemical substances due to secondary metabolites where most of them are bioactive constituents such as alkaloids, terpenoids, volatile oil, flavonoids and phenols that produce a definite physiological action in human body (Elujoba et al., 2005).

1.4.2 Traditional medicine in Sudan

Traditional medicine is the oldest form of health care in the world and is used in the prevention, and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases. traditional medicine is also variously known as complementary and alternative, or ethnic medicine, and it still plays a key role in many countries today(Abdullahi et al., 2011).

The medicaments used in traditional medicine are mostly derived from natural products. In traditional medicine, "clinical trials" have been conducted since ancient times. In the case of traditional Sudanes medicine, considerable experience and advances have been accumulated and developed over the past thousands of years with respect to methods of preparation, selection of herbs, identification of medicinal materials, and the best time for obtaining various different plants. Appropriate processing and dose regulation are urgently needed in traditional Sudanes medicine to improve drug efficacy and reduce drug toxicity. Considerable amounts of data have been acquired through clinical experiments, and in this way traditional medicine has assisted in the development of modern drugs.

Through its use of natural products, traditional medicine offers merits over other forms of medicine in such areas as the following: discovery of lead compounds and drug candidates; examining drug-like activity; and exploring physicochemical, biochemical, pharmacokinetic, and toxicological characteristics. If any form of traditional medicine is applied successfully, it may surprisingly assist in the development of new drugs, thereby resulting in many benefits, such as significant cost reductions.

Sudan has been home to indigenous civilization, such as Meroe, and road for others, namely pharaonic, Christian and Islamic civilizations. The country has been heavily influenced by fusion of different cultures. The immigrant Arab culture and

the neighboring cultures (mainly Egyptian and West African cultures) have strongly influenced Sudanese culture. However, there is a wide range of practices, which fall under the umbrella of traditional medicine.

Medicinal plants represent an important component of traditional medicine in Sudan and the flora of Sudan is relatively rich in medicinal plants corresponding to the wide range of ecological habitats and vegetation zones. These are coupled with ample inherited information in the field of medicinal plants and herbal traditional users which originally were unique blends of indigenous cultures of various nations (Khalid *et al.,* 1986).

Similar to other developing countries, traditional medical practices play an important role in Sudan. Herbal drugs are of major importance in Sudanese folk medicine (Elujoba et al., 2005)

In the past people depended exclusively on traditional medicine. They also used some wild plants for cosmetics and perfume by extracting the oils with primitive methods. In recent years, however, medicinal plants have represented a primary health source for the pharmaceutical industry. Large quantities are used for the preparation of infusions and decoctions both in the countries where traditional medicine is still of great therapeutic, social and economic importance, and in the production of important pharmaceutical products.

If these plants are cultivated on a large scale, they could represent an important source of hard currency, besides satisfying the market needs.

1.4.3 Albizia lebbeck **(L)Benth*:***

Description**:**

Albizia lebbeck(L)Benth is a fast growing tree with a spreading umbrella-shaped leaf and smooth grayish brown bark . The flowers, fruits, bark, leaves and seeds are all have medicinal value (Mohammed *et al.,* 2012). The *Albizia lebbeck* (L)Benth plant belong to the family fabaceae (formerly leguminosae), and sub-family Mimosae (Mishra et al., 2010). The plant has many common names such as

women tongue and rattle tree these names are derived from the noise made by the dry pods of the tree when they are being shaken by the wind (Ibraheem, 2007).The genus *Albizia*(L)Benth comprises approximately 150 species. These are mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa.

Albizia lebbeck (L)Benth is a fast-growing, medium-sized deciduous tree. *Albizia lebbeck* (L)Benth *seeds* are small, oblong, approximately 9 by 7 mm long and broad, compressed and light brown in color. *Albizia lebbeck*(L)Benth is an important medicinal plant.

1.2.4 Classification:

Kingdom: Plantae .

(unranked) : Angiosperms

(unranked) : Eudicots

(unranked) : Rosids

Order : Fabales

Family: Fabaceae

Subfamily: Mimosoideae

Tribe : Ingae

Genus : Albizia

Species:Albizia lebbeck

1.2.5 Traditional uses:

Traditional Medicine is applied successfully, it may surprisingly assist in the development of new drugs, thereby resulting in many benefits, such as significant cost reductions .*Albizia lebbeck* (L)Benth (Family Fabaceae) is a traditional medicinal Sudanese plant which is used in folk medicine. It is extensively used as folk medicine to piles and diarrhea , astringent , aphrodisiac , and used as brain tonic,for treating gonorrhea,and the seeds oil applied topically to cure leucoderma,scrofulous swellings (Sharma and Dubey , 2015)

Albizia lebbeck(L)Benth has active components such as isoquercetin, quercetin, polyphenols and saponins that have strong impact on hormonal and nervous system in the human body. As amild sedative, it wipes out the feelings of anxiety and stress. Albizia is helpful for people with chronic stress hormones in the body as it relaxes the mind. it,s also promotes mood by rebalancing hormones in the body and soothes nervous system that complicates the hormone levels. It is helpful for people with feelings of suicidal tendencies, experiencing irrational anger or thoughts. In ancient medicinal cultures, this herb assist the one to get peace.

decoction made with Albizia lebbeck(L)Benth is helpful for people with insomnia or sleeplessness.Besides soothing mind and nerves, it calms the body and provides long lasting and restful sleep.

Albizia lebbeck helps the people with allergies, asthma and chronic respiratory conditions by reducing inflammation in sinuses and tracts. It also lowers the impulse to cough or wheeze. It enhance respiratory health and speeds up recovery time.

Antioxidants present in *Albizia lebbeck* helps to eliminate free radicals in the body by scavenging harmful by products before mutation of cells or causing health problems.

Its bark and leaves possess antioxidant properties that acts outside the body and prevent scutaneous conditions such as blemishes, rashes, psoriasis, acne and wounds due to its antioxidant and anti-inflammatory properties that soothes the affected areas and lowers the appearance of irritation and blemishes.

