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كلية الدراسات العليا

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بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Phytochemical Constituents and Antimicrobial Activity of *Adansonia digitate* and *Tamarinds indica* leave extracts

المكونات الفيتوكيميائية والفعالية المضادة للميكروبات لمستخلصات أوراق التبلدي والعرديب

A Thesis Submitted In Partial Fulfillment for the Requirements of the Master Degree in Chemistry

By

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(B.Sc. honors, Chemistry)

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September 2022

إستهلال

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ١ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ٢ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ٣ ٱلَّذِي
عَلَّمَ بِٱلۡقَلَمِ ٤ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ٥ ﴾

صدق اللّه العظيم

سورة العلق: ـ الآيات (١ ـ ٥)

Dedication

I dedicate this work to

The soul of my mother,

My Father,

Brothers and Sisters

Acknowledgement

Praise to ALLAH Almighty for giving me strength, health, patience to complete this work. With sincere thanks and gratefulness, I would like to acknowledge my Supervisor **Dr. Mohamed Suleiman Ali Altoum** for his outstanding, knowledge encouragement, guidance, patience and constructive advice throughout this work. My special thanks for all staff of photochemistry department in National Centre for Research and the staff of the department of microbiology – unit of bacteriology in central laboratory for technical support.

Abstract

This study was conducted with the aim to extract and analyze the leaves powder of *Adansonia digitate* and *Tamarindus indica*, collected from forest on the south of Nyala in southern Darfour state, Sudan. Two methods of extractions from the leaves powder of *Adansonia* digitate and *Tamarindus indica* were performed; method 1: extraction by maceration using methanol solvent their yields were 8.3% and 15.6%, respectively. Method 2: successive extractions using soxhlet extractor from leaves powder of *Adansonia digitate* and *Tamarindus indica* using hexane, yielded 8.8% and 6.6 %, respectively. These extracts were subjected to phytochemical analysis *Adansonia digitate* showed the presence of alkaloids, Flavonoids, Tannins, steroids, Glycosides and carbohydrates. Whereas *Tamarindus indica* showed the presence of alkaloids, Tannins, Saponins, steroids, Glycosides and carbohydrates.

The antibacterial activity of methanol leaves extracts of *Adansonia digitate*, *Tamarindus indica* and their combined mixture (1:1) were studied in vitro against four bacterial strains namely *Saphylococcus aurus*, *Bacillus subtitles* (gram +ve bacteria) and *Escherichia coli*, *Pseudomonas aeruginosa* (gram –ve) ,using disc agar diffusion method. *Tamarindus indica* methanol leaves extract showed highest susceptibility against *Bacillus subtilis* at concentration (100mg/mL) with inhibition zone (22mm). *Adansonia digitate* methanol leaves extract show high susceptibility against *Bacillus subtilis* and *Pseudomonas aeruginosa* at concentration (100mg/mL), and (12.5mg/mL) respectively with inhibition zones of (20mm).the combined methanol leave extracts show high susceptibility against *Pseudomonas aeruginosa* at concentration (100mg/mL) with inhibition zone (20mm).

The GC-MS of Adansonia digitate leaves extract revealed the presence of 14 compounds Phenol,2-methyl-5-(1-methylethyl) (61.64%) and unsaturated fatty acid (9-Octadecenoic acid (Z)-, methylester(8.93 %) and Hexadecanoic acid, methyl ester(5.84%) for Adansonia digitate and 10 compounds for Tamarindus indica were completely identified and the major compounds were classified as unsaturated fatty acid including Hexadecanoic acid, methyl ester (35.01%) , Methyl stearate (25.68%) , Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester(22.73%).

المستخلص

أجريت هذه الدراسة بهدف استخلاص وتحليل مسحوق صفق نباتي التبلدي والعرديب ، التي تم جمعها من الغابات جنوب نيالا في ولاية جنوب دارفور غرب السودان. تم إستخدام طريقتين للحصول علي مستخلص مسحوق الأوراق للتبلدي والعرديب ؛الطريقة الأولي: الإستخلاص عن طريق الغمر بإستخدام مذيب الميثانول وكانت نواتجها علي النحو التالي 8.3% و15.6% علي التوالي. الطريقة الثانية : الإستخلاص المتتالي عن طريق جهاز السوكسيليت بإستخدام مذيب الهكسان وكانت نواتجها علي النحو التالي 8.8% و6.6% علي التوالي.

أجري المسح الكيميائي الاولى لمستخلصات الميثانول والهكسان عن وجود القلويدات ، الفلافونويدات التانينات، الستيرويدات ، الجليكوسيدات والكربوهيدرات لمستخلص أوراق التبلدي. بينما أظهرمستخلص أوراق العرديب وجود وجود القلويدات ، االصابونينات ، التانينات، الستيرويدات ، الجليكوسيدات والكربوهيدرات.

في هذه الدراسة تمت دراسة النشاط المضاد للبكتيريا لمستخلصات الميثانول من صفق التبلدي ، العرديب وخليطهما بنسبة (1:1) في المختبر ضد أربع سلالات بكتيرية الموجبة القياسية (البكتريا العصوية الرقيقة والبكتيريا العنقودية الذهبية) والبكتيريا السالبة القياسية (الإشريكية القلونية وبكتيريا الزائفة الزنجارية) وأجريت هذه الإختبارات بطريقة الإنتشارالقرصي .

أظهر مستخلص الميثانول من أوراق العرديب أعلي حساسية ضد البكتريا العصوية الرقيقة بتركيز (100 مغ/مل) مع منطقة تثبيط (22 مم). بينما أظهر مستخلص الميثانول من أوراق التبلدي أعلي حساسية ضد البكتريا العصوية الرقيقة وبكتيريا الزائفة الزنجارية بتركيز(100 مجم/مل) و (12.5 مجم/مل) على التوالي مع منطقة تثبيط (20 مم). فيما أظهر خليطهما بنسبة (1::1) أعلي حساسية ضد بكتيريا الزائفة الزنجارية بتركيز (100 مجم/مل) مع منطقة تثبيط (20 مم).

الGC-MS لمستخلص الميثانول من صفق التبلدي كشف عن وجود 14 مركب ،الفينول – 2 ميثيل-5-(1-ميثيل إيثيل) (61,64%) ، والاحماض الدهنية غير المشبعة ، حمض 9-أوكتاديكانويك(ز)-إستر الميثيل (8,93%) وحمض سداسي ديكانويك، إسترالميثيل(5,84%). وتم تحديد 10مركبات لمستخلص الميثانول من صفق التبلدي وصنفت المركبات الرئيسية على أنها أحماض دهنية غير مشبعة بما في ذلك حمض سداسي ديكينويك ،استر الميثيل (35,01%)، واستيرات الميثيل (25,68%) وحمض سداسي الكانويك، 2-هيدروكسي -(1-هيدروكسي ميثيل) إسترالإيثيل (22,73%).

List of Abbreviations

Ec	*Escherichia coli*
Pa	*Pseudomonas aeruginosa*
Sa	*Staphylococcus aureus*
Bs	*Bacillus subtilis*
ATCC	American Type Culture Collection
NCTC	National Collection of Type Culture
GC\MS	Gas Chromatography Mass Spectrometer
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony forming units
WHO	World health organization
MIC	Minimum inhibitory concentration
NIST	National Institute of Standards and Technology

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Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Natural products

A natural products are chemical compounds or substances produced by a living organisms. Natural products can also be prepared chemically (both semi-synthesis and total synthesis) and have played an important role in the development of organic chemistry by providing challenging synthetic targets. The term "natural product" has also been used commercially to refer to cosmetics, dietary supplements, and foods made from natural sources without the addition of artificial ingredients (Coleate and Molyneux, 1993). Natural products are, typically, defined in the field of organic chemistry as purified organic compounds isolated from natural sources and produced via primary or secondary metabolism pathways. In the field of medicinal chemistry, the definition is frequently narrowed to secondary metabolites (Coleate and Molyneux, 1993).

Natural plant products may also be useful in reducing the side effects of various chemotherapeutic agents and in extending life. The global interest in plant medicinal potential over the last few decades is thus quite logical (Kaushik and Dhiman, 2000). The use of a combination of natural products to treat diseases has produced a number of intriguing results, most notably the synergistic effects and pharmacological action of plant extracts from commonly therapeutic areas, most notably anti-infective, cardiovascular, and fields of anticancer (Martin and Ernst, 2003).

Another advantage of using plant-based medicines is that they have few side effects than synthetic medications. This could be because the active compounds found in plants are in lower concentrations than the human body requires (Tsuda et al., 2004).

Antibiotic resistance in bacteria is becoming a growing public health concern. Because of super resistant strains, most antibiotics on the market are failing to treat many bacterial infections. As a result, scientists have continued to look for new antibacterial agents, either by designing and synthesizing new agents or by searching for natural sources. Herbal medications, in particular, have seen a resurgence of interest due to the perception that plant preparations have a lower incidence of adverse reactions than synthetic antibacterial drugs. This, combined with lower costs, makes the search for natural therapeutics from plants an appealing option (Cock, 2008).

Infectious diseases have a negative impact on life quality and can lead to systemic and life-threatening diseases. Antibiotics have saved millions of lives and contributed to significant increases in life expectancy over the last century. However, the clinical efficacy of many existing antibiotics is being jeopardized by the emergence of multi-drug resistant pathogens, as well as the recent appearance of strains with reduced susceptibility and undesirable side effects of certain antibiotics; because of the increased bacterial resistance to antibiotics, toxic and harmful effects of, few common, antibacterial agents; therefore, there is an urgent need to study the biological properties of additional medicinal plants in order to create new antibiotics (Fernández et al., 2016).

1.2 Medicinal plants

Medicinal plants (also known as herbs, herbal medicines, pharmacologically active plants, or phytomedicinals) continue to be the most widely used form of medicine in the majority of countries. Over three-quarters of the world's population relies on raw plant products to meet their daily health care needs (Barrett and Kieffer, 2001).

The majority of plant materials collected are used to obtain fresh extracts from the entire plant or parts of it, (which could be) leaves, roots, flowers, or fruit. In the case of woody forms, the bark, roots, and other parts are mostly used (Rao and Arora, 2004).

Herbal medicine, also known as botanical medicine or phytomedicine, is the medicinal use of any plant's seeds, berries, roots, leaves, bark, or flowers. Botanical medicine, medical herbalism, herbology, and phytotherapy are all terms for herbal medicine. Humans' use of herbs and spices yields useful medicinal compounds (Tapsell, 2006).

With the development of phytochemistry and pharmaceutical chemistry, it has become easier to use active compounds isolated from plants or their synthetic equivalents in medicine. This is due to the fact that medicinal plants contain more chemical diversity and novelty than any other source (Cragg *et al*., 2006).

Africa has an enormously rich biodiversity and knowledge in the use of plants to treat various ailments. In fact, the WHO estimates that 80 percent of the population in Sub-Saharan Africa relies solely on traditional medicine derived from plants for their primary healthcare needs due to their availability, low cost, and socio-cultural background (Jayaweera, 1982).

1.3. Phytochemicals from plants

Phytochemicals are defined as chemical compounds produced by plants (Harborne, 1973). They are divided into two categories namely primary and secondary metabolites**.**

1.3.1 Primary metabolites

Primary metabolism refers to the processes that produce carboxylic acids of the Krebs cycle, α-amino acids, carbohydrates, fats, proteins, and nucleic acids, all of which are required for the organism's survival and well-being Fig (1.1). All organisms share the same metabolic pathways through which these compounds are synthesized and utilized (Torssell, 1990).

