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RESEARCH TITLE

Phytochemistry and Gas Chromatography of Active Ingredients Isolated from Selected Natural Products and Their Antibacterial Activity Against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

The increasing global demand for biologically active substances of plant origin has highlighted the potential of Sudan's rich but underexplored medicinal flora. This study investigates the phytochemical composition, gas chromatography profiles, and antibacterial activity of extracts from fenugreek (*Trigonella foenum-graecum*), cinnamon (*Cinnamomum verum*), and carob (*Ceratonia siliqua*) against *Escherichia coli* and *Staphylococcus aureus*. Plant samples were purchased from local markets in Wad Medani, Sudan, shade-dried, and ground for analysis. Phytochemical screening revealed that fenugreek contains tannins, saponins, alkaloids, and flavonoids; cinnamon contains tannins, saponins, and steroids; and carob contains tannins and saponins. Antibacterial testing, conducted using nutrient agar (NA) and eosin methylene blue (EMB) media, demonstrated inhibitory effects of all extracts at varying concentrations. Retention factor (RF) values were recorded for each plant extract using gas chromatography. Proximate analysis showed notable differences: fenugreek had high moisture content (30.54%) and low ash (9.49%), while cinnamon had low moisture (2.33%) and high ash (66.25%). The study concludes that fenugreek, cinnamon, and carob possess bioactive compounds with antibacterial properties and potential blood glucose-lowering effects. Further studies are recommended to isolate, identify, and formulate these active compounds for pharmaceutical use.

Key Words: Phytochemistry, Gas Chromatography, Antibacterial Activity, Fenugreek, Cinnamon, Carob, Escherichia coli, Staphylococcus aureus.

عنوان البحث

الكيمياء النباتية وكروماتوغرافيا الغاز للمكونات الفعالة المستخلصة من بعض المنتجات الطبيعية ونشاطها المضاد للبكتيريا ضد *Escherichia coli* و *Staphylococcus aureus*

المستخلص

لقد أبرز الطلب العالمي المتزايد على المواد الفعالة بيولوجيًا ذات الأصل النباتي الإمكانات الكبيرة للنباتات الطبية الغنية وغير المستغلة في السودان. تهدف هذه الدراسة إلى تحليل التركيب الكيميائي النباتي، وأنماط كروماتوغرافيا الغاز، والنشاط المضاد للبكتيريا لمستخلصات الحلبة (*Trigonella foenum-graecum*) ، والقرفة (*Cinnamomum verum*)، والخروب (*Ceratonia siliqua*) ضد *Escherichia coli* و *Staphylococcus aureus*. تم الحصول على عينات النباتات من الأسواق المحلية في مدينة ود مدني، السودان، ثم جففت في الظل وطُحنت للتحليل. كشفت الفحوصات الكيميائية النباتية أن الحلبة تحتوي على التانينات، الصابونين، القلويدات، والفلافونويدات؛ بينما تحتوي القرفة على التانينات، الصابونين، والستيرويدات؛ ويحتوي الخروب على التانينات والصابونين فقط. أظهرت اختبارات النشاط المضاد للبكتيريا باستخدام وسط الآغار المغذي (NA) وأغار الإوزين الأزرق الميثيلين (EMB) تأثيرًا مثبطًا لجميع المستخلصات بتراكيز مختلفة. تم تسجيل قيم عامل الاحتباس (RF) لكل مستخلص نباتي باستخدام كروماتوغرافيا الغاز. أظهر التحليل التقريبي فروقات ملحوظة؛ حيث احتوت الحلبة على نسبة رطوبة عالية (30.54٪) ونسبة رماد منخفضة (9.49٪)، بينما احتوت القرفة على نسبة رطوبة منخفضة (2.33٪) ونسبة رماد عالية (66.25٪). خلصت الدراسة إلى أن الحلبة والقرفة والخروب تحتوي على مركبات فعالة بيولوجيًا ذات خصائص مضادة للبكتيريا وقدرة محتملة على خفض نسبة السكر في الدم. ويوصى بإجراء المزيد من الدراسات لعزل وتحديد وتشكيل هذه المركبات الفعالة للاستخدام الدوائي.

الكلمات المفتاحية: الكيمياء النباتية، كروماتوغرافيا الغاز، النشاط المضاد للبكتيريا، الحلبة، القرفة، الخروب، Escherichia coli، Staphylococcus aureus.

CHAPTER ONE

1. INTRODUCTION

1.1. General Introduction

Medicinal plants possess effective medicinal properties and benefits, whether in one part of the plant or in some of its parts, for treating certain diseases (Fabricant and Fransworth, 2001). This can be one or more substances, which can have physiology function effects in treating disease. (FAO, 2018). Extracts of different plant parts have been reported to exhibit a variety of pharmacological activities such as antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, antimicrobial, anti-ulcer and wound healing effects (Afolayan and Meyer, 1995). Many modern drugs had got their origin from plant extracts (Craig, 1999). The Chinese book on roots and grasses treat 365 drugs (dried parts of medicinal plants), many of which are used even nowadays. The medicinal value of plants lies in some definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds, as well as Glycosides and Terpenes (Edeoga. *et al*., 2005). However, about 80% of the world population use medicinal plants as an alternative therapeutic prescriptive.

A microorganism is defined as a living thing that is so small it must be viewed with a microscope. Some microorganisms like viruses are so small they can only be seen with special electron microscopes Barry *et al* (1970).

Phytochemistry is the study of the chemicals produced by plants, particularly the secondary metabolites, synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental hazards (Dimitrova *et al.*, 2006).

Antibacterial agents will potentially reach the bedside over the next 8–10 years. While the pipeline features some innovative products, only a fraction of these are likely ever to come to market due to the high failure rate characteristic of the drug development process (Mothana and Lindequist, 2005).

1.2. Problem statement

The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are now in of great demand in the developing world for primary health care not because they are inexpensive, but also for better cultural acceptability, better compatibility with the human body and minimal side effects (Pal and Shukla, 2002).Among the herbs that are used medicinally are fenugreek, cinnamon and carob. They have an influential role in reducing blood glucose levels (Pal and Shukla, 2002).

1.3. Objectives

1.3.1. General objective

The main objective of the present work was to investigate the Phytochemistry and Gas chromatography of the active ingredients separated from some natural products and their antibacterial activity on *Escherichia coli* and *Staphylococcus aureus*

1.3.2. Specific objectives

1- To evaluate the antibacterial activates of the selected plant extracts on *E. coli* and *Staph. Aureus.*

2- To determine the phytochemical components of the selected plant parts.