Powdered *Albizia lebbeck* helps people with gastrointestinal problems. It binds water and food material by speeding up digestion process and keeps bowel movements regular.

Albizia lebbeck Bark is used as the treatment for boils and dysentery.In Sudan, leaves are used for night blindness; bark is used for dysentery and diarrhea; flowers are used for swellings, carbuncles, boils; seeds are used for diarrhea, piles and gonorrhea and roots are used for spongy and ulcerated gums.In Indo-China, seeds

and bark are used for diarrhea, dysentery, hemorrhoids; Flowers are applied as poultice to boils.The whole part is used to treat bites or stings from venomous animals.Leaves are used for ulcer, syphilis, cough, cold and respiratory problems. Roots are used to alleviate spasms and vitalize cardiovascular system.Apply the seed oil topically to cure leucoderma.Use the seeds to cure diarrhea, piles and scrofulous swellings. Bark eliminate toxins from the body, for eye problems; grind *Albizia lebbeck* leaves and using the cloth tie it on affected area. Let it remain for few minutes and to treat ear pain, extract the juice of Albizia lebbeck leaves and put few drops in ear.

1.2.6 Traditional remedies of *Albizia lebbeck*(L)Benth:

Mix the *Albizia lebbeck* leaves (fried in ghee) with honey and take it as a cure for cough . *Albizia lebbeck* seeds for piles, apply the grinded *Albizia lebbeck* seeds to the affected area. It helps to dry wart sin piles. Boil 200 ml of water with 10 gm of *Albizia lebbeck* bark till the volume reduces to one fourth.

Grind *Albizia lebbeck* leaves and mix it with honey and water. Drink this solution to treat urinary problems. Mix the *Albizia lebbeck* flower juice with black pepper and juice. Use this decoction for nasal instillation or oral medication for snake bites.

The fragrance of flowers treats migraine and headache.Leaves and flower juice is used to eliminate intestinal worms, It was also used to treat constipation.

1.2.7 Medical uses:

Traditionally plant is used as anti-asthmatic, anti-inflammatory, anti-fertility and anti-diarrhoeal, antiseptic, anti-dysenteric, anti-tubercular, leprosy, paralysis, helmenth infection (Folk Medicine at the H.E.J. 2013). Allergic rhinitis (Chulet et al.,2010). astringent, to treat the eye, psychoactive, flu, lung problems, pectoral problems, cough, gingivitis, abdominal tumors(Sesoltani et al., 2011). It is also used in the treatment of ringworms and wounds by washing the affected areas,

gonorrhea, leucorrhoea and other genital diseases (Hassan et al., 2007). Plant also shows cardio protective effects (Sasmal et al., 2013).

Piles and diarrhoea , astringent, aphrodisiac, and used as brain tonic, for treating gonorrhea, and the seed oil is applied topically to cure leucoderma, scrofulous swellings (Purendra and Teena , (2018).

According to phytochemical tests of *Albizia lebbeck* (L) Benth the presence of saponins, Flavonoids, glycosides, and tannins may be a rationale for the use of the plant in medicine preparations. Flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection. (Warrag et al.,2014). Terpenes are naturally occurring substances produced by a wide variety of plants and animals. A broad range of the biological properties of terpenoids is described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic activities. Terpenes are also presented as skin penetration enhancers and agents involved in the prevention and therapy of several inflammatory diseases. Moreover, a potential mechanism of their action against pathogens and their influence on skin permeability are discussed. The major conclusion is that larger-scale use of terpenoids in modern medicine should be taken into consideration.

Tannins are poly phenolic compounds that are broadly categorized into two major groups: hydrolyzable tannins, consisting of a central core of carbohydrate to which phenolic carboxylic acids are bound by ester linkage. The other type is condensed tannins, or proanthocyanidins, consisting of oligomers of two or more flavan-3-ols, such as catechin, epicatechin, or the corresponding gallocatechin. Tannins have a very high affinity for proteins and form protein-tannin complexes. The ingestion of a plant containing condensed tannins decreases nutrient utilization, protein being affected to a great extent, and decreases feed intake. On the other hand, hydrolyzable tannins are potentially toxic to animals. Consumption of feeds containing high levels of hydrolyzable tannins cause liver and kidney toxicity and

lead to death of animals. Oak and yellow wood poisonings are attributed to hydrolyzable tannins (Makkar et al., 2007).

Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative (Francis et al., 2002).

Flavonoids are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light, and are ubiquitous to green plant cells. The flavonoids are used by botanists for taxonomical classification. They regulate plant growth by inhibition of the exocytosis of the auxin indolyl acetic acid, as well as by induction of gene expression, and they influence other biological cells in numerous ways.

Flavonoids kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Yet, their toxicity to animal cells is low.

Flavonoids are major functional components of many herbal and insect preparations for medical use, which have been used since ancient times. The daily intake of flavonoids with normal food, especially fruit and vegetables is 1–2 g. Modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific

enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals.

Alkaloids many substances which interfere with the inflammatory response have been isolated from plants. Some alkaloids of vegetal origin which in the period of 1907 to 2000 were evaluated regarding a possible anti inflammatory activity. The alkaloids were classified in sub groups in accordance with their chemical structures and the pharmacological data were obtained from different experimental models. Of the 171 evaluated alkaloids, 137 presented anti-inflammatory activity, and among those, the isoquinoline type was the most studied. The carrageenin-induced paw edema was the most used model for evaluating the anti-inflammatory activity (Makkar et al., 2007).

A glycoside consists of two components, an aglycone (non-sugar) part and a sugar part. The aglycone portion may be of several different types of secondary metabolites, including coumarin, flavonoids or hydroxyanthracene. The sugar moiety is linked to the aglycone by a direct carbon to carbon bond (C-glycoside), or through oxygen to carbon bond (O-glycoside). Cyanide glycosides, release toxic hydrogen cyanide when cells are damaged and act as a defence mechanism (Filho et al., 2006).