1.3.2 Secondary metabolites

Secondary metabolites are chemical compounds produced by living organisms. Natural product research entails isolating these compounds in their purest form and investigating their structure, formation, use, and purpose in the organism.

Secondary metabolites appear to serve, primarily, as defense against predators and pathogens (Stahl *et al*., 1967).

Secondary metabolites, on the other hand, are non-essential to life but contribute to the species' fitness for survival. Secondary metabolites are also produced using other metabolic pathways than primary metabolites. These pathways are more characteristic for the particular family or genus and are related to the mechanism of evolution of species (Torssell, 1990).

Plant secondary metabolites are a diverse group of chemical compounds that are thought to be produced by the majority of plant species in response to biotic and abiotic stresses Fig (1.1). Historically, the term "secondary metabolites" has been used to refer to all compounds that are not "primary metabolites," that is, metabolites that are not required for an organism's growth, development, and reproduction (Fernandez *et al*., 2016). Terpenoids, alkaloids, sulfur-containing compounds, and phenolic compounds are the four major secondary metabolites in plants (Dillard and Bruce German, 2000).

The phytochemical content of medicinal plants were used to demonstrate and isolate the drug lead compounds and components from plant parts. The phytochemical properties of the plants, such as alkaloids, saponin glycosides, alkaloids, phytosterols, terpenoids, tannins, sterol, polyphenols, flavonoids, and anthranoids, can identify their unique biological activity. The majority of plant parts used for phytochemical property analysis were leaves, roots, barks, and fruits. The medicinal plants were studied for phytochemical constituents of ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water) extracts of various phytochemicals (Talema, 2020).

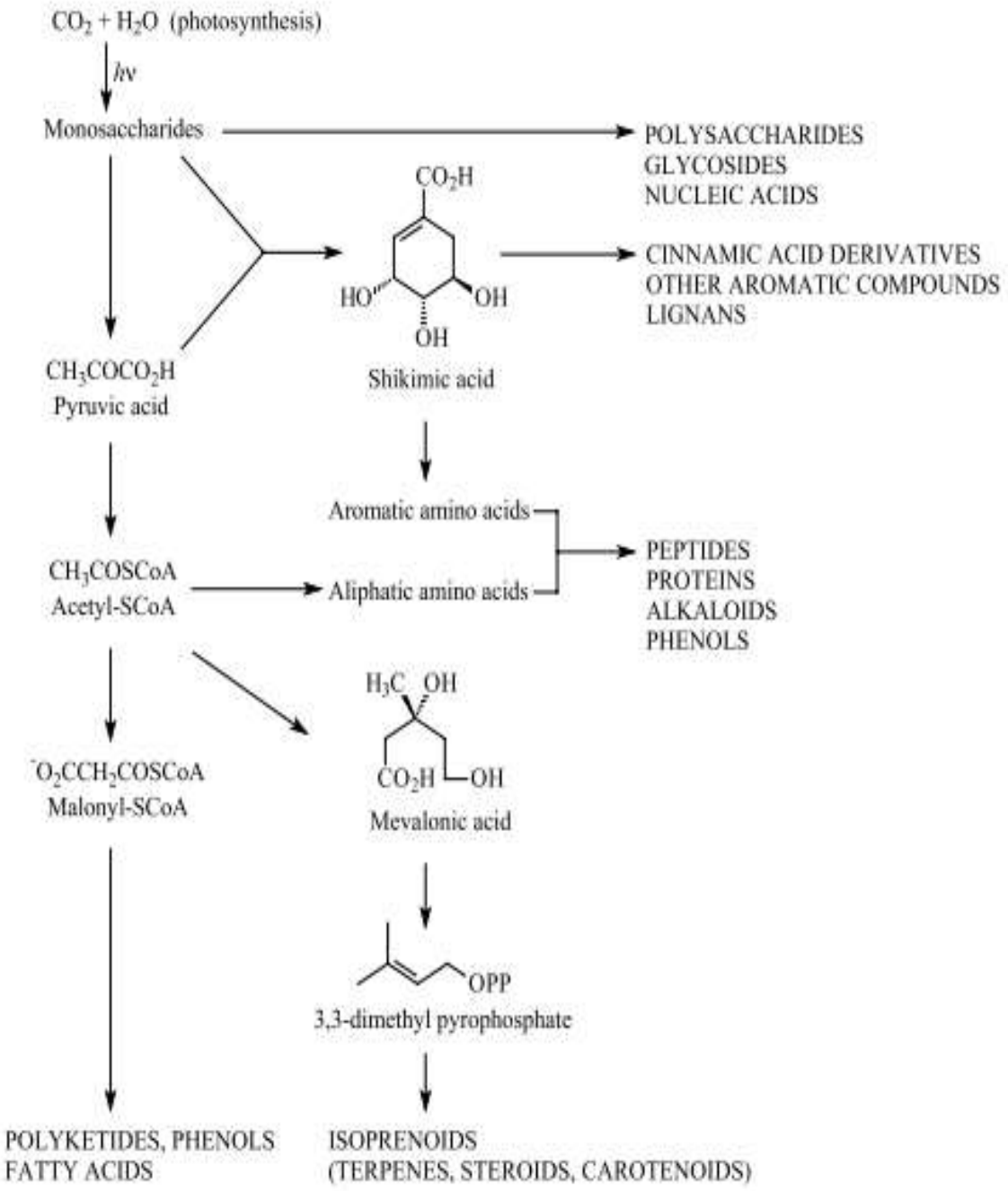

Figure (1.1). Primary and secondary metabolites.

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1.3.2.1 Phenolic compounds

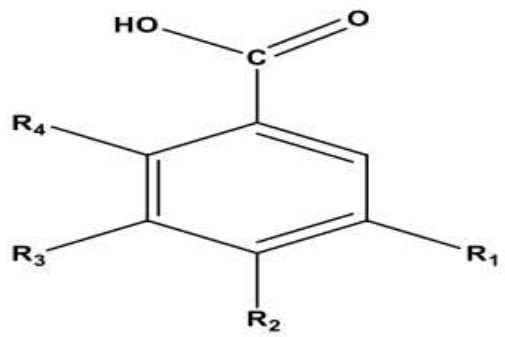
Phenolic compounds can be divided into several classes depending on the structure of the aglycone .The main phenolic subclasses in fruits are phenolic acids (hydroxybenzoic and hydroxycinnamic acids), coumarins, flavonoids and hydrolysable and condensed tannins (Hounsome *et al*., 2008).

Plants synthesize phenolic compounds during normal development and in response to stress conditions such as infection, wounding, UV irradiation, herbivores, and reactive oxygen species (Beckman, 2000). They are the most stable and powerful type of dietary antioxidants, with high in vitro, antioxidant capacity than vitamins and carotenoid antioxidants (Gardner *et al*., 2000).

1.3.2.1.1 Phenolic acids

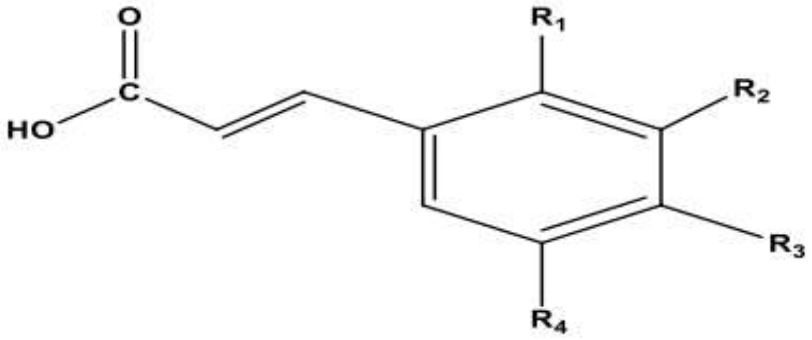
Phenolic acids are low-molecular-weight compounds classified into two groups: derivatives of benzoic acids with a C1-C6 backbone Fig (1.2.1) and derivatives of cinnamic acids with a C3-C6 backbone Fig (1.2.2). Only acid or alkaline hydrolysis or enzymes can liberate phenolic acids (Tsao, 2010).

Protocatechuic, vanillic, gallic, and syringic acids are examples of benzoic acids. Hydroxybenzoic acids are found in complex structures like hydrolyzable tannins (gallotannin*s* in mangoes and ellagitannins in red fruits such as pomegranate, strawberries, raspberries, and blackberries). Cinnamic acid is one of the most common phenolic acids found in many plants, including grapes, tea, green coffee beans, yerba mate, and others. Hydroxycinnamic acids, which are more common than hydroxybenzoic acids, are, primarily, composed of p-coumaric, caffeic, ferulic, and sinapic acids (Manach *et al*., 2004).



1. Salicylic acid (R_4 = OH; R_1 = R_2 = R_3 = H)
2. Gallic Acid (R_1 = R_2 = R_3 = OH; R_4 = H)
3. Gentisic acid (R_1 = R_3 = OH; R_2 = R_4 = H)
4. *p*-Hydroxybenzoic acid (R_2 = OH; R_1 = R_3 = R_4 = H)
5. Vanillic acid (R_1 = OCH_3; R_2 = OH; R_3 = R_4 = H)
6. Protocatechuic acid (R_1 = R_2 = OH; R_3 = R_4 = H)
7. Syringic acid (R_1 = R_3 = OCH_3; R_2 = OH; R_4 = H)

Figure (1.2.1).The basic formula and names of the main benzoic acids.



1. *o*-Coumaric acid (R_1 = OH; R_2 = R_3 = R_4 = H)
2. *m*-Coumaric acid (R_2 = OH; R_1 = R_3 = R_4 = H)
3. *p*-Coumaric acid (R_3 = OH; R_1 = R_2 = R_4 = H)
4. Caffeic acid (R_2 = R_3 = OH; R_1 = R_4 = H)
5. Ferulic acid (R_2 = OCH_3; R_3 = OH; R_1 = R_4 = H)
6. Sinapic acid (R_2 = R_4 = OCH_3; R_3 = OH; R_1 = H)

Figure (1.2.2). The basic formulas and names of the main cinnamic acids.

1.3.2.1.2 Flavonoids

Flavonoids are polyphenolic compounds that occurs naturally in plants. When it comes to the means of defense and communication that plants have evolved, flavonoids are one of the most important chemical classes of natural products. Flavonoids first appeared in green algae 500 million years ago, the result of a fusion of two biogenetic pathways, the cinnamate route and the ancient polyketide route. They have then become increasingly complex as plants have evolved (Swain *et al*., 1975).

Flavonoids along with carotenoids and chlorophyll, provide color to many species of flowers and fruits. They are only found in plants, where they are mostly found as glycosides, which are formed when one or more hydroxyl groups of phenols combine with reducing sugars (El Gharras, 2009).

Flavonoids are also linked to a variety of biological effects on health, such as antibacterial, anti-inflammatory, anti-allergic, and antithrombotic properties. The term flavonoid refers to polyphenolic compounds with the general structure C6-C3-C6 and two phenolic benzene rings A and C linked by a pyran ring B Fig (1.3)(Pyrzynska and Biesaga, 2009).

Flavonoids are the most abundant type of plant phenolic compound. According to the type of heterocycle involved, they are classified into six subclasses: flavonols, flavones, flavanones, isoflavonoids (isoflavones), flavanols, and anthocyanidins (Aharne and O'Brien, 2002; Manach *et al*., 2004).