3- To determine the proximate contents of the selected plant parts.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Plants as antimicrobial agents

Herbal medicine is the oldest form of medicine known to mankind. It was the mainstay of many early practiced form of medicine in the world today according to World Health Organization. Herbs have played an important part in our development. It provided us with food, medicine and cosmetic (Internet, 2015). Herbal medicine sometime referred to as herb slim or botanical medicine is the use of herb for their therapeutic, or medicine value. Herb is a plant or a plant part valued for its medicine, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substance that act upon their bodies. Throughout history, natural product from plant have played major sustaining role in the life of human, especially as food source and for medicinal products. Natural has provided mankind with folk medicines for centuries and continues to be the richest source of bioactive chemicals for development of modern drugs. Terrestrial plants in the particular were used as the basis of sophisticated traditional pharmacopoeias, as early as 260 Bc and some of the earliest Sudan and Chinese showed that plants were used for the preparation of hundreds of drugs covering almost impressive array of health problems and diseases (Osbourn and Lsnzothis, 2009). Medicinal and pharmaceutical science, through the development \of technology, have created milestone, plant-based systems continue to play and essential role in the healthcare of many communities. It was estimated by the world health Organization that approximately 4 billion people (80%) of the world's inhabitants rely mainly on traditional medicines for their primary healthcare (Farsworth, 1985). For the remaining 20% of the prescribed drugs contain extract or plant metabolites and an additional significant percentage of the market drugs have been development through studies employing natural products as the lead molecules (Craggy and Newman, (2005). Owing to their popular use as remedies for many infectious diseases, searches for plant containing antimicrobial substances were frequent (Betoni *et al*, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis and Ausubed, 2006). A number of phototherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability. Fewer side effects and reduced toxicity (Lee *et al*., 4 2007). There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; De Boer *et al*., 2005). Herbal medicine as a major component in all of the traditional medicine, noted that of the 19 plant – derived pharmaceutical medicines, about 74 percent was used in modern medicine in ways that correlated directly with their traditional uses as medicines by native culture. Substances derived from the plant, remain the basis for a large production of the commercial medication used today for the treatment of heart disease, high blood pressure, the powdered leaves of plants are known as the cardiac stimulant digitalis to the millions of heart patients, it keeps them alive worldwide (Khan *et al*., 2003).

2.2. Plant under test

2.2.1. Fenugreek

***Trigonellafoenum-graecum* L**. is a self-pollinating annual leguminous bean which belongs to Fabaceae family (Balch, 2003).

2.2.2. Scientific classification

Kindom;	Plantae	Family;	Fabaceae
Class:	Magnoliposide	Genus:	Trigonella
Order:	Fabales	Species:	*T.foenum- graecum (Lee, 2006)*

2.3. Plant description:

The Fenugreek (*Trigonellafoanum- graecum*) seeds sown in well prepared soil sprouts in three days. Seed grows erect, semi – erect or branched based on its variety and attains a height of 30 to 60 cm. It is has compound pinnate, trifoliate leaves, auxiliary white to yellow flowers, and 3 - 15 cm long thin pointed hoop – like beaked pods . Every pod contains 10-20 oblong greenish – brown seeds with unique hoop like groves (Srinivasan, 2006). Pods, number of seed in a pod, seed shape – size and plant height varies from one fenugreek variety to another (Balch, 2003). It is one of the most ancient medicinal herbs (Thomas *et al*., 2011).

2.4. Cinnamon

Cinnamon is a spice obtained from the inner bark of several trees from the genus Cinnamomum which belong to Lauraceae family, cinnamon has dark brown colour and astringent taste. It is used in both sweet and savoury (Fawze, 1987).

Fig. (2.1) Cinnamon

Source: ((Anon, 1989).

2.5. Origin

The origin of the genus cinnamon is South East Asia. Wild species are found in Equatoria in Africa and some other countries. The main producers of cinnamon bark and oil are China, Tanzania, Kenya Argentina, (Alshahat, 1992).

2.6. Botany

The genus cinnamon is large and ever-green tree more than 40 m in height, with strong and condenses branching. Stem diameter ranges between 0.5 m – 2.0 m in thickness and the bark is reddish. Leaves are leathery in appearance, opposite, simple, oval or oval rectangular in shape. The newly formed leaves are reddish green while the mature one is dark green in the upper surface and brighter the lower surface. 26 Flowers are white or white greenish, small in size. Fruits are rounded, smaller and with many rounded seeds, (Alshahat, 1992).

2.7. Carob

Ceratonia siliqua L. is an evergreen tree belongs to Leguminoseae (Fabaceae) family and Caesalpinaceae sub-family. It has wild and cultivated types. Turkey has a wide area for both types of carob bean. Carob tree is grown since antiquity in most countries of Mediterranean basin and it has an important value from economic and environmental point of view (Battle and Tous, 1997). Carob bean consist of 90% eatable part and 10% seed. The unripe pod is green and acrid, ripe one is brown and sweet. Carob fruit has high amounts and varieties of nutrients (Anonim, 2017).

2.8. Chemical composition

Proteins, amino acids and sugar contents were determined in pods at different harvesting stages. (Simsek 2017).

2.9. Carob Bean Products

Carob bean is also used for producing some commercial products. Powder (flour), syrup, locust bean gum and D-pinitol are the main examples of these products (Pazır and Alper, 2016).

2.10. Carob powder (flour)

Carob powder is produced by crashing, roasting and grinding of deseeded carob respectively (Yousif and Alghzawi, 2000). It can be named as 'functional ingredient' and promotes nutritional value of foods prepared with (Seczyk *et al*., 2016).

2.11. Uses

The carob is one of the most useful native Mediterranean trees. In producing countries, carob pods have traditionally been used as animal and human food and currently the main use is the seed for gum extraction. Carob pods provide fodder for ruminants (Louca and Papas 1973). and nonruminants (Sahle *et al*., 1992).

2.12. Microbial under test

2.12.1 *Staphylococcus aureus*

Members of the genus *Staphylococcus* (staphylococci) are gram-positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004).

2.12.2. Classification

Domain:	Bacteria	Family:	*Staphyiococcaceae*
Phylum:	Firmicutes	Genus:	*Staphylococcus*
Class:	Bacilli	Species:	*Aureus (Ryan and Ray, 2004).*
Order:	Bacillaes		

The Gram-positive cocci forms a heterogeneous collection of approximately 16 genera that colonize human. Features that they have in common are their spherical shape, their Gram strain reaction and the absence of end spores. There are two groups of bacteria, catalase- positive genera (*Staphylococcus*, *Micrococcus*, and *Stomatococcus*) and catalase–negative genera (*Streptococccus*, *Entrococcus* and related organism) as described by Murray *et al*. (1998). *Staphylococci* are included in the family *Micrococcaceae* and subdivided into three species. *S. aureus*. *S. pidermidis* and *S. aprofaiticus*. *Staphylococcus aureus* and related organisms are generally wide spread in nature their normal habitats being the skin, skin glands and mucus membranes of humans and other mammals (Devries, 1968). *Staphylococci* produce a variety of syndromes with clinical manifestation ranging from single pustules to sepsis and death. A lesion containing pus is the primary clinical finding; abscess formation is the typical pathological manifestation. Virulence of bacterial strain varies greatly (Devries, 1968).

Staphylococcus aurous is a persistent human pathogen that isresponsible for a wide range of diseases that vary in both clinical microenvironments in the human body. Although the diverse functionality of its surface-bound adhesions (microbial surface components recognizing adhesive matrix molecules) contributes to that ability, present models also suggest that the success of this organism as pathogen can be predicated by its capacity to manipulate and evade multiple host immune responses.The circulating complement system is a chief target of virulence factors produced by many pathogens. Because it is central in the amplification of all three complement pathways (classical, alternative andlectin-mediated activation), C3 represents a particularly likely target for organisms seeking to inhibit or modulate the essential complement response. S. aurous stimulates all three pathways of the complement system, and mice depleted of complement by treatment with cobra venom factor have been found to be more susceptible to S. aurous–induced septicemia than are untreated mice. Those results indicate that complement serves a principal function in the global immune response against S. aurous infection and suggest that the bacterium probably produces complement inhibitors that mimic the function of RCA proteins. Indeed, conditioned S. aurous culture medium contains at least one C3b-binding protein, which has-been identified as the 15.6- kilodalton extracellular fibrinogen-binding protein (Efb). Functional analysis has shown that recombinant Efbbinds to the thioester-containing C3d domain of C3b and inhibits the deposition of C3b onto sensitized surfaces and that S. aurousstrains incapable of expressing Efb are less virulent than a wild-type strain in amouse model of wound healing. Thus, attenuation of the complementresponse by S. aurous seems to be a critical mechanism ofimmunosuppression that is advantageous to its function as a human (Myszka, 1999; Janssen *et al*., 2005).