1.3 Essential oils

Essential oils are complex natural mixture of volatile and liquid aroma compounds from natural sources, isolated from plants usually by hydro-or steam distillation and by expression (citrus peel oils) .

The main constituents of essential oils terpenes including carbohydrates, alcohols, ethers, aldehydes, esters and ketones – are responsible for the fragrant and biological properties of aromatic and medicinal plants. Due to these properties, since ancient times spices and herbs have been added to food, not only as flavouring agents but also as preservatives. For centuries essential oils have been isolated from different parts of plants and also are used for similar purposes. Essential oils cover a broad spectrum of activities.

Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticancerogenic properties. Others are biocides against abroad range of organisms such as bacteria, fungi, viruses,protozoa, insects and plants (Charai, M (1996).

The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenou layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant. Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelica archangelica), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated Factors, climatic, seasonal and geographical conditions, harvest period and extraction techniques (Pannizi et al., 1993).

The yield of oils from the plants can also be affected by the stages of the plant growth. Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators. The oxygenated molecules of essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers. These properties were established after the oils have been extracted from the plant materials (Pannizi et al., 1993).

1.3.1 Essential oil remedies

The diverse uses of essential oils in talk medicine are discussed briefly below:

i-Burns: lavender essential oil is mixed with aloe Vera to treat burns.

ii-Bug bites: lavender oil is applied for bug bites and stings.

iii-Bruises: essential oil like lavender oil as a hot compress to treat bruises or other wounds

iv-Motion sickness: peppermint; lavender and ginger oil are usually applied to reduce motion sickness.

v-Colds: Some drops of oil of oregano and frankincense are extremely useful for colds.

vi- Neck pain: peppermint, cypress and ginger oils with cayenne paperand coconut oil are the bases is for a homemade pain relieving muscle rub.

vii- Headache relief: A combination lavender oil and peppermint oil is apply to temples to help with headaches and migraines.

viii-Cough or sinusitis: Eucalyptus essential oil is known for its powerful ability to fight coughs and open airways.

ix- Broken bones: To support healing of broken bones, helichrysum, firand cypress essential oils are used.

x- Indigestion: Ginger, peppermint and fennel essential oils support digestion and healing leaky gut.

xi. Bronchitis and asthma: A homemade vapor rubs is prepared by combining eucalyptus, peppermint and coconut oil.

xii.**Bruises:**Levender and frankincense oils are treated with
hot water and applied to affected area.

xiv.Memary: Bergamot, peppermint or grapefruit seed essential oils may increase concentration during the day.

xv.Sore Feet: Peppermint oil with Epsom salt is added to a warm water foot bath.

xvi. Teeth grinding: lavender is placed on the bottom of the feetand behind ears before bed.

xvii. Eczema and psoriasis: To treat eczema, psoriasis or red dry skin, a mixture of lavender essential oil with Shea butter is applied externally.

xviii. **Improving circulation**: Grapefruit essential oils added to warm a bath water to improve circulation.

xix. Balance of blood sugar: peppermint and cinnamon essential oils are in haled to reduce appetite and balance blood sugar.

xx.fatigue: Peppermint oil is inhaled before a workout to reduce fatigue

xxx.Fever: Some drops of eucalyptus, peppermint and lavender essential oils are added to a cool cloth to sponge the body.

xL: Hangover symptoms: Some drops each of juniper berry cedar wood, grapefruit, lavender, rosemary and lemon oil are added a warm bath.

L: Arthritis relief: Some drops of wintergreen, cypress and lemongrass oils are mass aged of into affected areas.

Lx.Ringworm: Some drops of tea tree oil are combined with coconut oil and massage over the affected area twice a day.

Lxx. Head lice treatment: Some three drops of thyme, lavender and eucalyptus oils are applied to scalp. The head is covered with a shower cap and left on for 30 minutes.

Lxxx. Blistered skin: Some drops of tea tree oil are mixed with drops of unscented oil and applied to the blistered area up to five times per day.

xc. Sunburn: Acombination of lavender or chamomile oil with coconut oil is applied to the skin with a cotton ball to reduce swelling and pain.

xcix. Immune system: Some drops oregano oil is mixed with carrier oil and rubbed on the bottom of feet.

c. Weigh loss: Grapefruit, ginger and cinnamon oil and take as a supplement three times daily to support metabolism (Bu‘Lock(1965)).

1.3.2 Extraction of essential oils

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process. The oil droplets being stored in the oil glands or sacs can be removed by either accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are;

1.3.2.1 Solvent extraction

Most flowers contain too little volatile oil to undergo expression , their chemical compounds are too delicate and easily denatured by the high heat used in steam distillation . Instead , a solvent such as hexane or supercritical carbon dioxide is used to extract the oils . Extracts from hexane and other hydrophobic solvents are called concretes , which are a mixture of essential oil , waxes, resins , and other lipophilic (oil – soluble) plant material (Benzie 2003).

Highly fragrant concretes contain large quantities of non –fragrant waxes and resins , hence , another solvent , such as ethyl alcohol ,which is more polar in nature ,is used to extract the fragrant oil from the concrete .

The alcohol solution is chilled to -1800 (0 0F) for more than 48 hour which causes the waxes and liquids to precipitate out . The precipitates are then filtered out and the ethanol is removed from the remaining solution by evaporation , vacuum purge ,or both ,leaving behind the absolute oil (Benzie 2003).

Supercritical carbon dioxide is used as a solvent in supercritical fluid extraction . This method has many benefits including (Knekt, (2008).avoiding petrochemical residues in the loss of some " top notes " when steam distillation is used . It does not yield absolute oil directly. The supercritical carbon dioxide will extract both waxes and essential oils that makeup the concrete (Benzie 2003).