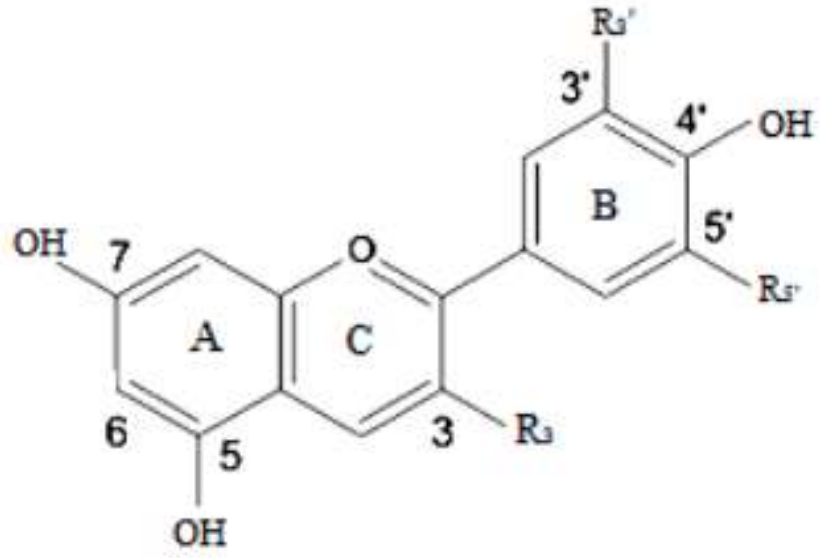


Figure (1.3). Basic flavonoid structure.

1.3.2.1.3 Tannins

Tannins are oligomeric, polyphenolic compounds with high molecular weights that accumulate, naturally in many plants as byproducts of secondary plant metabolism (Caygill and Mueller-Harvey, 1999). They greatly vary in structure between plant species, but most tannins have one thing in common: they precipitate protein. Tannins are classified chemically into two groups: hydrolysable tannins Fig (1.4.1) and condensed tannins Fig (1.4.2).Hydrolysable tannins are polyesters of sugars (mostly glucose) and gallic or ellagic acids that are, generally, thought to be harmful to animal nutrition. Condensed tannins are flavan-3-ol polymers. They are also known as proanthocyanidins because they form colorful anthocyanidins when heated in the presence of acid (Serrano *et al*., 2009).

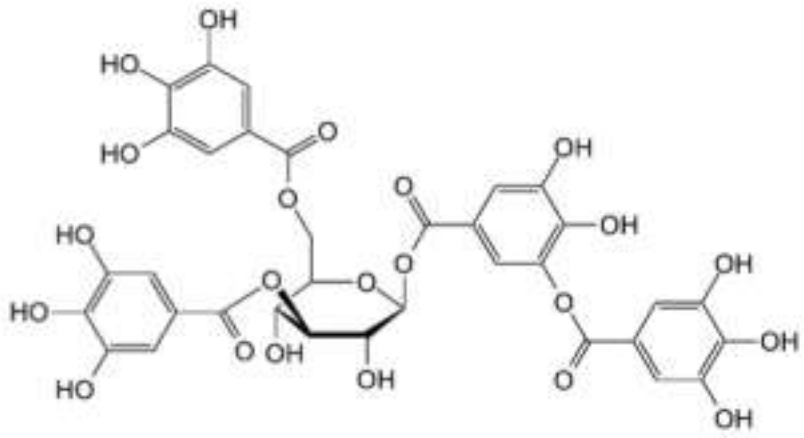


Figure (1.4.1). Structure of hydrolyzable tannins.

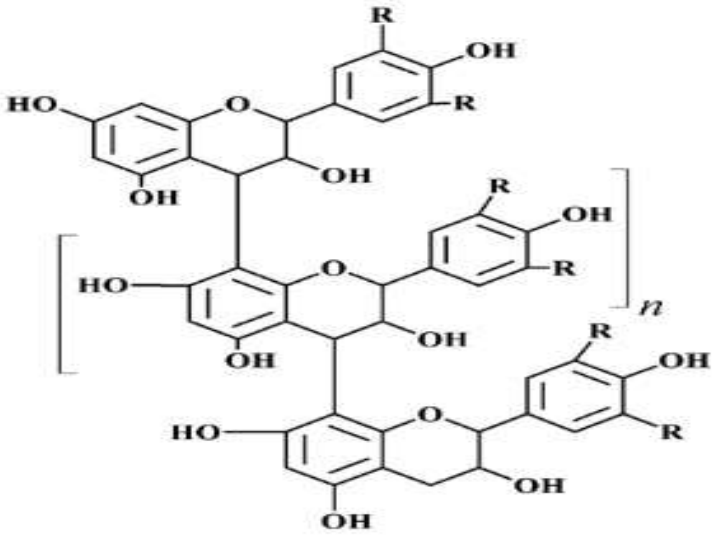


Figure (1.4.2). Structure of a condensed tannin.

1.3.2.2 Alkaloids

Alkaloids Fig (1.5) are chemical compounds produced by plants' secondary metabolism. They are alkaline substances because they all contain, at least one nitrogen atom. The majority of alkaloids are N-heterocyclic compounds (sometimes called true alkaloids). Other nitrogen-containing alkaloids include amino acid-derived alkaloids (protoalkaloids), peptide and cyclopeptide alkaloids, and polyamine alkaloids.The final group of alkaloid-like substances

includes compounds with steroid, purine, and terpene (Waisser and Karel, 2001).

Some alkaloid bioactive components, such as morphine and cordine, have been shown to be effective not only against bacterial and fungal pathogens, but also against trypanosomes and plasmodia (Freiburghaus *et al*., 1996). Some alkaloids found in dietary food materials have also been found to have a bacterial and antidiarrheal effect in the small intestines, where they have the ability to intercalate with microbial genetic material (Ghoshal et al., 1996).

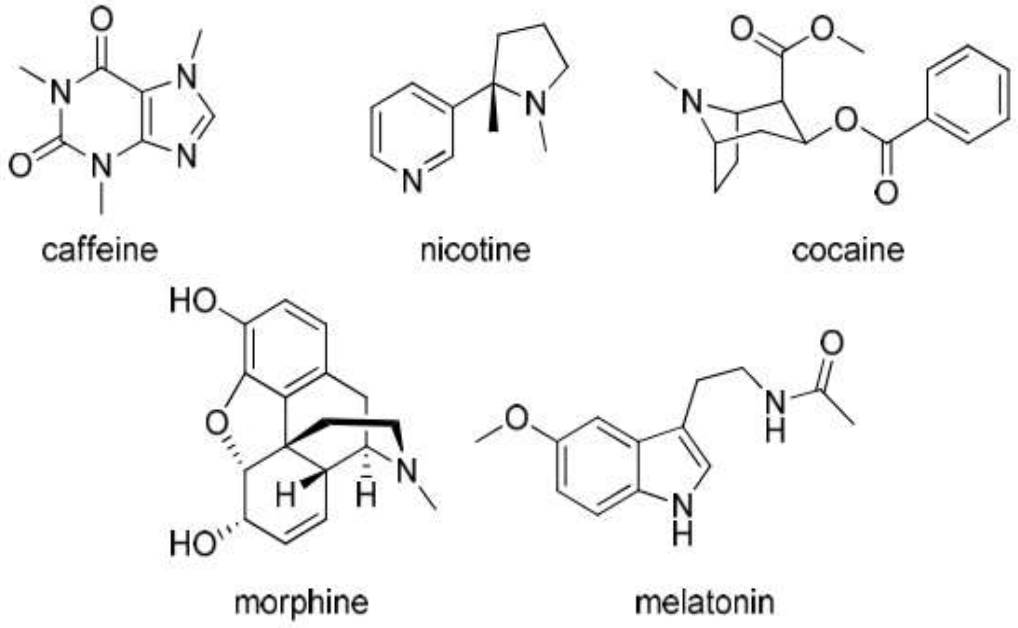


Figure (1.5). Chemical structure of some widely known alkaloid.

1.3.2.4 Terpenes and steroids

Terpenes is the generic name for a class of natural products composed of isoprene (isopentenyl) units Fig (1.6). The term may also refer to the terpenoids, which are oxygen derivatives of these compounds. Wallach developed the theory that provided the first conceptual framework for a common structural relationship among terpenes in 1887 after conducting structural investigations on several terpenes (Tirillini *et al*., 1999).

Terpenes, according to his theory, are made up of one or more isoprene (2-methyl-1,3-diene) units joined together head to tail. Ruzicka's formulations of the biogenetic isoprene rule in the 1950s refined Wallach's idea by emphasizing mechanistic considerations of terpene synthesis in terms of electrophilic elongations, cyclizations, and rearrangements (Kawabata *et al*., 1990).

Terpenoids are classified into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes based on the number of C5 isoprene units present in the molecule. Steroids are C30 derived from terpenoids, which are frequently thought to be a separate class of metabolites. Steroids are synthesized from squalene (C30; one of the first steps in sterol synthesis is the formation of squalene from two C15 farnesene molecules) and triterpenes of the terpenoid synthesis pathway (Dick *et al*., 2012).

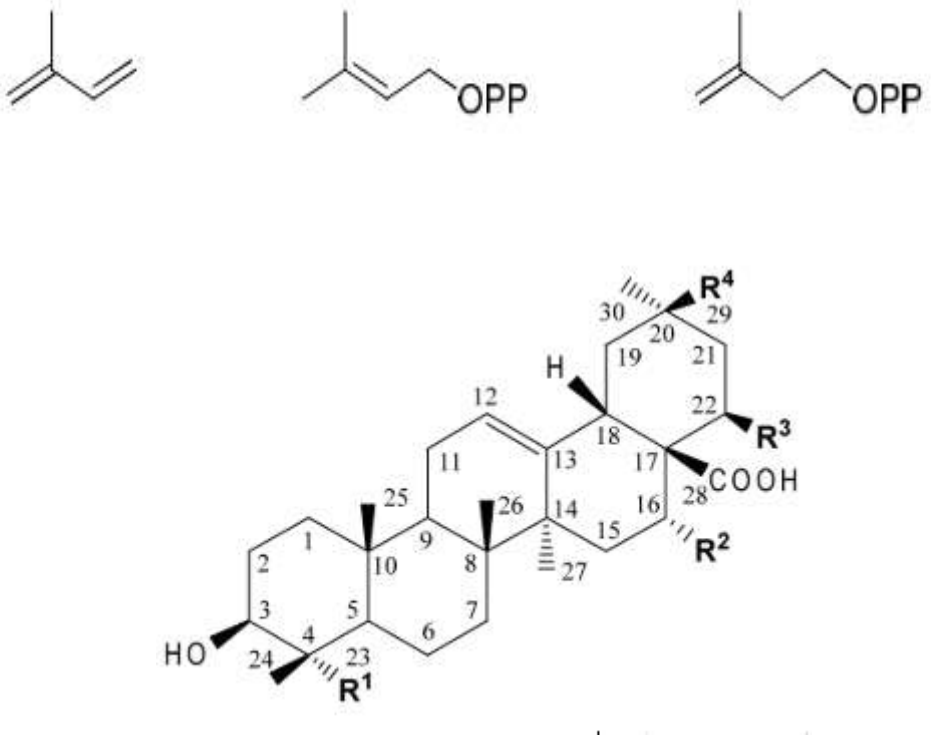


Figure (1.6). Molecular structures of isoprene, dimethylallyl diphosphate, isopentenyl diphosphate and carbon skeleton of triterpenes (30C).