2.12.3. *Escherichia coli (E. coli)*

Domain;	Bacteria	Family:	Enterobacteriaceae
Class:	Gammaproteobacteria	Genus;	*Escherichia*
Order:	Enterobacteriales	Species;	*Coli (Sherris, 1984).*

E. coli a gram negative rod-shaped bacterium that is commonly found in the lower intestine. Most *E. coli* strains are harmless, but some can cause serious food poisoning in humans (Vogt and Dippold, 2005). The harmless strains are part of the normal flora of the gut (Bentley and meganathan, 1982). *E. coli* are not always confined to the intestine, and their ability to survive for brief period outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology. *E. coli* was discovered by the German pediatrician and bacteriologist Theodor Escherichia in 1885, and is now classified as part of the Enterbacteriaceae family of gamma-proteobacteria (Feng *et al*., 2002).

2.13. Phytochemicals

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome (Jalal *et al*., 1982).

Compounds in plants (apart from vitamins, minerals, and macronutrients) that have a beneficial effect to the human body are termed phytonutrients. There are over 10,000 of them, and they have effects such as antioxidant, boosting the immune system, anti-inflammatory, antiviral, antibacterial, and cellular repair. Highly colored vegetables and fruits tend to be highest in these chemicals, but tea, chocolate, nuts, flax seeds, and olive oil are all excellent sources as well. Various families of plants tend towards certain families of phytonutrients, for example, orange foods tend to have the caretenoid group (Brown and Arthur, 2001; Papp *et al*., 2007).

2.14. Chromatography

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation (Vogel *et al.,* 2003).

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive (Vogel *et al*., 2003).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in College of Sciens and Food analysis Laboratory, Faculty of Engineering and Technology, University of Gezira, in Auguse, 2021

3.2. Plant source

Various plants fenugreek, cinnamon and carob these plants used in this study were obtained from Wad Medani market. Bacteria were obtained from the food Science and medical Microbiology laboratory, Faculty of Engineering and Technology, University of Gezira.

Nutrient Agar modes Bacteria *(Staphylococcus aureus)* and Eosin Methylene Blue (EMB) modes Bacteria (*E.coli*) from the medical microbiology laboratory Gezira University laboratory.

3.3. *Escherichia coli* and *Staphylococcus aureus*

Escherichia coli and *Staphylococcus aureus* were obtain and investigated at the Medical Laboratory University of Gezira.

3.4. Microbial source

The two bacterial isolates (*E. coil* and *Staphylococcus aureus*) were obtained from the laboratory of the plant pathology center, Faculty of Agriculture Sciences Technology, University of Gezira.

3.5. Media

The media used in this study were prepared locally, using chemicals from Oxoid Corporation substances. The media include the followings:

3.6. Bacteriological media *(Staphylococcus aureus)*

3.6.1. Nutrient Agar:

The medium composition was as follows: (g/L)

Lap- lemoco powder	1.0	Sodium chloride	5.0
Yeast extract	2.0	Agar	15.5
Peptone	5.0		

3.7. Preparation of the Media

Twenty-eight grams of an already prepared medium (Himedia Ltd.), were used per 1000 ml of distilled water. The medium was dispensed into flask (250 ml), and autoclaved at 121^0 C (151 b/in2) for 15 minutes, then poured into sterile Petri dishes, which were allowed to solidity and kept inverted into a refrigerator before use

3.8. The Cup –plate agar diffusion (Inhibition zone) method

This method was used using Nutrient Agar (NA). In this method 2ml of a standardized bacterial cell suspension (10×10^5) of *E. coli* or of *Staphylococcus aureus* were thoroughly mixed with 200ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidity at room temperature for 24 hours. Sterile Whatsman glass fiber disc (No.5) were saturated with the some natural products, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72hours and the inhibition zones were measured as described by Barry *et al.*, (1970).

3.9. Bacteriological media *(E. coil)*

3.9.1. Eosin Methylene Blue (EMB)

This medium was used for isolation and maintenance of bacteria *E. coli*, and for other experiments whenever needed.

3.10. Secondary metabolites compounds

3.10.1. Saponins

Saponins are compounds whose active portions form colloidal solution in water, are produced lather on shaking and precipitated cholesterol. They occur as glycosides (glycosides and glycones) which are tri-terpenoid or steroidal structures. They are a combination of lipophiclic (fat – soluble) a glycones at one end of the moleaule and hydrophilic (water soluble) sugars at the other end gives them the ability to lower surface 9 tension producing the characteristic detergent or coap- like effect on membranes and skin (Takechi and Tanki 1995).

3.10.2 Tannins

Tannins represent the largest group of polyphenols they are widely distributed in the bark of trees, insect galls, leaves steam and fruit. Tannins were originally isolated from the bark and insect galls of oak tree (Scalbert, 1991).

3.10. 3 Flavonoids

These compounds occur as yellow and white plant pigments (latin flavus = yellow). Rutin was discovered in (Rutagraveolens) in 1842. It was later became known as vitamin B (permeability factor). Flavonoids occur both in Free State and as gluosicles. Their chemical structure is based on a C_{15} skeleton consisting of two benzene rings connected by a three – carbon chain , that is $C_6 – C_3 – C_6$. The three carbon chain is generally closed to form a heterocyclic ring (the C – ring) flavonoids are products of both the shikimic acid and acetate path way, being formed by the condensation of a phenyl propanoid precursor with three malonyl coenzyme A units (Scalbert, 1991).

3.10. 4 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolate in 1805 from thopium poppy pap aversomniferum. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, commonly isolated from the plants of the Ranuculaceae, or buttercup (Atta and Rahman, 1995). Are commonly found to have antimicrobial properties (Omulokoli *et al.,* 1997).

3.10.5 Steroids

A steroid is an organic compound with four rings arranged in a specific configuration. Examples include the dietary lipid cholesterol, the sex hormones estradiol and testosterone (Lednicer, 2011), and the anti–inflammatory drug dexamethasone (Rhen, 2002). Steroids have two principal biological functions: certain steroids (such as cholesterol) are important components of cell membranes which alter membrane fluidity. Many steroids are signaling molecules which activate steroid hormone receptors (Bowen, 2001).

3.10.6 glycosides

This group of compounds is considered as one of the most important of naturally occurring products (digitoxin, ouabain, etc.,). These drugs are used in medicine primarily to increase the tone, excitability and the contractility and the contractility of them exert a diuretic action due to the increased renal circulation. These glycosides occur in small amounts in the seeds, Fenugreek, Cinnamon and Ceratonia or barks of plants of wide geographical distribution, particularly of the families: Apocynacea, Asctepiadaceae, liliaceous and Ranunculaceae. The glycolgens group of this glycoside is chemically related to bile acids and sterols and possessed the steroidal structure with the tetra cyclic carbon skeleton, which is largely saturated. In addition to the tetra cyclic carbon (Steroidal portion) there is an of the steroidal carbon skeleton. Glycoside is the17unsaturated lactones

ring attached to C In a number of these cardio active glycosides the4and C3of hydroxyl groups at Cpresence aglycones have additional hydroxyl groups at their positions (Harbone, 1998).