Subsequent processing with liquid carbon dioxide , achieved in the same extractor by merely lowering the extraction temperature , will separate the waxes from the essential oils .This lower temperature process prevents the decomposition and Denaturing of compounds . When the extraction is complete, the pressure is reduced to ambient and carbon dioxide reverts to a gas , leaving no residue . Supercritical carbon dioxide is also used for making decaffeinated coffee. Although it uses the same basic principles, it is a different process because of the difference in scale (Benzie 2003).

This method involves the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used for extraction. For instance, leaves, roots, fruits are extracted with benzene with or without mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and color. After the extraction, the solvent is removed by distillation under reduced pressure leaving behind the semi solid concentrate, this concentrate is extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil.

1.3.2.2 Enfleurage

This process is applicable to flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odorless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind

only the essential oil; hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

1.3.2.3 Cold pressing

Another method of extracting essential oil that has not found high application in scientific research is cold pressing. It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

1.3.2.4 Steam distillation

This is the most common method of extracting oils and is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel.

steam is added and passed through the plant that contains the plants aromatic molecules or oils. Once upon, the plant releases these aromatic molecules and in the state, the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapors. As they cool, they condense and transform into a liquid state.

1.3.2.5 Hydro distillation

The technique involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapor and oil are removed through a water cool condenser.

1.3.2.6 Hydro diffusion

Hydro diffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant

material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma of the plants

1.3.2.7 Microwave assisted process (MAP)

The MAP process uses microwave to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants. this technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction. This technique has also been applied for the extraction of saponins from some medicinal plants (Safir et al., 1998).

1.3.2.8 Carbon dioxide extraction

In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition of the oil.

1.3.3 Chemical Composition of Essential oils

In general, pure essential oils can be subdivided into two distinct groups of chemical constituents the hydrocarbons which are made up almost exclusively of terpenes(mono terpenes,sesquterpenes and diterpenes) and the oxygenated compounds which are mainly esters ,ketones,alcohol,phenols and oxides.

The two main purposes of analyzing essential oils are:

(i) To identify and quantify as many constituents as possible.

(ii) To evaluate the quality of the oils and detect any possible adulteration that may affect their usage.

Analysis of essential oils is generally performed using Gas chromatography (qualitative analysis) and Gas chromatography-mass spectroscopy (qualitative analysis).Gas chromatography analysis is a common Confirmation test.

1.3.4 Gas Chromatography Analysis (GC)

Gas chromatography analysis is a chemical instrument used for separating chemicals in a complex sample and provides a representative spectral output. The gas chromatography instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak. The time elapsed between injection and evaluation is called "Retention time".

The sample is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separate before eluting from the column end. The detector measure different compounds as they emerge from the column.

1.3.5 Gas Chromatography-Mass Spectroscopy Analysis (GC/MS)

GC-MS a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures (Skoog et al., 2007). GC-MS instrument consists of two main components. The gas chromatography separates different compounds in the sample into pulses of pure chemicals based on their volatility (Oregon State University, 2012)by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog et al., 2007). Spectra of compounds are collected as they exit a chromatographic column by the mass

spectrometer, which identifies and quantifies the chemicals according their mass-to charge ratio (m/z). (Oregon State University, 2012)

After the sample has passed through the GC, the chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions.

This is important because the particles must be charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. This information is sent to the computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that travelled through the filter. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent, so that it can be put into various uses (Kagawa et al., 2003).

1.4 Skin problems

Skin disease is a common ailment and it affects all ages from the neonate to the elderly and cause harm in number of ways (Marks 2006)

The ability of Plants to grow on different types of soils rich in microorganisms are as a result of their potential to produce wide range of selective anti-bacterial compounds that are capable of wading off potential microbial invaders (Cammune *et al.,* 1992).Medicinal plants have been used for centuries as remedies for human and animal diseases because of their therapeutic values. Hence, plant derived drugs remain an important resources especially in many countries of the world to combat diseases.

Approximately 60-80% of the world s population still relies on traditional edicines for the treatment of common illness (WHO, 2002). It is estimated that there are 250,000 to 500,000 species of plants on earth (Borris, 1996). There is a

growing interest in correlating phytochemical constituents of plant with its pharmacological activity.

There are more than a thousand conditions that may affect the skin but most skin diseases can be categorized into nine common types (three of them):

1.4.1 Bacterial infections

Such infections are caused by a variety of bacteria, the most common types being staphylococci and streptococci. Bacteria may infect the topmost layers of skin, the follicles, or the deeper layers of skin. If not treated correctly, these infections may spread throughout the body. Examples include impel folliculitis, cellulitis and lyme disease. Bacterial infections are better treated with antibiotics.

1.4.2 Fungal infections

Harmless fungi are always present on surface of the skin. Infection occurs when these organisms enter into the body. These infections are usually superficial, affecting the skin, hair, nails and include athlete's foot, lock itch and ringworm. However, in people with suppressed immune system or who have been taking antibiotics for long period -, the fungi may spread to deep within the body, causing more serious disease.

1.4.3 Tumors and cancers

These growths arise when skin cells begin to multiply faster than normal. Not every skin growth is cancerous. Some tumors are harmless and will not spread. Skin cancer is the most common of all the cancers, affecting 800,000 Americans each year. It is caused, in 90% of cases, by sun exposure. The three types of skin cancers are basal cell cancer (the most curable), squamous cell cancer (which may grow and spread) and malignant melanoma (the most deadly form). Prevention involves protecting the skin against damaging ultraviolet rays. Early detection helps to improve the chances of a cure. Regular self-examinations are, therefore, recommended.

1.4.4 Plants for skin disease:

Natural drugs from the plants are gaining popularity because of several advantages such as often having fewer side-effects, better patient tolerance, being relatively less expensive and acceptable due to a long history of use. Besides herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. For these reasons several plants have been investigated for treatment of skin diseases ranging from it chink to skin cancer.