1.3.2.5 Saponins

Saponins are one of the most abundant groups of naturally occurring glycosides. They are mostly found in plant species, but they are also found in some lower marine animals (Hostettmann and Marston 1995). Saponins are distinguished by their rather they have ability to form stable, soap-like foams in aqueous solution, complex molecular structure, which consists of water-soluble (hydrophilic) sugars attached to a lipid-soluble triterpene or steroid triterpene. Hydrophilic part provides a number of physicochemical properties, including wetting, emulsifying, and foaming properties, as well as being surface active as a detergent. Saponins have been used in the production of a wide range of everyday products, including toothpaste, shampoos, and cosmetics, due to their unique properties (Shi *et al*., 2004).

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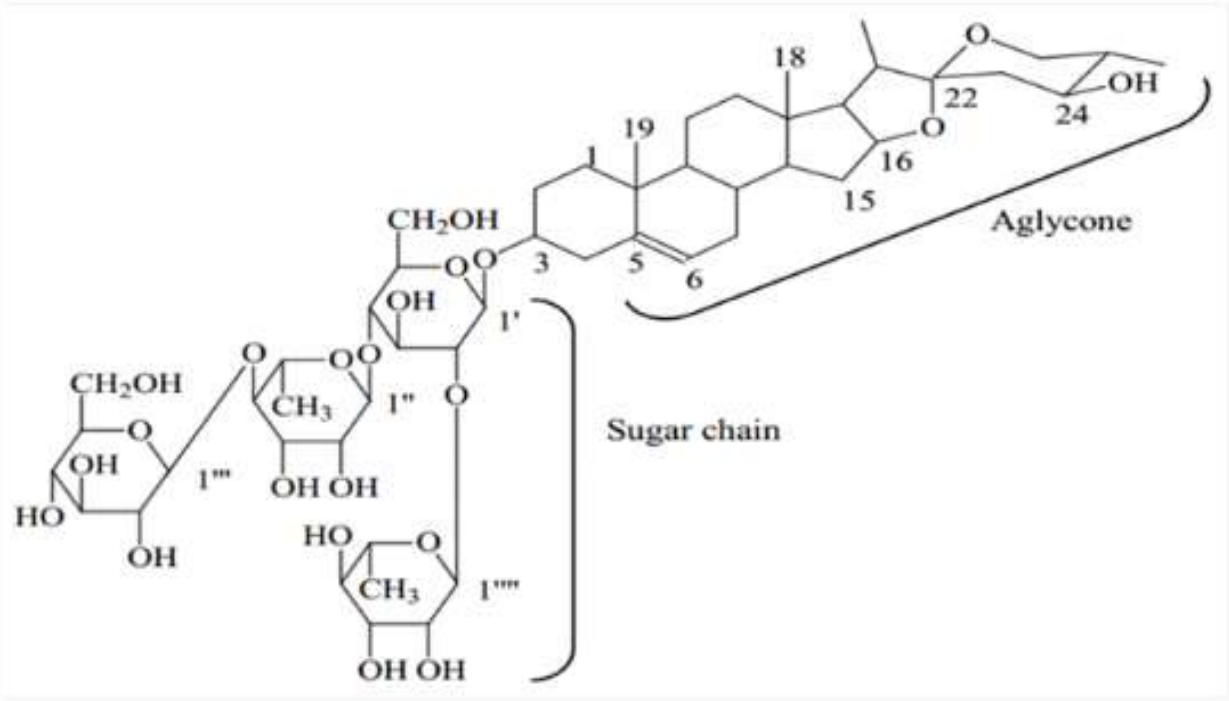

Figure (1.7) .the chemical Structure of steroid saponin.

1.3.2.6 Cardiac Glycosides

Cardiac Glycosides Fig (1.8) are complex in the same plant, and while the majority of them are toxic, many have pharmacological activity, particularly to the heart. They are used to treat congestive heart failure by inhibiting the Na+/K+-ATPase pump, resulting in positive ionotropic effects and electrophysiological changes. This improves heart muscle strength and systolic concentration against congestive heart failure (Ogunwenmo *et al*., 2007). They are also used to treat atrial fibrillation, to flatter, and as emetics and diuretics (Harborne, 1973).

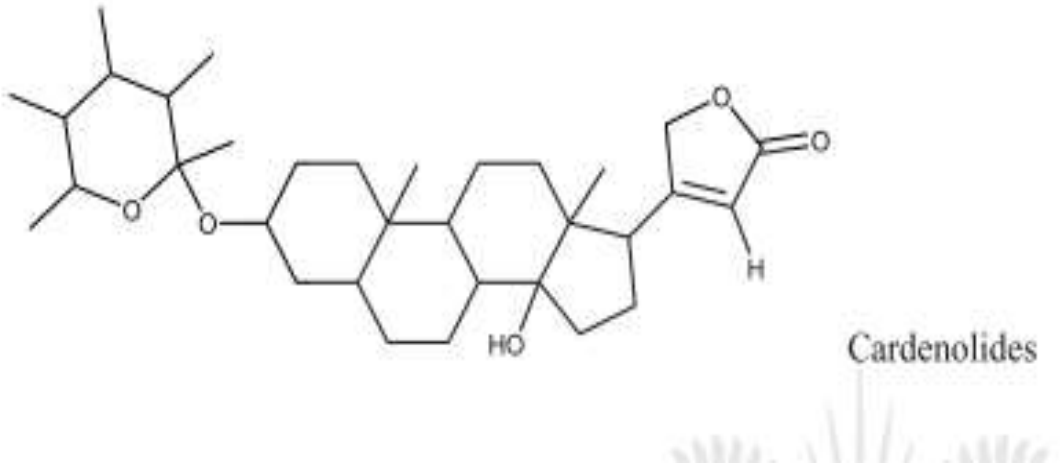

Figure (1.8). Structure of cardiac glycoside.

1.4 Natural Products as antibacterial agents:

Antibacterial agents are substances that inhibits bacterial growth and infection. Antibacterial agents attack bacterial metabolic processes such as protein synthesis, interfere with bacterial cell wall synthesis and function, or target bacterial DNA (Anderson *et al*., 2012).

The emergence and re-emergence of lethal infectious diseases caused by pathogenic microorganisms, such as influenza viruses, cholera, and hepatitis B, is regarded as a global crisis (Morens and Fauci 2013; Natarajan *et al*., 2014). Antimicrobial resistance in bacteria has increased over the years, making

treatment of bacterial infections difficult and complicated by antibiotic resistance (Tenover, 2006).

Furthermore, there have been numerous health issues associated with currently, available synthetic antimicrobial agents, necessitating the development of new, broad-spectrum, more active, and safe antimicrobial agents (Natarajan *et al*., 2014).

Bacterial resistance to antimicrobial agents can be acquired through de novo mutation or through the acquisition of resistant genes from other organisms (Tenover, 2006). Bacterial resistance could be caused by the production of enzymes that destroy the antibacterial drug, the development of efflux systems that prevent the drug from reaching its intracellular target, the modification of the drug's target site, and the development of an alternative pathway that by passes the drug's action (Tenover, 2006).

Plant extracts have been used to treat infectious diseases caused by pathogenic fungi, viruses, and bacteria (Buwa and Van Staden 2006), and plant materials continue to be an important natural source in combating various diseases around the world (Natarajan *et al*., 2014). The ability of plants to synthesize de novo antimicrobial agents in response to microbial attack has led to the exploration of medicinal plants in the hope of developing new antibacterial drugs (Natarajan *et al*., 2014). Natural drugs can be either original natural or synthetic products based on natural plant structures. They can also be derived or chemically synthesized from natural sources (Demain and Sanchez, 2009). Pefloxacin, norfloxacin, ciprofloxacin, and levofloxacin are examples of natural antibiotics derived from the alkaloid quinine, an active compound found in the plant *Cinchona succirubra* (Demain and Sanchez, 2009).

1.4.1Type of tasted organism

Antibacterial activity of *Adansonia digitate* and *Tamarindus indica* were evaluated against the standard pathogenic microorganisms shown below:

Table (1.1): Type of tasted organism.

No	organisms	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeruginosa*	G-ve
4	*Escherichia coli*	G-ve

1.4.1.1 *Bacillus subtilis*

B. subtilis is a Gram-positive, facultative aerobic, sporulating bacteria that is commonly found in soil as well as in the gastrointestinal tracts of humans and ruminants. *B. subtilis* is normally thought to be non-pathogenic; however, it has been linked to food-borne illnesses causing diarrhea, nausea, and vomiting, and is associated with rice dishes served in oriental restaurants, and its infection is self-limiting (Prescott *et al*., 2008).

B. subtilis produces "subtilism," an extracellular enzyme that catalyzes the breakdown of proteins into polypeptides and has been shown to be a potent occupational allergen (Prescott *et al*., 2008; Chandra & Khan, 2013).

1.4.1.2. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive cocci that belongs to the family *Micrococcaceae* and is part of the genus *Staphylococcus*. *S. aureus* is non-motile and non-capsulate, and it occurs in groups but also singly and in pairs (Cheesbrough, 2006).

It is characterized as catalase positive and coagulase, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow *staphylococci* (Stark, 2013).

S. aureus is a commensal bacterium as well as a human pathogen. *S. aureus* colonizes approximately 30% of the human population, causes device-related infections, and causes a variety of skin and soft tissue infections ranging from the benign (e.g., impetigo and uncomplicated cellulitis) to the immediately life threatening (Tong *et al*., 2015).

1.4.1.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a large genus of Gram-negative, aerobic rod bacteria in the *Pseudomonadaceae* family. It is found on the skin of some healthy people as well as in soil, water, and vegetation (Todar, 2004). It is pothogenic only when introduced into areas devoid of normal defenses, such as skin and mucous membrane disruption following direct tissue damage. The bacterium attaches to and colonizes mucous membranes or the skin, then invades locally and causes systemic disease. *P. aeruginosa* infections that are the most serious include wound and burn infection, malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (Bodey *et al*., 2007).

1.4.14. *Escherichia coli*

E. coli is the most well-known and important species of the genus *Escherichia*, which is one of the most common members of the *Enterobacteriaceae* family. E. coli is a Gram-negative, motile rod; some strains are capsulated, aerobic, and facultatively anaerobic. The ideal temperature for growth is 36-37 ℃. It is naturally found in the intestine, soil, and water and is the most common pathogen isolated from cystitis patients (Cheesbrough, 2006).

Gastrointestinal tracts. *E. coli* is the most common cause of urinary tract infection in humans, causing at least five different types of gastrointestinal diseases. The presence of one or more virulence factors, such as invasiveness factors, heat-liable and heat-stable enterotoxins, verotoxins, and colonization factors, is generally responsible for pathogenicity.Detection of specific

virulence factors or a serotype associated with a virulence factor is usually used to identify pathogenic strains (Akinnibosunet *al*., 2008).

1.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing, commonly referred to as in vitro testing, aims to ascertain how well an antibiotic inhibits the growth of a bacterial isolate in a laboratory setting. A highly defined approach is used in antimicrobial susceptibility testing to provide repeatable and trustworthy results. There have been numerous methods developed and they are categorized into two main groups: diffusion and dilution based techniques Depending on the testing method utilized either a qualitative or quantitative result will be determined (Balouiri *et al*., 2016).

1.5.1 Diffusion based techniques

1.5.1.1 Disk diffusion test

The Kirby-Bauer antibiotic testing method was first introduced by Bauer and colleagues in 1966 (Bauer *et al*., 1966). On agar plates inoculated with the test microorganism, antibiotic-impregnated discs are positioned an upward-sloping gradient of medication concentration is created as the antibacterial agent diffuses radially into the agar, starting near the disk and waning outward (Matuschek *et al*., 2014).