3.11. Thin layer chromatography (TLC)

The TLC plates were made by mixing the adsorbent (silica gel)' containing small amount of calcium sulphate (gypsum) as an inert binder, with twice the volume of distilled water (Van Sumere *et al*., 1965). The mixture was spread as slurry on previously cleaned glass micro – plates (20x20 cm dimension) using a TLC spreader of o.5 mm think layer, The plate was set to be air dried at room temperature for 30 minutes and activated at 110^{o} C in an oven for 30 minutes. Samples of red and white Roselle petals powders, were drawn with capillary tubes and spotted on stationary phase (pre – coated silica gel plate) in a line at about 2cm from the bottom. The TLC plates were developed in saturation chamber using in mobile phase solvent system composed of (hexane and acetone) in ratio of 80:20 (volume by volume), respectively. Prior to development, the solvent was poured into chromatography tank, covered and allowed to saturate at room temperature 28^{o} C± 2^{o} C) for 30 minutes. The plate loaded with the samples was then carefully placed into the chromatography tank. At the end of the chromatography development, the plate was removed out and left to dry at room temperature (Kotze and Eloff, 2002). The separated spots were visualized under (Ultra- Violet light UV265 nm). The plates were then exposed to iodine vapor. Distances between the spot and the end of the mobile phase were measured. And the refraction factor RF) Value were calculated and recorded according to the distance that that the spot moves in respect to point that the solvent reached. The thin layer chromatography was run to determine the active ingredients in Roselle petals (in term of spots) in white and red types.

Rf value = $\frac{\text{The distance driven by component (cm)}}{\text{The distance driven by solvent}}$

3.12. The cup –plate agar diffusion (Inhibition zone) method

This method was used, using Nutrient Agar (NA). In this method 2ml of a standardized bacterial cell suspension (10×10^{5}) of *E. coli* or of *Staph. aureus* were thoroughly mixed with 200 ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidity at room temperature for 24 hours. Sterile Whatsman glass fiber discs (No. 5) were saturated with the extract then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry *et al* (1970).

13.13. Approximate analysis tests

3.13.1. Moisture content

The moisture content was determined according to the standard method of the Association of Official Analysis Chemists (AOAC, 2010). A sample of 10 g was weighed into a pre-dried, then the sample was placed into an oven at 105^{o}C until a constant weight was obtained. After drying, the covered sample was transferred to desiccators and cooled to room temperature before reweighting. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

$$\text{Moisture content (\%)} = \frac{(ws - wd) \times 100\%}{\text{Sample weight (g)}}$$

where:

Ws = weight of sample before drying.

Wd = weight of sample after drying.

3.14. Crude protein content

The protein content was determined in all samples by micro-Kjeldahl method using a copper sulphate-soduimsulphate catalyst according to the official method of the AOAC (2003). A sample of

10 g was accurately weighed and transferred together with, 4gm NaSo4 of Kjeldahl catalysts and 25 ml of concentrated sulphuric acid into aKjeldahl digestion flask was placedinto a Kjeldahl digestion unit for about 2 hours until a colorless digest was obtained and the flask was left to cool at room temperature. The distillation of ammonia was carried out into 25 ml boric acid (2%) by using 20 ml sodium hydroxide solution (45%) finally. The distillate was titrated with standard solution of HCl in the presence of 2-3 drops of bromocreasol green and methyl red as an indicator until a brown reddish color was observed. The crude protein was calculated as follows:

$$\text{Crude protein (\%)} = \frac{\text{(No. of mls (0.1N) HCl)} \times F \times DF \times 0.014 \times 100\%}{\text{Sample weight (g)}}$$

where:

N: normality of HCl

F: protein conversion factor = 6.25

3.15. Fat content

Fat content was determined according to the official method of the AOAC (2003). A sample of 5 g that previously extracted with hexane, was weighed into an extraction thimble and covered with cotton then, the sample and a pre-dried and weighed extraction flask containing about 100 ml hexanes were attached to the extraction unit (Electro thermal, England) and the extraction process was conducted for 16 hrs. At the end of the extraction period, the flask was disconnected from the unit and the solvent was redistilled later, the flask with the remaining crude ether extract was put in an oven at 105 ^{o}C for 3hours, cooled to room temperature in a desiccators. Reweighed and the dried extract registered as fat content according to the following formula:

$$\text{Fat content (\%)} = \frac{(W2 - W1) \times 100\%}{W3}$$

where:

W2 = weight of the flask

W1 = weight of the empty flask

W3 = initial weight of the sample

3.16. Total carbohydrates

Total carbohydrates were calculated by difference according to the following equation: total carbohydrates = 100% - (moisture% + protein% + Fat% Ash %)

3.17. Crude fiber content

The crude fiber was determined according to the official method of the AOAC (2003). About 2 g of a defatted sample was placed into a conical flask containing 200 ml of H_2SO4 (0.26 N). The flask was then, fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digested sample was filtered (under vacuum) through a porcelain filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23N), solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20 ml diethyl ether. Finally, the crucible was dried at 105^{o}C (overnight) to a constant weight, cooled, weighed, at 550-600 ^{o}C until a constant weight was obtained and the difference in weight was considered as crude fiber and calculated as:

$$\text{Crude fiber (\%)} = \frac{(W1 - W2) \times 100\%}{\text{sample weight (g)}}$$

where:

W1= weight of sample before ignition (g)

W2= weight of sample after ignition (g)

3.18. Phytochemical screening of Carob, Fenugreek and Cinnamon

3.18.1. Test for saponins

A weight of 5 g of the dried plant powder were extracted with 20 ml ethanol (50%) and filtered. Aliquots of the ethanolic extract (10 ml each) were evaporated to dryness under reducing pressure. The residue was dissolved in distilled water (4 ml) and filtered. The filtrate was vigorously shaken; if a voluminous froth was developed and persisted for almost one hour, this indicates the presence of saponins (Harborne, 1998).

3.18.2. Test for tannins

The dried powder of the plant materials (5 g) were extracted with ethanol (50%) and filtered. Chloride reagent (5%, w/v in methanol) was added, and the appearance of a green color which changes to a bluish black color or precipitate indicates the presence of tannins (Balbaa, 1974).

3.18.3. Test for flavonoids

A known weight (20 g) of the dried powder were macerated in 1% of hydrochloric acid (50 ml) over night, filtered and the filtrate was subjected to the following test:

a. A known volume (10 ml) from each filtrate was rendered alkaline with sodium hydroxide (10% w/v); if a yellow color was formed that might indicate the presence of flavonoid.
b. Shinoma Test: 5 ml from each filtrate was mixed with concentrated hydrochloric acid (1ml) and magnesium turning was added. The formation of a red color is an indication for the presence of flavonoid (Harborne, 1998).

3.18.4. Test for alkaloids

The dried powder of each of the plant seeds (5 g) was extracted with ethanol (5%) and filtered. Aliquots from the ethanol extract (10 ml each) were mixed with hydrochloric acid (20 ml 10% v/v), and filtered. The filtrate was alkaline with ammonium hydroxide and extracted with successive proteins of chloroform. The combined chloroform extract was evaporated and dryness, the residue was dissolved in hydrochloric acid (2 ml 10% v/v) and tested with Mayer reagent, and Dragendroffs reagent, respectively. If a precipitate was formed, it is an indication for the presence of alkaloids (Balbaa, 1974).

3.18.5. Test for steroids

A weight of 200 mg of each plant material was taken in 10 ml chloroform and then filtered. In 2ml acetic anhydride and small amount H_2SO_4 was added, appearances of blue green ring indicate presence steroids (Harborne, 1984).

3.18.6. Test for glycoside

Keller-Kiliani test: 5 ml of the extract was treated with 1ml of glacial acetic acid containing 1drop of ferric chloride solution, this was under played with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates the presence of deoxysugar characteristic of cardenolides (Harbone, 1998).