1.4.5 Antibacterial activity of essential oils

Bacterial pathogens and their control are a serious problem in agriculture practice. spraying with antibiotics and copper compounds, usually suggested to control bacterial diseases, have never been satisfactory. Furthermore, antibiotics are forbidden in many countries and copper compounds, because of their general toxicity, exert a negative impact on both yield and the environment. As an alternative strategy to prevent the spread of diseases, natural compounds of plant are being tested for their antimicrobial activity. Naturally occurring biologically active plant products can be a source of new pesticides or serve as templates for new, more effective compounds (Kalama, 2003).

Investigations of aromatic and medicinal plants enable finding plants producing effective essential oils that have already found a considerable range of applications .Various essential oils are biocides against a broad range of organisms such as bacteria, fungi I, viruses, protozoa, insects and plants. In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial Properties against bacteria and fungi. There is vast diversity among aromatic and medicinal plants (Bhusita ,2005).

Antimicrobials can be grouped according to the micro organism they act primarily agent. They can also be classified according to their function. Agents that kill microbes are called microbicidal; while those that merely inhibit their growth are called biostatic .The use of antimicrobial medicines to treat infection is known as

antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis31.The science dealing with the study of the prevention and treatment of diseases caused by micro-organisms is known as medical microbiology. Its sub disciplines are virology (study of viruses), bacteriology (study of Bacteria), mycology (study of fungi), physiology (study of algae) and protozoology (study of protozoa).

For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders.

The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses),bactericides (killing bacteria), algicides (killing algae) or fungicides (killing fungi) (Robert,1962).

The antibacterial agents are classified in three categories:

(i) Antibiotics and chemically synthesized Chemotherapeutic agents.

(ii) Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives)

(iii) Immunological products (Thompson,(1972) .

The main classes of antimicrobial agents are disinfectants (―nonselective antimicrobials‖ such as bleach). which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics are applied to living tissue and help reduce infection during surgery and antibiotics tend to destroy microorganisms within the body. Antibacterial are used to treat bacterial infections. The drug toxicity to human and other animals from antibacterial is generally considered low. Prolonged use of certain antibacterial can decrease the number of gut flora, which may have a negative impact on health.

Antibacterial are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infection.(Tripathi, 1994) .

1.4.6 Anti microbial Activity by using diffusion method.

. PRINCIPLE. In these method the substance to be assayed is allowed to diffuse through solid, inoculated culture medium. If the substance being assayed is a bacteriostatic or bactericidal agent, a zone of inhibition results. If the substance is a growth factor, a zone of growth zone of exhibition develops. The size of the zone, either of inhibition or growth, is a function of the concentration or in certain circumstances, the amount of the substance being assayed. This function can be expressed as a linear relationship between the size of the zone and the logarithm of the concentration of the substance . By measuring the distance the substance diffuses, as evidenced by growth or lack of growth of the test organism, and comparing it with that of a known standard preparation, the potency of the sample may be calculated.

1.5 Objectives

The present study is aim to:

- Extract the *Albizia lebbeck* seeds oil by using soxhlet and n-hexane as solvent.
- Characterize the chemical profile presents in *Albizia lebbeck* seeds oil by using GC.MS analysis.
- Phytochemical screening of it is extracts (petroleum ether, distilled water).
- Predict the antimicrobial activity of the *Albizia lebbeck* seeds oil using diffusion method.

Chapter Two

Materials & Methods

CHAPTER TWO

MATERIALS and METHODS

2. Materials:

- *Albizia lebbeck*(L.) Benth seeds

-Chloroform $CHCl_3$

- Petroleum ether

- Boron tri Floride (BF_3).

- Wagner's reagent(Iodine in Potassium Iodide 2.5gm iodine is dissolve in 12.5gm of potassium iodide KI_2).

- Lead Acetate $(CH_3COO)_2Pb$).

- Dilute Hydrochloric acid HCl.

- Distilled water H_2O.

-Sodium Hydroxide NaOH.

- Concentrated (H_2SO_4).

- n-hexane.

-Methanol (CH_3OH).

-Ferric Chloride $FeCl_3$.

-Fehling' Solution.

-Micro Organism **(***Bacillus subtilis* , *Staphylococcus aureus* , *Pseudomonas aeroginosa* , *Escherichia coli Aspergillusniger* , *Candida albicans*)

-All the material from (loba chemie) company (India)

2.1 Apparatus:

- Soxhlet.

2.2 Instrument:

-Gas Chromatography Mass Spectrometry (GC/MS).

Instrument information:

Detector: Mass spectrometer

Model: GC/MS-QP2010Ultra

Company: Shimadzu

Country: Japan

Column: Rtx-5MS…Length (30m)…Diameter (0.25mm)…Thickness (0.25μI)

Carrier gas: Helium

2.3 Sample collection and identification

The plant *Albizia lebbeck* (L)Benth (locally named Degen Albash) Seeds was collected from Khartoum state, Omdurman.

The botanical classification of the plant was confirmed at the Medicinal & Aromatic Plants and Traditional Medicine Research Institute, Khartoum, Sudan.

2.4 Preparation of plant Material

The seeds were removed from their pods and ground to powder by using grinder- machine and stored to extraction.

The petroleum ether and water extracts were prepared by extracting 50g of pulverized sample with 125mL of solvent under continuous agitation using shaker apparatus for 3 days (maceration method). The extracts were filtered and solvent were evaporated under room temperature for 24 hours. The different extracts were preserved in refrigerator till time of use.

2.5 Phytochemical screen:

Phytochemical examinations were carried out for all the extracts as per the standard methods. Phytochemical screening for constituents was undertaken using standard qualitative methods as described by (Harborne, 1984). The extracts were evaluated qualitatively for the presence of alkaloids, flavonoids, glycosides, phenols, saponins, tannins and terpenes.

2.5.1 Alkaloid test:

In dry and clean test tube 1 ml of the extract were added and 3 drops of dilute Hydrochloric acid then it were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown /reddish precipitate indicates the presence of alkaloids.