Depending on the bacterium's susceptibility to an antimicrobial agent, bacteria only grow up to the highest concentration they can tolerate, resulting in a zone of inhibition where no bacterial growth occurs. To the nearest millimeter, the inhibitory zone's diameter is measured (Clsi, 2018; Eucast, 2018).

1.5.2 Dilution based techniques

1.5.2.1 Agar dilution

A conventional bacterial inoculum of 1.0×10^4 CFU is spotted onto the surface of Mueller-Hinton agar plates, which have been prepared using repeated antibiotic dilutions. The MIC is the plate with the lowest antimicrobial concentration on which no growth is seen (Clsi, 2015).

1.5.2.2 Broth dilution

A series of antimicrobial concentrations made in Mueller-Hinton broth make up a broth dilution. Microtiter plates or large tubes (macro broth dilution) can be used for this procedure (micro broth dilution). In both human and animal diagnostic laboratories, the test of broth microdilution is frequently utilized.

In order to inoculate commercially available 96 well microliter plates, a bacterial inoculum produced to a density of McFarland 0.5 standard is added to broth media, resulting in a final inoculum of 5.0×10^5 CFU/ml .Using a manual (mirror box) or automated viewing apparatus, the MICs are identified by looking for either a lack of turbidity or a lack of a cell pellet in each well (Jorgensen and Ferraro, 2009; Clsi, 2015).

1.6 Gas Chromatography-Mass Spectrometry (GC-MS)

Fundamentals of GC-MS: Complex organic and biological mixtures are analyzed using GC/MS, which combines the two analytical methods of GC and Mass Spectrometry. There are two primary parts to the GC-MS instrument. By circulating an inert gas (mobile phase), which transports the sample, the gas chromatography part separates various components in the sample into pulses of pure chemicals based on their volatility. The mass spectrometer gathers compound spectra as they leave a chromatographic column, identifying and quantifying the substances based on their mass-to-charge ratio (m/z). The computer can then be used to store and analyze these spectra (Skoog *et al*., 2007).

1.7 Plants under study

1.7.1 *Adansonia digitate*

The origin of the common name "baobab" is unknown. Most scientists believe it is derived from the Arabic word "buhibab," which means "fruit with many seeds" (Diop *et al*., 2005). The genus *Adansonia* is named after the botanist Michel Adanson (1727-1806) (Esterhuyse *et al*., 2001). Because of the shape of the leaves, the species name *digitata* (hand-like) was chosen. Eight baobab

species have been identified worldwide, with six of them being endemic to Madagascar, the genus *Adansonia's* presumed center of evolutionary origin. The African species *Adansonia digitata* is found throughout Sub-Saharan Africa's savanna woodlands (Wickens and Lowe, 2008).

The only species that is not native to Africa is *Adansonia gibbosa* (A.Cunn.) Guymer ex D.A.Baum, which is native to Australia (Drake, 2006; Wickens and Lowe, 2008).

1.7.1.1 Scintific Classification of *Adansonia digitate*

Kingdom : *Plantae*

Division : Tacheophyta

Class : *Magnoliopsida*

Order : *Malvaless*

Family : *Malvaceae*

Genus : *Adansonia*

Species : *digitata*

Botanical name: *Adansonia digitate* (Rahul *et al*., 2015)

1.7.1.2 Botincal Description of Baobab Tree

Baobab (*A. digitata L.),* a *Malvaceae* family tree, is found throughout the hot, arid regions of tropical Africa. It is a deciduous, massive, and majestic tree up to 25 m tall that can live for hundreds of years. The trunk is swollen and stout, up to 10 m in diameter, tapering or cylindrical and abruptly bottle-shaped, and frequently buttressed. Branches are dispersed irregularly and in large numbers. Smooth, reddish brown to grey bark that is soft and fibrous. The tree has a dense lateral root system that terminates in tubers. The leaves are foliate and alternate. Young tree leaves are frequently simple. The mature leaf size can reach 20 cm in diameter. Flowers are large and showy, pendulous, solitary or paired in leaf axils. Flower buds are globose (occasionally ovoid). The baobab tree's fruit hangs singly on long stalks and has an ovoid, woody, indehiscent shell 20 to 30 cm long and up to 10 cm in diameter. The shell contains

numerous hard, brownish seeds that are round or ovoid in shape and up to 15 mm long, embedded in a yellowish-white, floury acidic pulp. The ripe fruit pulp is naturally dehydrated, powdery, whitish in color, and has a slightly acidulous flavor (Kaboré *et al.*, 2011).

1.7.1.3 Distribution and occurrence of baobab

It is found in parts of western, north-eastern, central, and southern Africa, as well as Oman and Yemen in the Arabian Peninsula, Asia. According to Sidibe and Williams (2002), the African baobab grows naturally in most of the countries south of the Sahara and is particularly associated with drier savanna or a minimum of 300 mm of annual rainfall. However, forest areas associated with human habitation exist. The plant grows in Africa at latitudes 16°N and25° S in areas with less than one day of frost per year, with extensions of its distribution into forest areas associated with human habitation. The plant grows in Africa at latitudes of 16o N and 25o S in areas with no more than one day of frost per year (Kamatou, *et al*., 2011).

outside of Africa, including northern Australia and a number of Asian countries such as India, Sri Lanka, Indonesia, and the Philippines, as well as parts of the Middle East and the West Indies (Sidibe and Williams, 2002).

In Sudan, they are mostly found as monumental individuals or in clumps on rocky outcrops, which give this area a distinct character. In Kordofan, Darfur, and the Blue Nile, baobabs form belts (Darr *et al*., 2016).

1.7.1.4 Chemical Constituents

The leaves have a high concentration of essential amino acids, minerals, and vitamin A. It contains (in dry weight) 13-15 percent protein, 60-70 percent carbohydrate, 4-10 percent fat, 11 percent fiber, and 16 percent ash. The energy value ranges from 1180 to 1900 kJ/100g, with 80 percent of the energy being metabolizable. The leaves also have a significant amount of mucilage, which when hydrolyzed yields galactouronic acid and glucouronic acid, as well as small amounts of galactose, rhamnose, glucose, and arabinose. In addition, bark

contains fat, calcium, copper, iron, and zinc. Furthermore, betulinic acid was isolated from the bark, whereas the leaf only yielded taraxerone and lupeol and baurenol acetate (terpenoids).The dry baobab fruit pulps have high carbohydrate, energy, calcium, potassium (very high), thiamine, nicotinic acid, and vitamin C values (very high). The pulp of the baobab fruit is high in mucilage, pectins, tartarate, and free tartaric acids. The tartarate content gives rise to the name "cream of tartar tree." Fructose, saccharose, and glucose content provide sweetness to the pulp. Fruit pulps are also acidic due to the presence of organic acids such as citric, tartaric, malic, succinic, and ascorbic acid. The pulp is high in calcium and vitamins B and C when eaten raw (De Caluwé *et al*., 2010).

1.7.1.5 Ethno Medicinal Uses

- Diarrhea and dysentery
- Promote granulation
- Sickle cell anemia
- Bronchial asthma
- Dermatitis
- To treat Hiccoughs in infants and children
- Diminishing the heat and quenching the thirst
- Substitute for cinchona bark
- Laxative
- Source of cream of tartar
- To treat fatigue and insect bites
- Baobab oil is used on inflamed gums and to ease diseased teeth
- The baobab bark was exported to Europe for use as a fever treatment (De Caluwé 2010).

1.7.2 *Tamarindus indica*.

Tamarind fruit was thought to be produced by an Indian palm at first, as the name tamarind comes from the Persian word 'tamarihind,' which means 'date of India.' Its Sanskrit name, 'amlika,' denotes its long presence in the country. Morton (1987) attributed its origin to the Indian Brahmasamhita scriptures between 1200 and 200 BC, but others believed it was indigenous to the drier savannahs of tropical Africa, from Sudan, Ethiopia, Kenya, and Tanzania westward through Sub-Sahelian Africa to Senegal. The origin of the tamarind tree is now thought to be Madagascar. It is thought to have been introduced to South and Southeast Asia, where it has become naturalized in many areas. It is now widely planted in semiarid Africa and South Asia, including Bangladesh, India, Myanmar, Malaysia, Sri Lanka, Thailand, and several African, Australian, Central American, and South American countries (Rao and Mathew 2012).

1.7.2.1 Scientific Classification of *Tamarindus indica.*

Kingdom : *Plantae*
Subkingdom : *Tracheobionta*
Superdivision : *Spermatophyta*
Division : *Magnoliophyta*
Class : *Magnoliopsida*
Subclass : *Rosidae*
Superorder : *Rosanae*
Order : *Fabales*
Family : *Fabaceae*
Subfamily : *Caesalpiniaceae*
Tribe : *Detarieae*
Genus : *Tamarindus*
Species : *Indica* (Wagh and Bhagure 2012).

1.7.2.2 Botonlogical description of *Tamarindus indica*

Tamarindus indica is an evergreen tree in the family that can *Fabaceae* grow up to 25m tall and 7.5m in diameter, with a canopy covering up to 12m radius. The tree is sturdy, gracefully sloping down at the ends, and wind-resistant; the stem bark is dark gray and heavily fissured. The leaves are 7.5-15cm long, fine feathery, and pinnate, with 10-20 pairs of oblong leaflets. Although the leaves are essentially evergreen, they may be shed briefly during the dry season in very dry regions. Flowers are inconspicuous, borne in small racemes, 5-petalled (2-petalled reduced to bristles), and yellow with orange or red streaks. The pink color of the flower buds is due to the outer color of the four sepals, which are shed when the flowers open (Popenoe, 1974; Morton, 1987; Pamploma-Roger, 1999).

The fruits are hanging pods that are 15-20cm long and 2-3.3cm in diameter, with a yellow-brown or reddish-brown flesh or pulp covering the seeds inside. The pulps of mature dry fruits dehydrate to a sticky paste that is surrounded by a few coarse strands of fiber that extend lengthwise from the stalk. The pod typically contains 1-12 fully formed hard, glossy-brown, squarish seeds that measure about 1.1-1.25cm in diameter and are enclosed in a parchment-like membrane (Morton, 1987; Pamploma-Roger, 1999).

1.7.2.3 Geographical Distribution of Plant

Tamarind grows naturally throughout Asia up to 500 m in elevation, from Burma to Afghanistan. It is distributed continuously in the Indian subcontinent's southern and central regions (which have similar wet and semi-arid climatic characteristics to tropical regions) (Dash *et al*., 2014). It is also found in small patches in northern India. *T. indica* is commonly found in woodlands in Africa and is well adapted to arid and semiarid environments. It is primarily a tropical tree that can withstand temperatures as high as 47°C but is extremely sensitive to frost (Coronel, 1991).

1.7.2.4 Chemical composition

In the Leaves Pulps contains invert sugar, citric acid, pipecolic acid nicotinic acid, 1-malic acid, volatile oils (geraniol, limonene) , pipecolic acid, lupanone , lupeol , orientin , isoorientin , vitamin B3, vitamin C , vitexin, isovitexin , benzyl benzoate (40.6%), cinnamates, serine, pectin, beta alanine, proline, phenylalanine, leucine, potassium, 1-malic acid, tannin, glycosides. Fruits contain Furan derivatives and carboxylic acid. Phlorotannins, apple acid, grape acid, succinic acid, citric acid, tartaric acid, pectin, invert sugar. Seeds contain Campesterol, β-amyrin, β-sitosterol, palmitic acid, oleic acid, linoleic acid and eicosanoic acid. The Mucilage, arabinose, xylose, galactose pectin, glucose and uronic acid was also found.Stem bark contain Tannins, saponins, glycosides, peroxidase and lipids (Zohrameena *et al*., 2017).