3.19. Statistical analysis

The statistical analysis was done by using ANOVA, EXCEL 2010 Anova: Single Factor

CHAPTER FOUR

4. RESULTS

The effect of the different concentration of the Fenugreek, Cinnamon and Ceratonia extracts on both the number of colonies of E. coli were receded on Table (4.1- 4.3). Table (4.1) showed the effected on *E. coli* the results showed that the higher concentration were highly effected. The Fenugreek extracts were found to decrease the number of colonies from 88 to 1and from 24 to 1 at the higher 75 and 100%), concentration respectively (Table, 4.1). The effect of the different concentration of Cinnamon and Ceratonia extracts on *E. coli* are showing in Table (4.2- 4.3). The results indicated that the extracts are less effected compared to the Fenugreek extracts the Cinnamon and the Ceratonia

extracts both gave 14 similar of control at the higher concentration (100%).

Table (4.1) Effects of different concentrations of the Fenugreek on the number of colonies of *E. coli*

Concentration	Time (Hours)				Mean
	6	12	18	24	
0.0%	62	80	88	90	80
25%	50	68	76	80	68.5
50%	30	60	62	54	51.5
75%	7	10	12	18	11.75
100%	1	1	1	1	1

Result on table (4.1): were showing the effect of the Fenugreek on the number of colonies of *E.coli* they gave 7cm at the75% concentration and1 cm at the higher concentration (100%) compared to 90 cm at the control treatment (0.0%)

ANOVA

Source	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7065.167	4	1766.292	1.883169	0.146129	2.776289
Total	29575.6	28				

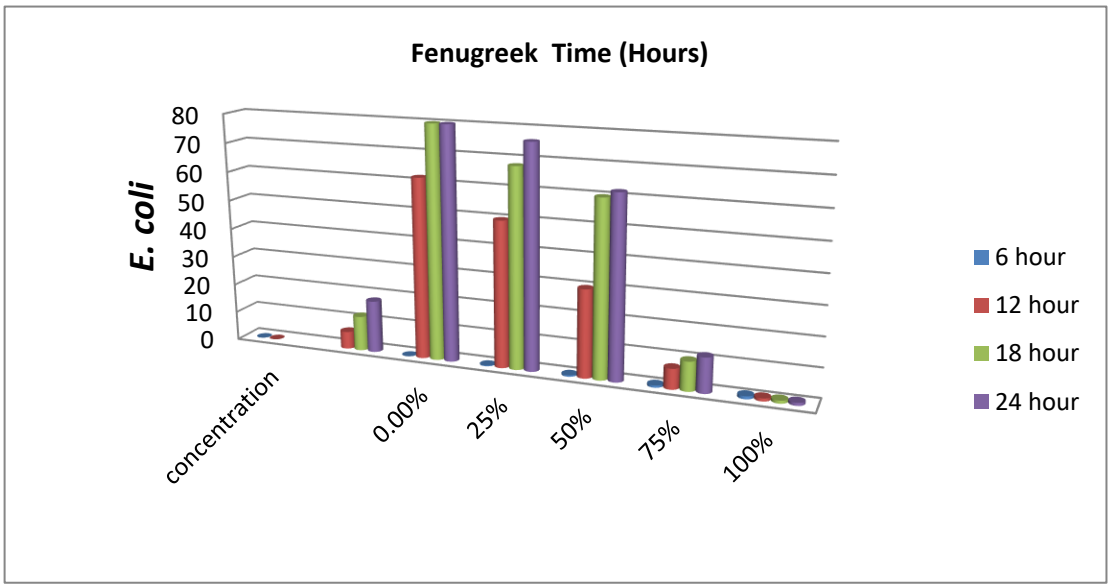

Fig. (4.1) Effects of different concentrations of the Fenugreek on the number of colonies of *E. coli*

Table (4.2) Effects of different concentrations of the Cinnamon on the number of colonies *E. coli*

Concentration	Time (Hours)				Mean
	6	12	18	24	
0.0%	4.2	2.4	4.2	4.2	3.75
25%	8.4	7.7	8.0	8.1	8.05
50%	9.4	9.8	10.0	10.0	9.8
75%	10.5	11.5	11.6	11.6	11.3
100%	2	2.7	2.7	2.7	2.52

Table (4.2):) Effects of different concentrations of the Cinnamon on the number of colonies *E. coli* they gave11.6cm at the 75% concentration and and 2.52 cm at the higer concentration (100%) compared to 4.2 cm at th control treatment(0.0%)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	298.0787	4	74.51967	3.052122	0.036288	2.776289
Within Groups	585.9767	24	24.41569			
Total	884.0553	28				

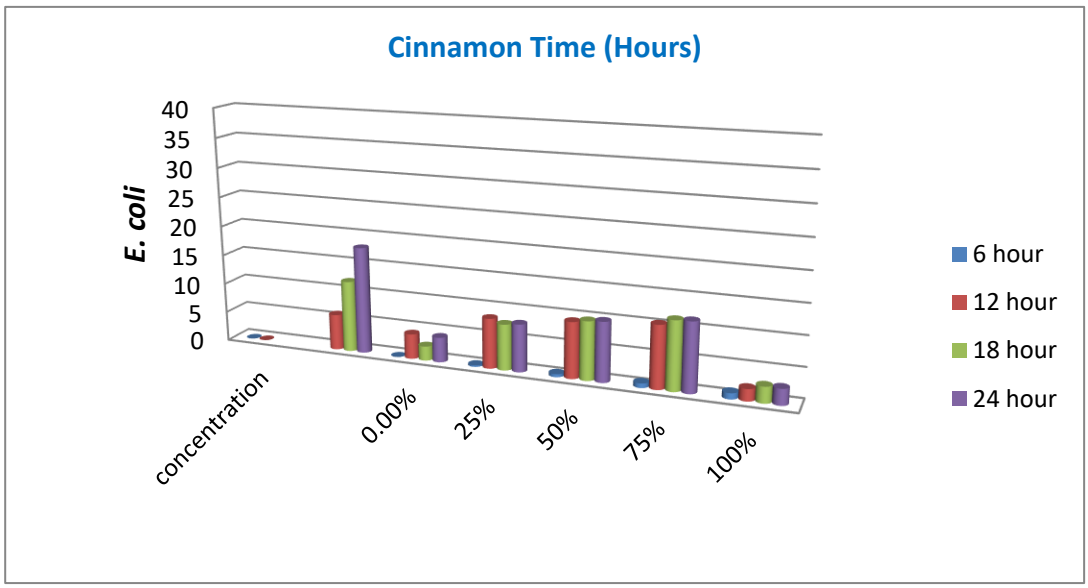

Fig. (4.2) Effects of different concentrations of the Cinnamon on the number of colonies *E. coli*

Table (4.3) Effects of different concentrations of the Ceratonia on the number of colonies *E. coli*

concentration	Time (Hours)				Mean
	6	12	18	24	
0.0%	4.5	4.5	4.5	4.5	4.5
25%	7.4	7.7	8.0	8.01	7.77
50%	9.4	9.8	10.0	10.0	9.8
75%	10.5	11.5	11.4	11.4	11.2
100%	10	10	10.3	10.5	10.2

Result onTable (4.3)) Effects of different concentrations of the Cinnamon on the number of colonies *E. coli* were showing the effect on Ceratonia results were similar they gave 11.5% cm at the 75% concentration and 10.5 cm at the higher concentration (100%) compared to 4.5 cm at the control treatment (0.0%)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	392.99	4	98.248	6.076	0.0015	2.7762
Within Groups	388.01	24	16.167			
Total	781.014	28				