2.5.2 Detection of Terpenoids (Salkowski test)

0.5ml of each of the extract was added 2ml of chloroform and then 3ml of the concentrated H_2SO_4 was carefully added to from a layer. A reddish brown colour of the interface was indicating the presence of terpenoids / steroids.

2.5.3 Detection of Saponins

Froth Test: 0.5ml Extracts were diluted with 5ml of distilled water. The solution was shaken vigorously and observed for the stable persistent froth. Formation of 1 cm layer of foam was indicating the presence of saponins.

2.5.4 Detection of Tannins

Five per cent Ferric Chloride solution (5% $FeCl_3$) were added drop by drop to 2-3ml of the extract. A dark green colored precipitate indicates the presences of tannins.

2.5.5 Detection of Glycosides

Two and half milliliter (2½ ml) of 50% H_2SO_4 was added to 5cm3 of the extracts in a test tube. The mixture was heated in boiling water for 15 minutes. It was then cooled and neutralized with 10% NaOH, 5ml of Fehling's solution was added and the mixture was boiled. A brick red precipitate was observed which indicated the presence of glycosides.

2.5.6 Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of 10% ferric chloride solution. Formation of bluish black color was indicating the presence of phenols

2.5.7 Flavonoids :

Lead Acetate test: In dry and clean test tube 1 ml, of extract was treated with, few, drops of lead acetate solution. Formation of, a yellow, precipitate indicates the presence of flavonoids.

2.6 Extraction of oil from seeds of *Albizia lebbeck*(L)Benth:

Powdered seeds of *Albizia lebbeck* (100g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil

was kept in the fridge at 4^{o}C for further manipulation. The percentage of oil was calculated as follow:

Seeds oil % = (volume of oil / plant (g)) ×100(v/w)…….

2.7 GC-MS Analysis of Essential Oil

The qualitative and quantitative analysis of *Albizia lebbeck* essential oil was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japan's Shimadzu Company, with capillary column (Rtx-5MS- (30m) in length, diameter (0.25mm) and thickness (0.25µl). The sample was injected by using split mode, helium as the mobile phase (carrier gas) passed with flow rate 1.69 ml/min, the temperature program was started from 50 °C reaching 280 °C as final temperature degree with a temperature program rate of 7 ºC/min, starting at three minutes and finishing at forty minutes , The injection port, ion source and interface temperature were (280, 200 and 250) °C, respectively.

The sample was analyzed by using scan mode to identify chemical composition of the essential oil in the range of m/z 40-550 mass to charge and the total run time was 40 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

The percentage of each compound was based on the peak area divided by the total area of component peaks. The instrument was connected to a computer coupled with special software that was used to analyze the data and the results were recorded.

2.8 Biological activity

2.8.1Antimicrobial assay

Albizia lebbeck seeds oil was screened for antimicrobial activity against five standard human pathogens (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*) using the disk diffusion method .

2.8.2 Preparation of bacterial suspensions

One ml. aliquots of 24 hours broth culture of the test organisms were distributed onto agar slopes and incubated at 37^{o} C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce suspension containing about 108- 104 colony forming units per ml. The suspension was stored in refrigerator at 4^{o}C until used. The average number of viable organism per ml of the

Saline suspension was determined by means of the surface viable counting technique (Miles and Misra,1938).

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volume (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature to dry, and then incubated at 37^{o} C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.8.3 Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25^{o}C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

2.8.4 Testing of antibacterial activity:

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^{8}cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.8.5 Testing of antifungal activity

The above mentioned method was adopted for antifungal activity, but instead of nutrient agar dextrose agar was used. Samples were used here by the same concentrations used above.

Chapter Three

Results & Discussion

CHAPTER THREE

RESULTS and DISCUSSION

3. The yield of *Albizia lebbeck* (L)Benth seed Oil:

For 100g of the pulverized *Albizia lebbeck*, it was found that the yield only 3.5ml equivalent to 3.5% v/w of the constituent was oil. The color of the oil obtained from the *Albizia lebbeck* was yellowish green.

This result is good agreement with the results of (Mondira, 2016) who reported that **"**the percentage yield of fixed oil from ***Albizia lebbeck*** (seeds) extracted by sovlent extration method (soxhlet) was 4.7% v/w".

3.1 Phytochemical Screening

Table (**3.1.1**) Below showed a phytochemical screening of ***Albizia lebbeck*** *(*seeds) extracts by using Petroleum ether and distilled Water as solvents respectively in order of polarity.

Constituent	Solvent	
	Petroleum ether	Water
Alkaloids	+	+
Tannis	_	+
Flavnoid	+	+
Terpenes	+	_
Glycoside	+	+
Saponins	_	+
Phenols	+	+

Key:(+) present ; (-)absent.

The phytochemical screening of chemical constituents in *Albizzia lebbeck* study showed that seeds were rich in flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity. The presence of flavonoids in the present study support the opinion of (Mousallamy , 1998) who noted that flavonoids in *Albizzialebbeck* seeds. Also,