1.7.2.5 Ethno Medicinal Uses

- The leaves and bark were used as wound healer.
- Fruit pulp and leaves are used as anti-malarial agent.
- Flowers, leaves, bark and fruit pulps were used as aphrodisiac.
- Fresh bark or stem used as abdominal pain reliever.
- Fruit pulp with lemon or milk and leaf juice were used as anti-diarrheal and anti-dysentery agent.
- Bark and leaves are used as anti-asthmatic and anti-tussive.
- Leaves or fruits are used as anti-measles and against mumps.
- Leaf and bark decoctions were used as hepato-protective.
- Leaves are used as anti-diabetic.
- Fruits are used as antibacterial.
- Bark is used as anthelmintic.
- Fruits were used as preservative.
- Antihypertensive.
- Anti-stomachic, and
- Nephroprotective (Milind 2012).

1.8 Objectives of the study

i) To prepare methanol and hexane leave extracts of *Adansonia digitate* and *Tamarindus indica.*

ii) To perform general phytochemical screening for methanol hexane leave extracts *Adansonia digitate* and *Tamarindus indica.*

Iii) To analyze the methanolic leave extracts using GC-MS spectroscopy.

vi)To assess antibacterial activity of methanolic leave extracts of *Adansonia digitate* and *Tamarindus indica* and their combined.

Chapter Two

Materials and Methods

Materials and methods

2.1 Materials

The leaves of *Adansonia digitate* and *Tamarindus indica* were collected from Nyala in southern sudan. The leaves were washed with water to removed dust then the leaves were dried in air and shade at room temperature for 3 days, and made into powder. The plants were kindly authenticated by Aromatic and Medicinal plant institute- Khartoum – Sudan.

2.1.1 Instrumentations:

GC-MS technique model (GC/MS-QP2010-Ultra) from Japans, Shimadzu Company, Column Rtx-5MS length (30m), Diameter (0.25mm), Thickness (0.25 μm).Weighing analytical balance, oven, soxhlet extractor, rotatory evaporator, water bath and heater.

2.1.2 Chemicals:

Methanol, chloroform, hexane, hydrochloric acid, sulfuric acid, potassium iodide, ferric chloride, glacial acetic acid, ammonia solution, mercuric chloride, iodine, All chemicals used in the study were analytical grade and used without further purification.

2.2 Methods

2.2.1 Extraction methods:

2.2.1.1 Preparation of methanol extract:

100g of the plant sample were coarsely powdered using mortar and pestle and transferred into a beaker and a solution of 80% methanol (500 mL) was added. The contents of the beaker were left at room temperature for three days with frequent shaking. The extract was filtered and clear solution was evaporated, and the residual extract was dried, weighed and the %yield was calculated.

2.2.1.2 Preparation of hexane extract:

100g of the plant sample were coarsely powdered using mortar and pestle and successively extracted with hexane using soxhelt extractor apparatus. Extraction

carried out for about four hours till the colour of solvents at the last siphoning time returned colourless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extracts were allowed to dry in Petri dishes till complete dryness.

2.2.2 Phytochemical screening

Phytochemical analysis for constituents of the extracts for qualitative detection of alkaloids, flavonoids, tannins and saponins was carried out on the extracts with few modifications were detected using standard procedures as described by (Wang *et al*., 2014).

2.2.2.1 Detection of alkaloids:

-Mayer's test: One mL of the extract was acidified with 1% HCl, few drops of Mayer's reagent (Mercuric chloride solution: 1.36 g in 60 mL distilled water, Potassium iodide solution: 5 g in 10 mL distilled water and the two solutions were combined and then diluted with distilled water up to 100 Ml) was added, appearance of turbidity indicates the presence of alkaloids.

-Wagner's test: 2mL of Wagner's (1.27 g iodine and 2 g of potassium iodide in 100 ml distilled water) added to One mL of the extract, and the reaction mixture was observed for the formation of reddish-brown precipitate indicates the presence of alkaloids.

2.2.2.2 Detection of tannins

2 ML of the extract were stirred with about ten ml of distilled water and filtered. Few drops of 10% ferric chloride solution were added to two ml of the filtrate. Occurrence of a black, black and green, blue green indicates the presence of tannins.

2.2.2.3 Detection of glycosides

1mL of solvent extract was dissolved in 2mL of glacial acetic acid containing one drop of $FeCl_3$ solution and 1mL of concentrated H_2So_4 . A brown ring obtained at the interface indicated the presence of cardenolides.

2.2.2.4 Detection of saponins

Two mL of the extract were boiled with five mL of distilled water, filtered; about three mL of distilled water was further added to the filtrate and shaken vigorously for about 50 minutes. Frothing which persist for thirty minutes indicates the presence of saponins.

2.2.2.5 Detection of triterpenes and sterols (Salkowski test):

A few drops of concentrated sulfuric acid were added to 2 mL of extract dissolved in 6 mL of chloroform two layers were formed, the upper green color indicates the presence of sterol and the middle red brown ring indicated the presence of triterpenes.

2.2.2.6 Detection of flavonoids

Lead acetate test: 1mL extract was added to 1mL of 10% lead acetate. It gently shaken. A muddy brownish precipitate indicates the presence of flavonoids.

Ferric chloride: 1ml extract was added to 10% l_3 . The mixture was shaken. A wooly brown precipitate indicates the presence of flavonoid.

2.2.2.7 Detection of carbohydrates

Molish's test:To 2ml of extract , two drops of alcoholic solution of α-naphthol were added. The mixture is well shaken and a few drops of concentrated sulfuric acid were added slowly along the sides of the test tube. The appearance of violet ring at the junction indicates the presence of carbohydrates.

2.2.3 Antibacterial activity materials

2.2.3.1 Bacterial microorganisms:

Staphylococcus aureus ATCC 25923 (Gram +ve bacteria)

Bacillus subtilis NTCC 8236 (gram +ve bacteria)

Escherichia coli ATCC 25922 (Gram -ve bacteria)

Pseudomonas aeruginosa, NCTC (Gram – ve bacteria)

*ATCC: American Type Culture Collection Rockville, Meryl and USA.

*NCTC: National Collection of Type Culture, Colindale England.

2.2.3.2 Media:

Mueller Hinton Agar.

2.2.3.3 Preparation of reference strains of bacteria

One-mL aliquots of 24 h broth culture of tested organisms were aseptically added to Nutrient agar slopes) and incubated at 37 **C** for 24 h. The bacterial growth was harvested and washed off by addition of sterile normal saline. The harvest was suspended in a suitable volume of normal saline to produce a suspension containing about 10^8 -10^9 colony forming units per ml (CFU/mL). The suspension was stored in the refrigerator at 4 **C** till used. The average number of viable organisms per ml of the stock suspension was determined by surface viable counting technique (Miles and Misra, 1938).

2.2.3.4 Testing of extracts for antibacterial activity against standard organisms:

Sterile cotton swab was dipped into the bacterial test suspension matched with 0.5 McFarland standard to inoculate entire surface of Mueller Hinton agar plate. Wells or cups of 10 mm were made with a sterile cork borer in the inoculated agar plates. 200 µL volumes from different concentrations (100%, 50%, 25%, 12.5% and 6.25) of methanol leaves extracts of *Adansonia digitate* and *Tamarindus indica* and their combined mixture (1:1) were poured, directly, into the wells using automatic pipettes. The plates were allowed to stand for 30 min in room temperature for extract diffusion to take place and incubated in an incubator in an upright position at 37**C** for 24 hours. After incubation inhibition zone diameters were measured in millimeter (Mohammed, 2015).

2.2.4 Spectroscopic Methods

2.2.4.1The GC/MS Method

Analysis of samples were carried out using GC/MS technique model (GC/MS-QP2010-Ultra Shimadzu) with capillary column (Rtx-5MS- (30m) in length, diameter (0.25mm) and thickness (0.25µm). The sample was injected using split mode, helium as the mobile phase (carrier gas) passed at a 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 10 **C**/min, starting at three minutes and finishing at twenty eight minutes, the injection port, ion source and interface temperature were 280, 200 and 250 **C** respectively. The samples were analyzed using scan mode to identify chemical composition of the sample in the range of m/z 40-550 charges to ratio and the total run time was 28 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.

2.2.5 Data analysis

All collected data were analyzed using Microsoft Office Excel 2016.

Chapter Three

Results and Discussion

Chapter Three

Results and discussion

3.1 The yields of leave extracts of *Adansonia digitate* and *Tamarindus indica:*

The leaves of *Adansonia digitate* and *Tamarindus indica* were extracted using different solvents and methods: maceration by methanol and successively using suxelet extraction by hexane.

Table (3.1): shows extract % yields of Adansonia *digitate* and *Tamarindus indica* extract by using maceration method.

No	extract	weight	% yield
1	*Adansonia digitate*	8.26g	8.26
2	*Tamarindus indica*	15.58g	15.58

Table (3.2): shows extract % yields of *Adansonia digitate* and *Tamarindus indica using* soxhlet/hexane method.

No	extract	weight	% yield
1	*Adansonia digitate*	8.8g	8.8
2	*Tamarindus indica*	6.6g	6.6

3.2 Phytochemical screening result of *Adansonia digitate* and *Tamarindus Indica* Leave extracts

Photochemical screening of *Adansonia digitate* and *Tamarindus indica* Leaves extracts were carefully carried out using methods described in section (2.2.2). The results presented in Table (3.3) summarize the classes of natural compounds present in the plant.

Table (3.3): General phytochemical screening of *Adansonia digitate* and *Tamarindus indica* Leaves extracts.

No	Chemical component	Reagent	Observation	*Adansonia digitate*		*Tamarindus indica*	
				Methanol extract	Hexane extract	Methanol extract	Hexane extract
1	Alkaloids	Mayer's	Precipitate or turbidity	+	-	+	-
		Wagner's	reddish-brown precipitate	+	-	+	-
2	Flavonoids	Lead acetate	muddy brownish precipitate	+	-	-	-
		Ferric chloride	-	-	-	-	-
3	Tannins	Ferric chloride	Blue-black or green color	+	-	+	-
4	Saponins	Froth test	1cm foam hight	-	-	+	-
5	Steroids	Salkowsky	the upper layer green color	+	-	+	-
6	Triterpenes	Salkowsky	-	-	-	-	-
7	Glycosides	conc. H2SO4	A brown ring obtained	+	-	+	-
8	carbohydrates	Molish	Violet ring	+	-	+	-

Key: + positive, – Negative.

Table (3.3): shows phytochemical screening of *Adansonia digitate* and *Tamarindus indica* Leaves extracts. Only methanolic extract show phytochemical content however *Tamarindus indica* methanolic extract is avoid of flavonoids, triterpenes and *Adansonia digitate* methanolic extract is avoid of saponins and

Triterpenes. Hexane extract for both plant don't contain any phytochemicals.

3.3 Bioassay of the methanolic extracts of *Adansonia digitate*, Tamarindus *indica* leaves and their combined:

Different concentrations of the several of methanolic extracts of *Adansonia digitate* and *Tamarindus indica* leaves and their combined were carefully assessed for their anti-bacterial activity against four strains table (3.4) below.