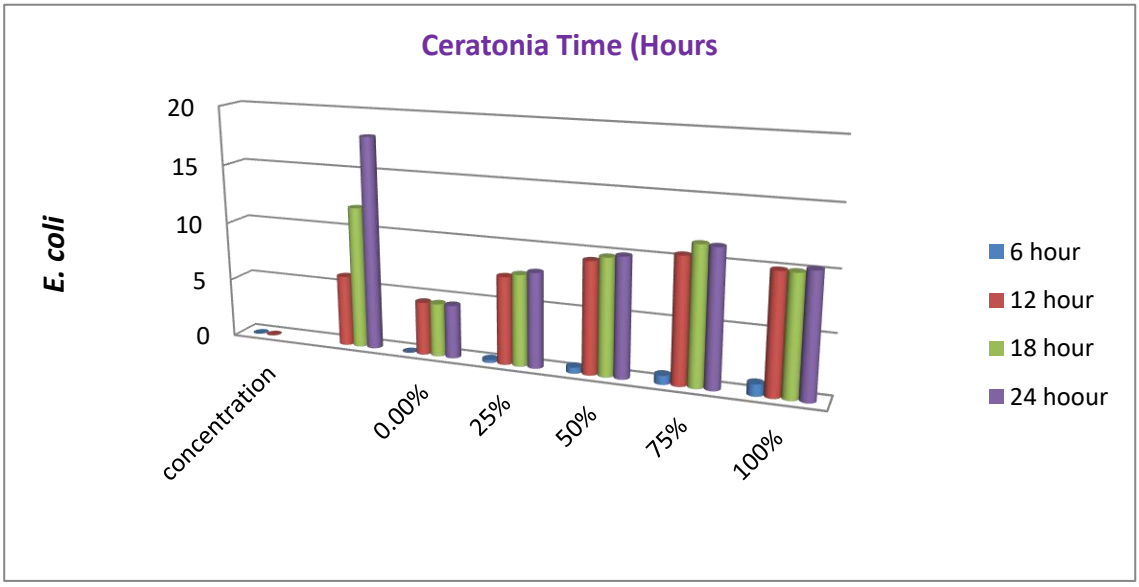

Fig. (4.3) Effects of different concentrations of the Ceratonia on the number of colonies *E. coli*

The effect of the leaf extracts of the three plant (Fenugreek, Cinnamon and Ceratonia) on the number of colonies of the bacteria S. auras are showing Table (4, 5, and 6) respectively. The extracts of the Fenugreek, Cinnamon and Ceratonia found effective in reducing the number of colonies of S. auras. However the extracts of Ceratonia were less effective allowing 12.7 colonies to grow (Table,33) compared to that of Fenugreek and Cinnamon which allowed only 10.7 and 9.9 colonies to grow (Tables,5 and 6)

Table (4.4) Effects of different concentrations of the Fenugreek on the number of colonies *Staphylococcus aureus*

Concentration	Time (Hours)				Mean
	6	12	18	24	
0.0%	5.1	5.1	5.1	5.1	5.1
25%	7.4	7.7	8.0	8.01	7.77
50%	9.4	9.8	10.0	10.0	9.8
75%	8.5	8.5	9.6	10.6	9.3
100%	4	4.7	4.7	4.7	4.52

Result on table (4.4): were showing the effect of the Fenugreek on the number of colonies of *S.aureus* they gave 10.6 cm at the75% concentration and4.52 cm at the higher concentration (100%) compared to 5.1 cm at the control treatment (0.0%)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	315.59	4	78.898	4.413	0.0081	2.7762
Within Groups	429.03	24	17.876			
Total	744.62	28				

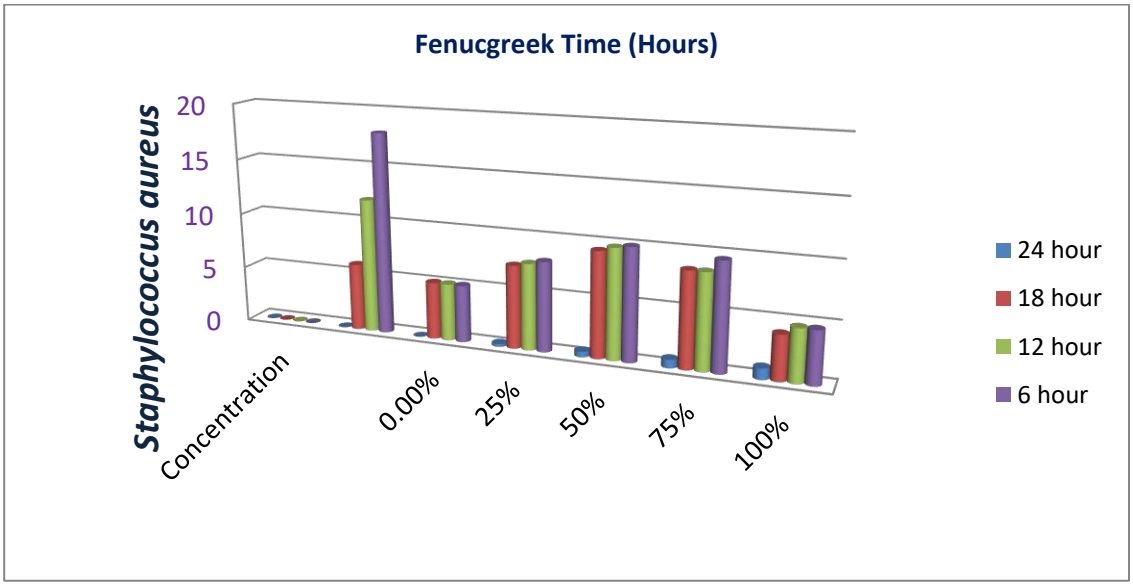

Fig. (4.4) Effects of different concentrations of the Fenugreek on the number of colonies *Staphylococcus aureus*

Table (4.5) Effects of different concentrations of the Cinnamon on the number of colonies of *Staphylococcus aureus*

concentration	Time (Hours)				Mean
	6	12	18	24	
0.0%	4	4	4	4	4
25%	6.4	6.7	7.0	7.01	6.77
50%	9.4	9.8	10.0	10.0	9.8
75%	10.5	11.5	11.6	11.6	11.3
100%	9.9	9.9	9.9	9.9	9.9

Result on table (4.5): were showing the effect of the Cinnamon on the number of colonies of *S.aureus* they gave 11.6 cm at the75% concentration and 9.9 cm at the higher concentration (100%) compared to 4 cm at the control treatment (0.0%)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	370.539	4	92.634	5.1772	0.00375	2.7762
Within Groups	429.428	24	17.8928			
Total	799.968	28				