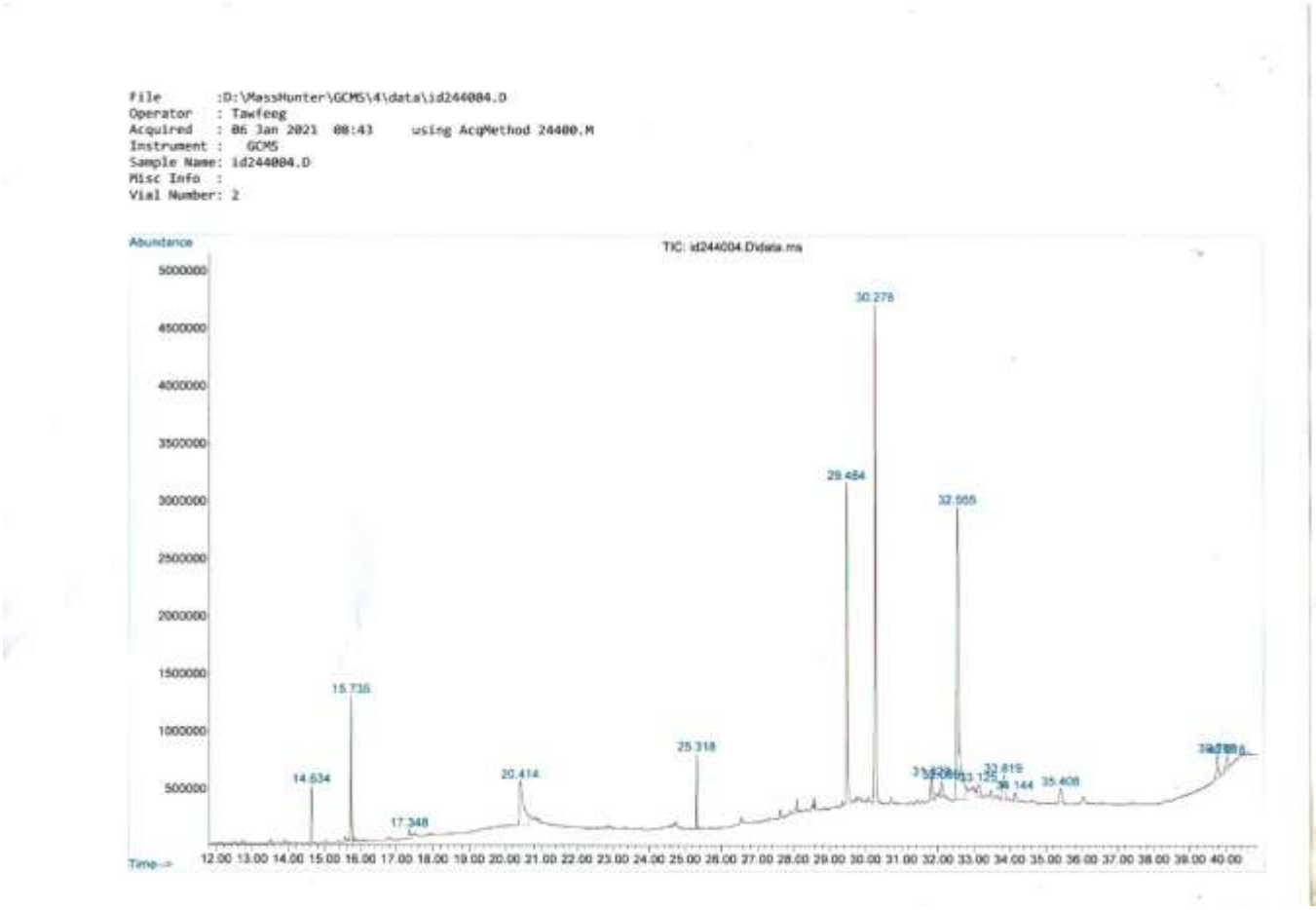
the presence of saponin support the observation of (Ueda , 2003) who reported that saponin in *Albizzia lebbeck* seeds. Tannins and saponins were found to be present.

The Aqueous extract possess higher amount of phytochemical constituents compared to the Petroleum ether extract.The latter showed the absent of saponins and Tannis. However in the aqueous extract absent only of terpens. The presence of seven phytochemical constituents in *Albizia lebbeck (*seeds) indicates its richness in chemical constituents . Hence it indicates its herbal potentials in medicinal applications and partially confirmed its traditionally claimed therapeutic properties. This study is in agreement with the results of (Yasmin Hassan,*et* (2020) who reported "the presence of saponins, tannins and terpenes may be a rationale for the use of the plant in medicine preparations". This is because saponins are active agents with soap like properties". Tannin when present helps in healing of wounds and also has antimicrobials properties (Trease and Evans, 1996) .In addition Flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection (Okwu, and Omodiromiro,2005).

3.2 Chemical Composition by GC-MS

Table (**3.2.1**) list the main components of the oil of *Albizia lebbeck* seeds by the (GC/MS) technique.

Compound Name	Area,%
9,12-Octadecadienoicacid,methyl ester	**25.81**
Chondrillasterol	**5.969**
Docosenoic acid,methyl ester	**7.663**
n-Hexadecanoic acid,methyl ester	**0.668**
Bis(2-ethyl hexyl)phathalate	**2.891**
9-Octadecenoic acid,methyl ester	**15.27**
Ethyl p-methoxycinnamate	**1.954**
Tricosanoic acid,methyl ester	**1.447**
Stigmasterol	**1.228**
Eicosenoic acid,methyl ester	**27.80**
9,10-Anthracenedion1,4-bis(butylamino)	**1.431**
Methyl- 13,16- Docosandienoate	**2.514**
Heptadecenoic acid,methyl ester	**0.652**
Tetracosanoic acid,methyl ester	**1.718**
Phytyl linoleate	**1.965**
Phytyl stearate	**1.001**



Fig(3.2.2): GC/MS chromatogram of *Albizia lebbeck* seeds oil

The *Albizialebbeck* (seeds) oil was analyzed using GC-MS technique. Identification of compounds from oil was based on comparison of their mass spectra with the, computer library of NIST or Wiley Registry of Mass Spectral Data. GC-MS of the oil of *Albizialebbeck* detection and identification of sixteen (16) compounds (Table 3.3.1),which provided a rich source of unsaturated fatty acids (79.71%), Total Saturated fatty acids(16.43%) .

Eight (8) fatty acids were determined, Among them 5 fatty acid were found to be the unsaturated and the rest of the fatty acids were found to be the saturated fatty acids. Some of unsaturated found to be polyunsaturated fatty acids. Linoleic acids, Oleic acid were found to be the most abundant, while palmitic acid was found to be in the least amount.