Table (3.4): show Antibacterial activity of different concentration of the methanolic extracts of leaves against standard organisms.

Plant name	Conc. / mg/ml.	MIZD			
		Ec	Ps	Sa	Bs
Adansonia digitate	100	19	12	15	20
	50	12	15	12	17
	25	11	19	10	15
	12.5	-	20	-	11
	6.25	-	15	-	-
Tamarindus indica	100	13	18	19	22
	50	12	16	17	19
	25	11	17	15	17
	12.5	-	19	12	15
	6.25	-	18	11	14
The combined	100	15	16	18	19
	50	13	15	16	18
	25.5	11	17	13	17
	12.25	-	20	11	15
	6.25	-	19	-	12

Key: Bacterial species PS = *Pseudomonas aeruginosa*, Sa= *Staphylococcus aureus*, Bs= *Bacillus subtilis*, Ec= *Escherichia coli*

> 18mm (MIZD) =very active. 13-18mm (MIZD) = active.

12-9mm (MIZD) = partially active. < 9 mm (MIZD) = inactive.

MIZD = Minimum inhibitory Zone diameter.

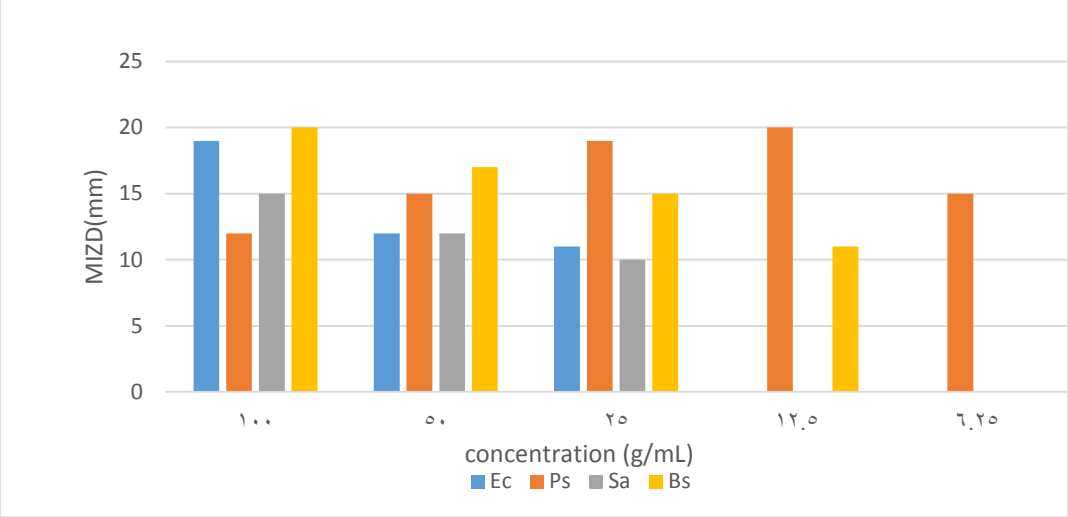

Figure (3.1). Antibacterial activity of methanolic extract of *Adansonia digitate* leaves.

Fig (3.1) illustrates the antibacterial activity of *Adansonia digitate* methanol leaves extract. *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, were show high susceptibility at high concentration (100mg/mL) with inhibition zone (20mm), (19mm) and (15mm) respectively. Whereas *Pseudomonas aeruginosa* was show high susceptibility at low concentration (12.5mg/mL) with inhibition (20mm).

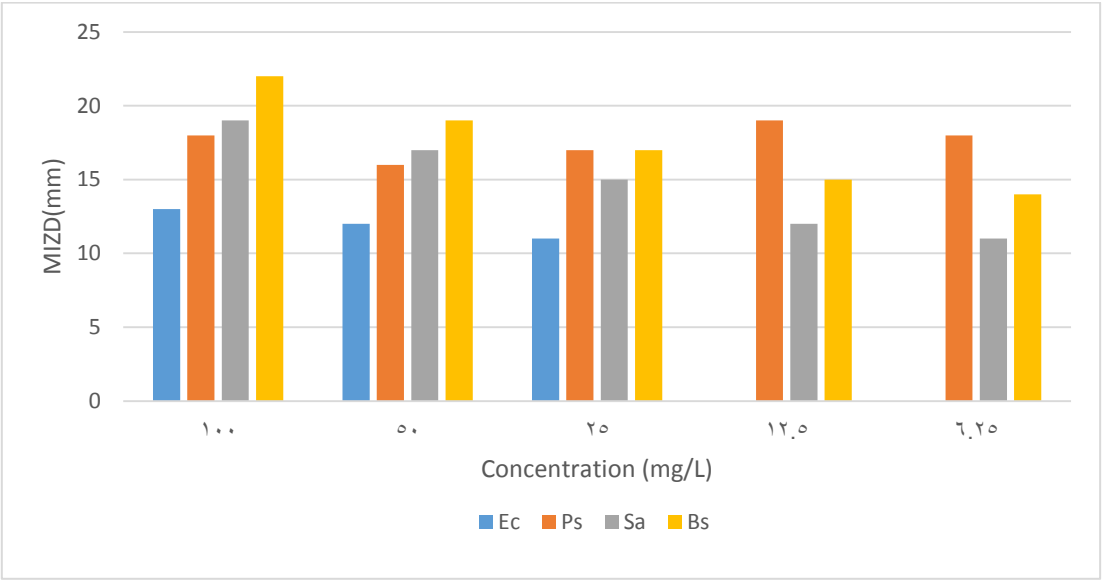

Figure (3.2). Antibacterial activity of methanolic extract of *Tamarindus indica* leaves.

Fig (3.2) illustrates the antibacterial activity of *Tamarindus indica* methanol leaves extract. *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, were show highest susceptibility at high concentration (100mg/mL) with inhibition zone (22mm), (19mm) and (13mm) respectively. Whereas *Pseudomonas aeruginosa* was show high susceptibility at low concentration (12.5mg/mL) with inhibition (19mm).

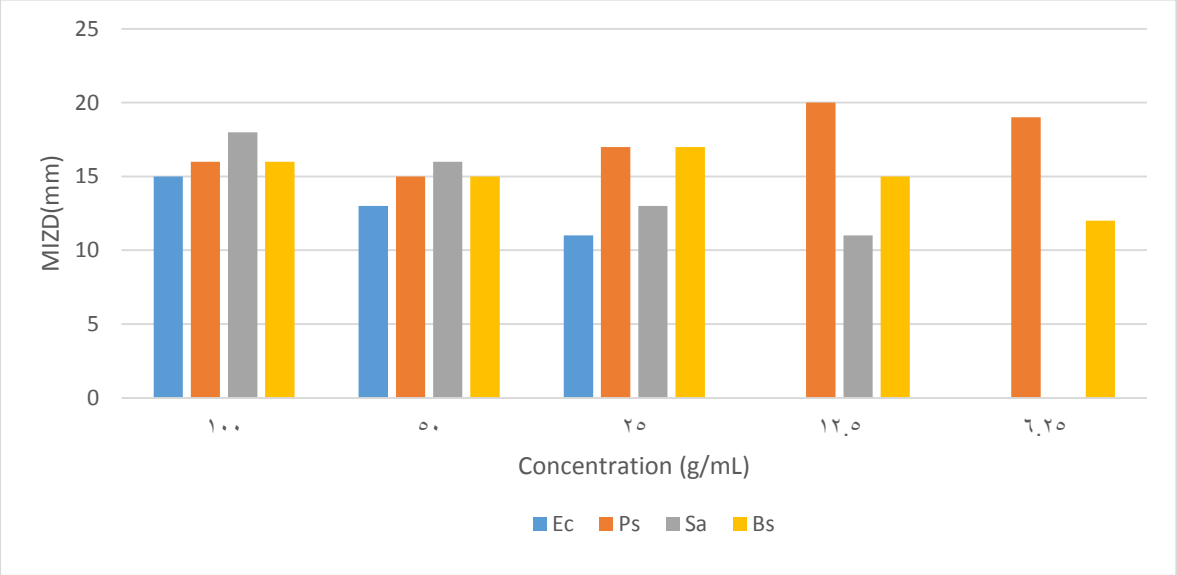

Figure (3.3). Antibacterial activity of the combined mixture (1:1) of methanolic extract of *Adansonia digitate Tamarindus indica* leaves.

Fig (3.3) illustrates the antibacterial activity of combined mixture (1:1) of methanolic extracts *adansonia digitate* and *Tamarindus indica* leaves. Staphylococcus aureus and Escherichia coli, were show highest susceptibility at high concentration (100mg/ml) with inhibition zone (18mm), (15mm) respectively. Whereas Bacillus subtilis and Pseudomonas aeruginosa were show high susceptibility at low concentration (25mg/ml) and (12.5mg/ml) with inhibition zone (17mm), (20mm) respectively.

3.4 Chromatographic analysis

3.4.1 Composition of *Adansonia digitate* methanolic leave extract

Figure (3.4) and Table (3.5) shows the chemical composition of *Adansonia digitate* methanol leave extract that were analyzed by gas chromatographic mass spectroscopic analysis (GC/MS). 14 compounds were identified.

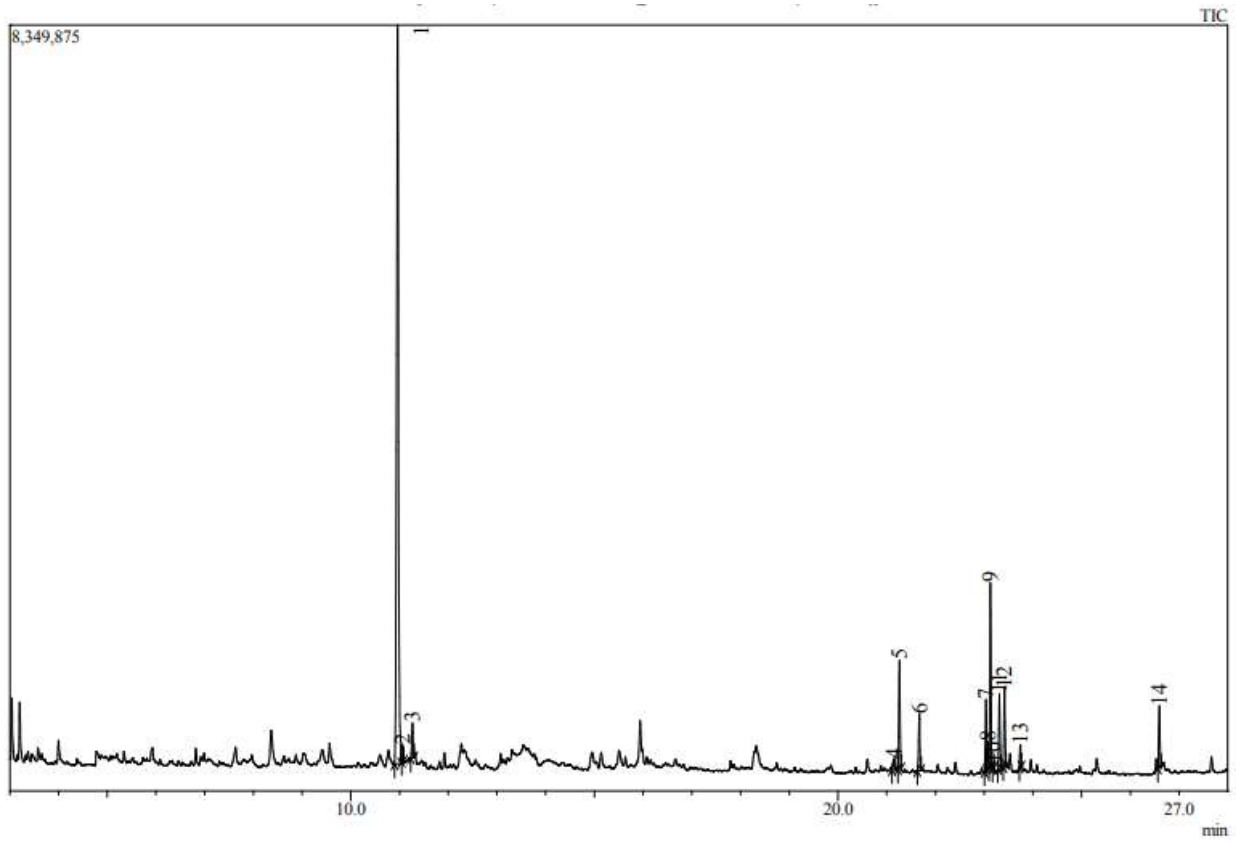

Figure (3.4). The GC/MS analysis of methanolic extract of *Adansonia Digitate* leaves.