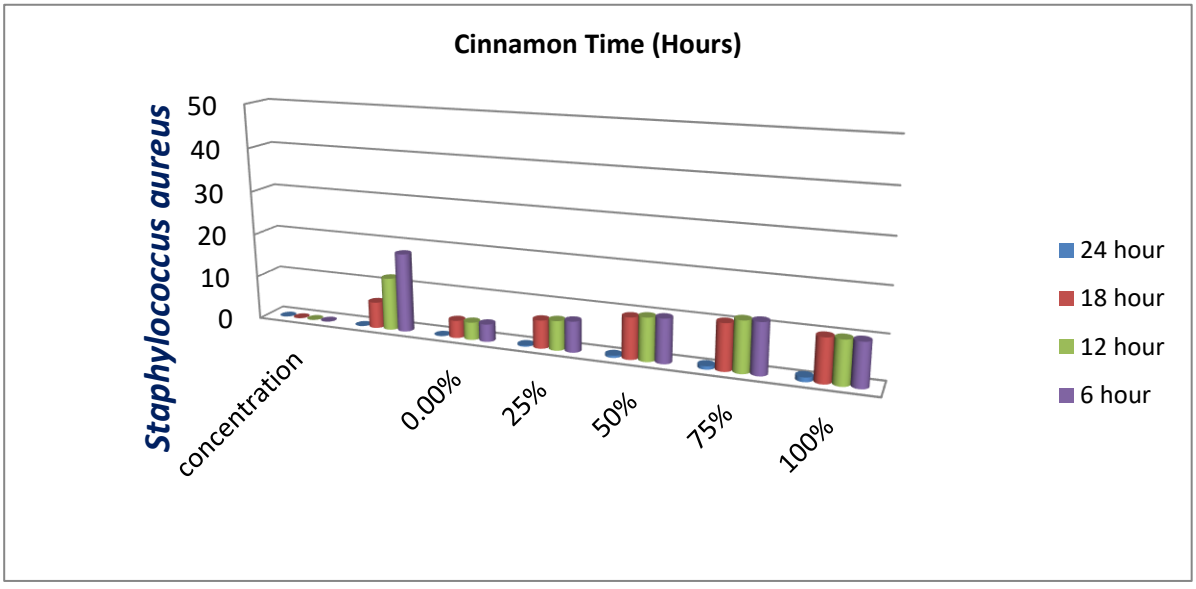

Fig. (4.5) Effects of different concentrations of the Cinnamon on the number of colonies of *Staphylococcus aureus*

Table (4.6) Effects of different concentrations of the Certonia on the number of colonies of *Staphylococcus aureus*

concentration	Time (Hours)			
	6	12	18	24
0.0%	6	6.1	6.1	6.1
25%	7.3	7.6	8.0	8.02
50%	9.2	9.5	9.9	10.0
75%	10.5	11.3	11.6	11.6
100%	12	12.7	12.7	12.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	445.86	4	111.44	7.618	0.0004	2.776
Within Groups	351.12	24	14.630			
Total	796.98	28				

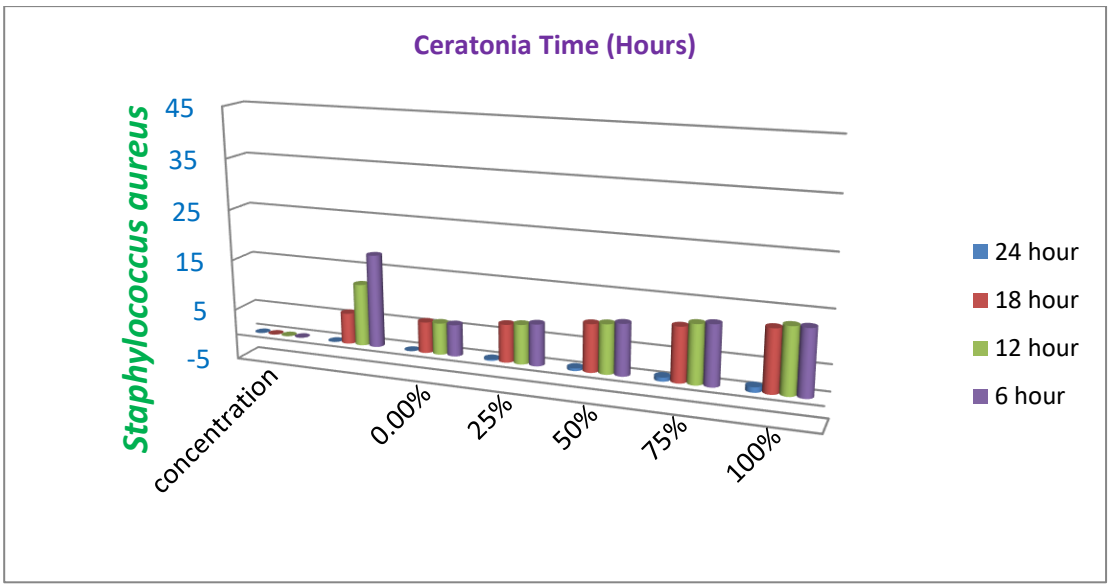

Fig. (4.6) Effects of different concentrations of the Certonia on the number of colonies of *Staphylococcus aureus*

The effect of the different concentration of Fenugreek, Cinnamon and Ceratonia extract on the inhibition zone of *E. coli* are shown in table (7 and 8) the results indicated that extracts are very effective increasing the inhibition from 5.5 to 6.7 at the 8th day of incubation.

Table (4.7) Effects of different concentrations of the Fenugreek on the inhibition zone of *E. coli*

Concentration	Days			
	2	4	6	8
0.0%	5.5	5.5	5.5	5.5
25%	7.4	7.7	8.4	9.8
50%	8.4	8.8	9.0	9.0
75%	9.5	10.5	11.2	10.2
100%	6	6.7	6.7	6.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	184.55	4	46.137	8.9935	0.0002	2.8951
Within Groups	97.472	19	5.1301			
Total	282.02	23				

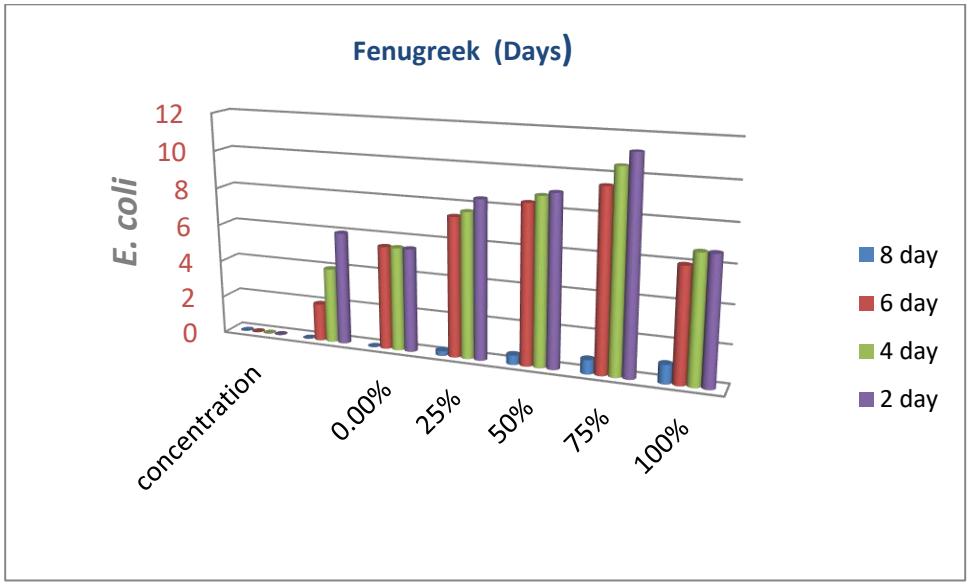

Fig. (4.7) Effects of different concentrations of the Fenugreek on the inhibition zone of *E. coli*

Table (4.8) Effects of different concentrations of the Cinnamon on the inhibition zone of *E. coli*

Concentration	Days			
	2	4	6	8
0.0%	6.5	6.5	6.5	6.5
25%	8.4	7.7	8.4	8.1
50%	9.6	9.7	10.5	10.5
75%	9.9	10.4	11.9	11.9
100%	10	10.7	10.7	10.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	277.19	4	69.29	12.488	1.24E-05	2.776
Within Groups	133.18	24	5.55			
Total	410.37	28				