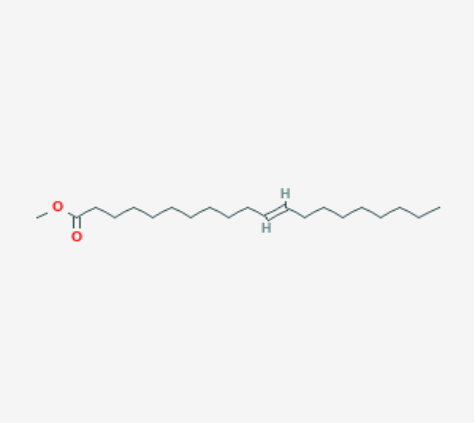
The compounds acquire high percentage in *Albizialebbeck*(L.) Benth seeds oil were Eicosenoic acid, methyl ester (27.80%), 9, 12-Octadecadienoic acid (25.81), 9-Octadecenoic acid (15.27%), Docosenoic acid, methyl ester

(7.663%), Chondrillasterol (5.969%) (sterol lipids has antibacterial properties), while other compounds (Phytyl linoleate (1.965), n-hexadecanoic acid,methyl ester (0.688%), stigmasterol (1.228%)(plays an important role in the regulatory and tissue rebuilding mechanism).

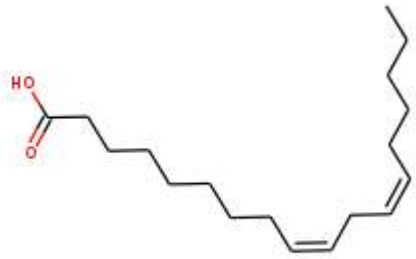
This analysis of *Albizia lebbeck* seeds revealed some of the chemical components and diverse range of bioactive molecules with high therapeutic value making *albizia lebbeck* being a rich source of different types of medicines and plays an important role in drug development programs in the pharmaceutical industry and perfume industry.

This study shows an agreement with the results of (Teena *et al*., (2018), Maria Ellenita (2014), Rayan *et al.* (2020) and Mondira *et al.*, (2016) whom reported, the major component of *Albizia lebbeck* seeds were 9, 12-Octadecadienoic acid,methyl ester , Eicosenoic acid, methyl ester, 9-Octadecenoic acid,methyl ester, Docosenoic acid, methyl ester .

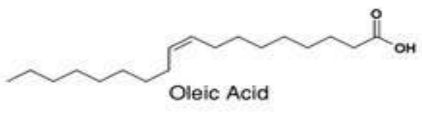
The present study is contrary to the results of (Muhammad *et al*., 2012) who concluded, analysis of the seeds oil sample from Pakistan showed that is the Eicosenoic acid was a minor component (7.3%) while in our in study Eicosenoic acid is a major component (27.80%) as showed GC-MS results above.



Ecosenoic acid

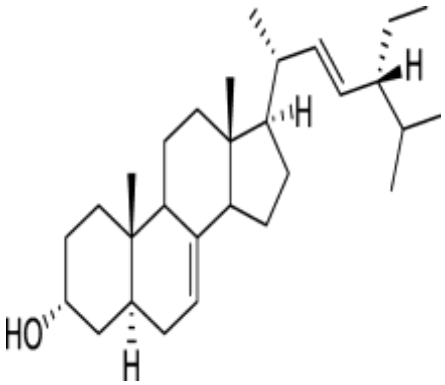


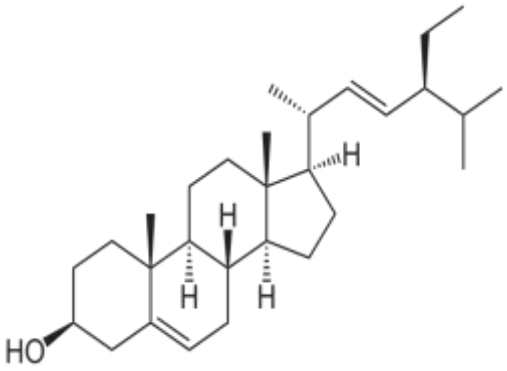
9,12-Octadecadienoic acid



Oleic Acid

9-Octadecadenoic acid





Chondrillesterol **Stigmaserol**

Fig (3.2.3): *Albizia lebbeck* seeds oil constituents

3.3. Biological activities

3.3.1.Antimicrobial Activity

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table-3. The results were interpreted in terms of the commonly used

terms (results<9mm: inactive; 9-12mm: partially active; 13-18mm: active; results>18mm: very active).

Table-3, represented the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

3.3.2. Table-(3.3.2): Antimicrobial activity of *Albizialebbeck*(L.) Benth seeds oil.

Plant Name	**Standard bacterial strains Conc.(100mg/ml)**				
	Gram-negative		**Gram-positive**		**Fungal species**
Albizialebbeck(L.) Benth seeds *oil*	***E.c***	***Ps.a***	***S.a***	***B.s***	***C.a***
	15	10	15	12	10

Standard bacterial strains used ;*S.a* =*Staphylococcus aureus* ,B.s = *Bacillus subtilis* . E.c = *Escherichia coli, Ps.a = Pseudomonas aeruginosa.*

The oil of *Albizia lebbeck* seeds showed activity against *Escherichia coli,, Staphylococcus aureus.*However,it gave partial activity against *Pseudomonas aeruginosa , Bacillus subtilis* and *Candida albicans*.

Figure (3.3.3)- Inhibition Zone against Staphylococcus Aureus

Figure (3.3.4) Inhibition Zone against Bacillus subtilis

Figure (3.3.5)- Inhibition Zone against Pseudomonas aeruginosa

Figure (3.3.6)- Inhibition Zone against Escherichia Coli

Figure (3.3.7)- Inhibition Zone against Candida albicans

Conclusion

According to the results as shown in GC-MS , phytochemical screening and antimicrobial activity Suggest that *Albizia lebbeck* seeds have ability to treat and control infection , and considered as good source for antibacterial agent in the future. Further study may be need in the field of biological activities with different solvents system and different extraction methods.

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