Table (3.5): Composition of *Adansonia digitate* methanolic leave extract

Peak#	Name	R.Time	Area	Area%
1	Phenol, 2-methyl-5-(1-methylethyl)-	10.961	21769758	61.64
2	Phenol, 2,3,5,6-tetramethyl-	11.060	302273	0.86
3	2-Methoxy-4-vinylphenol	11.266	702259	1.99
4	14-Octadecenal	21.145	259895	0.74
5	Hexadecanoic acid, methyl ester	21.262	2061629	5.84
6	n-Hexadecanoic acid	21.670	1145183	3.24
7	9,12-Octadecadienoic acid, methyl ester	23.038	1262398	3.57
8	9,12,15-Octadecatrienoic acid, methyl ester, (	23.078	221696	0.63
9	9-Octadecenoic acid (Z)-, methyl ester	23.128	3152567	8.93
10	7-Hexadecenoic acid, methyl ester, (Z)-	23.185	49981	0.14
11	Phytol	23.312	1309598	3.71
12	Methyl stearate	23.417	1472119	4.17
13	Octadecanoic acid	23.742	372549	1.05
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	26.596	1237610	3.50
			35319515	100.00

Phenol, 2 -methyl-5-(1-methylethyl)- (1) was expected to be the major compound from GC/MS analysis of the *Adansonia digitate* methanol leaves extract (61.64 %), followed by 9-Octadecenoic acid (Z)-, methyl ester (2)(8.93) and finally Hexadecanoic acid, methyl ester (3) (5.84%) .

3.4 .2 composition of *Tamarindus indica* methanolic leave extract.

Figure (3.5) and Table (3.6) show the chemical composition of methanol leave extract of *Tamarindus indica* that were analyzed by gas chromatographic mass spectroscopic analysis (GC/MS). 10 compounds were identified.

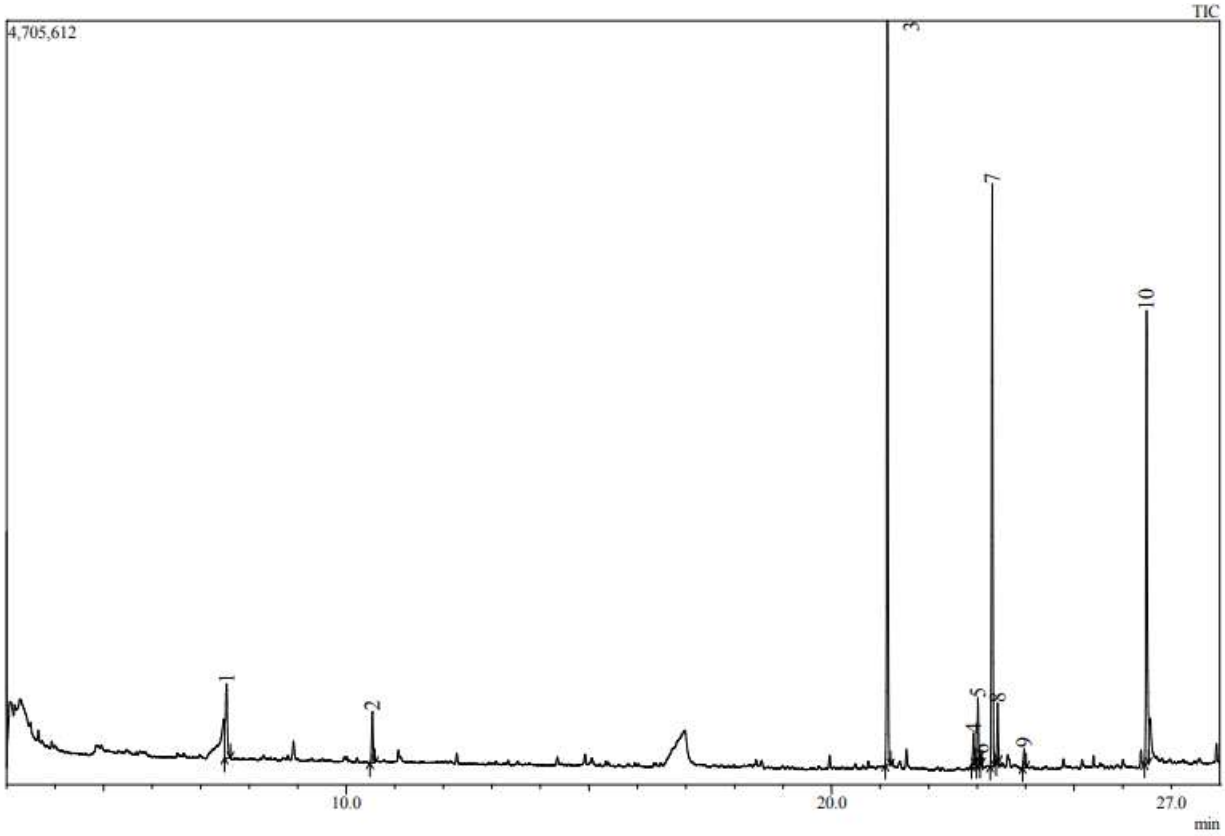

Figure (3.5).the GC/MS analysis of methanolic extract of *Tamarindus indica* leaves.

Table (3.6): Composition of *Tamarindus indica* methanolic leave extract.

Peak#	Name	R.Time	Area	Area%
1	Undecane	7.534	1314741	5.68
2	Carbamic acid, N-[1,1-bis(trifluoromethyl)pr	10.538	626640	2.71
3	Hexadecanoic acid, methyl ester	21.151	8101607	35.01
4	13-Heptadecyn-1-ol	22.926	330872	1.43
5	10-Octadecenoic acid, methyl ester	23.016	690623	2.98
6	13-Docosenoic acid, methyl ester	23.078	80847	0.35
7	Methyl stearate	23.310	5943298	25.68
8	3,4-Oxazolidinedicarboxylic acid, 2-(1-methy	23.421	573867	2.48
9	Butanal, ethylhydrazone	23.970	218221	0.94
10	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	26.491	5259256	22.73
			23139972	100.00

Hexadecanoic acid, methyl esteracid (1)(35.01%) was expected to be the major compound from GC/MS analysis of the Tamarindus indica methanol leaves extract , followed by Methyl stearate (2) (25.68%) and finally Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3) (22.73%) .

3.5 Conclusion

It is concluded that:

- The phytochemical components of methanolic extract of *A. digitata* leaves revealed the presence of alkaloids, Flavonoids, Tannins, steroids and Glycosides.
- Phytochemical components of methanolic extract of *T.indica* leaves are revealed the presence of alkaloids, Tannins, steroids, Glycosides and saponins.
- The antibacterial activity of methanolic extracts of *Adansonia digitate*, *Tamarindus indica* leaves and their combined combined mixture (1:1) revealed that the plants can be used as antibiotic therapeutic agents. The results showed that the methanolic leave extracts were effective against the tested organism. *Tamarindus indica* methanolic leave extract Show high susceptibility against *Bacillus subtilis* at concentration (100 mg/ml) with inhibition zone (22mm).
- Identification of the compounds on the methanol extracts of *Adansonia digitate*, *Tamarindus indica* leave using GC/MS (Gas chromatography /Mass Spectroscopy) technique.

3.6 Recommendations

- Due to the good antibacterial effects of methanolic extracts of *Adansonia digitate*, *Tamarindus indica* leaves and their combined combined mixture (1:1), it may be recommended as a natural antibacterial agent.
- The crude leaves extracts and isolates could be evaluation for other biological activities like antimalarial. Anti-inflammatory, antileshmenial.etc...
- Phytochemicals in leaves extracts of *Adansonia digitate*, *Tamarindus indica* may be isolated and their structures elucidated via spectroscopic tools (UV, IR, 1HNMR and MS).

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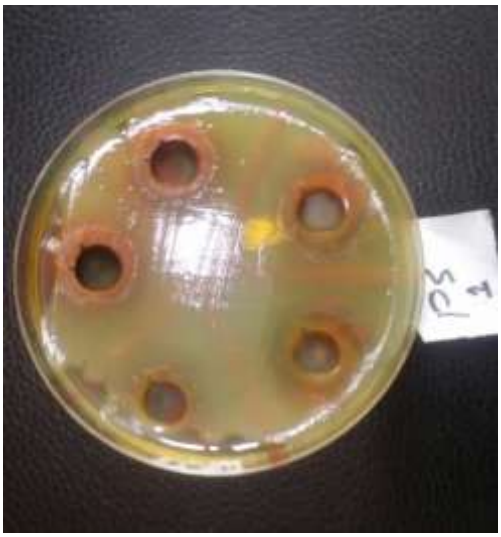
Appendix

(1)



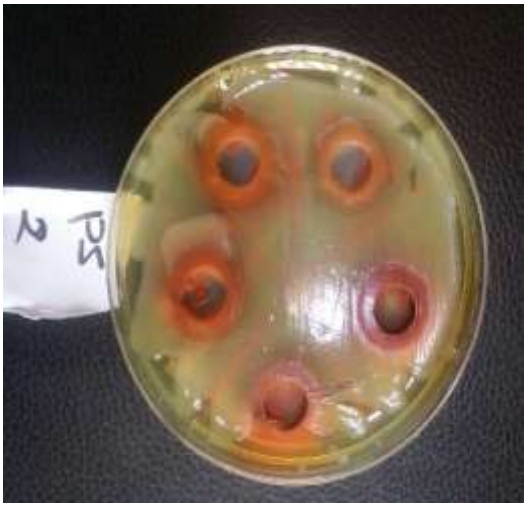
(2)

(3)

(4)

Plates (3.1). Inhibition zone of methanolic extract of *Adansonia digitate* leaves against *Escherichia coli (1), Pseudomonas aeruginosa (2), Staphylococcus- aureus (3), Bacillus subtilis (4).*

(1)



(2)

(3)

(4)

Plates (3.2). Inhibition zone of methanolic extract of *Tamarindus indica* leaves against *Escherichia coli (1), Pseudomonas aeruginosa (2*), *Staphylococcus- aureus (3), Bacillus subtilis (4).*

(1)

(2)

(3)

(4)

Plates (3.3). Inhibition zone of the combined mixture (1:1) of methanolic extract of *Adansonia digitate Tamarindus indica* leaves against *Escherichia coli (1), Pseudomonas aeruginosa (2), Staphylococcus aureus (3), Bacillus subtilis (4).*