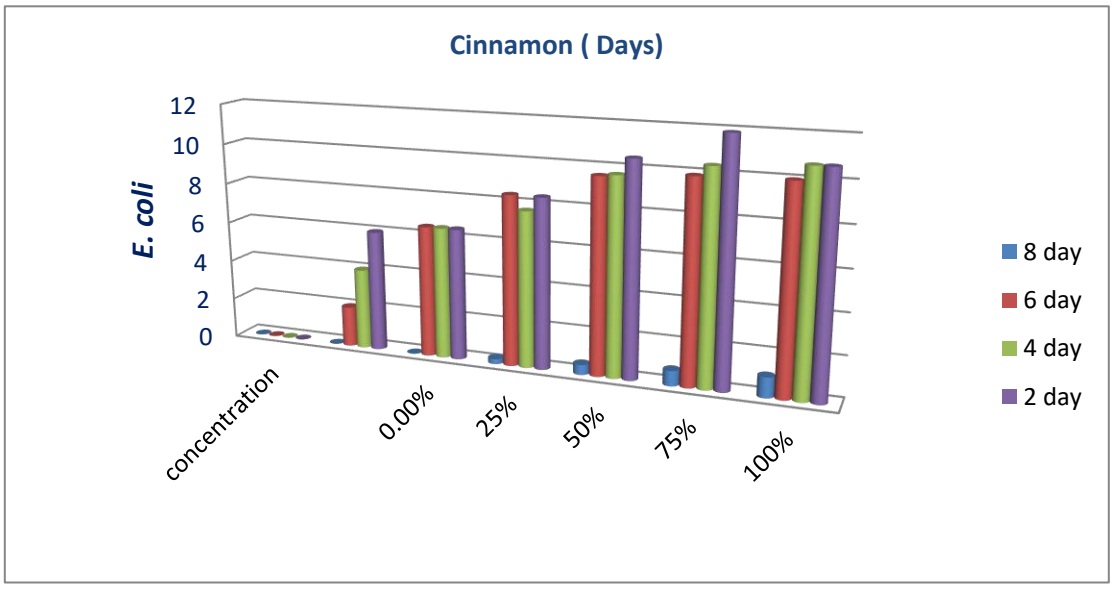

Fig. (4.8) Effects of different concentrations of the Cinnamon on the inhibition zone of *E. coli*

Table (4.9) Effects of different concentrations of the Ceratonia on the inhibition zone of *E. coli*

Concentration	Days				Mean
	2	4	6	8	
0.0%	4.2	4.2	4.2	4.2	4.2
25%	8.4	7.7	8.0	8.1	8.05
50%	9.4	9.8	10.0	10.0	9.8
75%	10.5	11.5	11.6	11.6	11.3
100%	12	12.7	12.7	12.7	12.52

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	183.56	4	45.890	5.329	0.004	2.895
Within Groups	163.60	19	8.610			
Total	347.16	23				

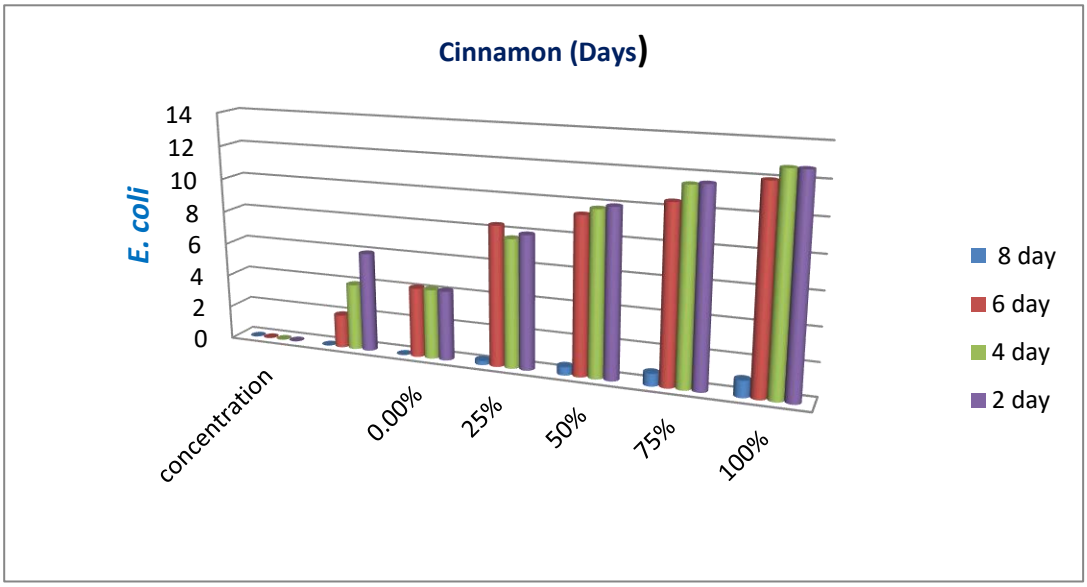

Fig. (4.9) Effects of different concentrations of the Ceratonia on the inhibition zone of *E. coli*

The effect of the different concentration of Fenugreek, Cinnamon and Ceratonia extract on the inhibition zone of *E. coli* are shown in table (10 and 11) the results indicated that extracts are very effective increasing the inhibition from 5.1 to 9.7 at the 8th day of incubation.

Table (4.10) Effects of different concentrations of the Fenugreek on the inhibition zone of *Staphylococcus aureus*

concentration	Days				Mean
	2	4	6	8	
0.0%	5.1	5.1	5.1	5.1	5.1
25%	7.4	9.8	8.0	8.01	830
50%	9.4	8.5	10.0	10.0	9.47
75%	8.5	10.5	8.6	10.6	9.55
100%	9.2	9.7	9.7	9.7	9.57

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	224.63	4	56.158	11.86	1.85E-05	2.776
Within Groups	113.58	24	4.732			
Total	338.22	28				

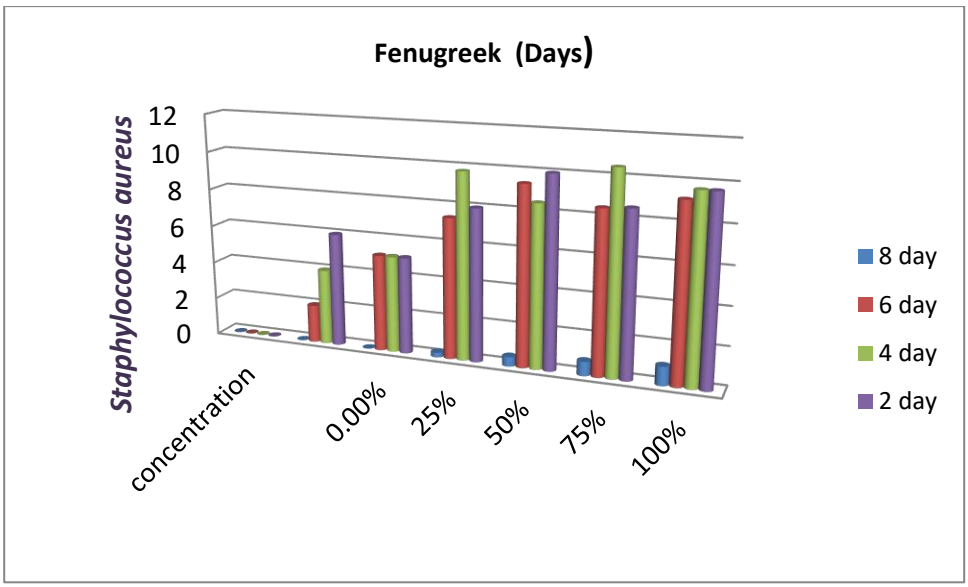

Fig. (4.10) Effects of different concentrations of the Fenugreek on the inhibition zone of *Staphylococcus aureus*

Table (4.11) Effects of different concentrations of the Cinnamon on the inhibition zone of *Staphylococcus aureus*

concentration	Days				Mean
	2	4	6	8	
0.0%	6.1	6.1	5.1	5.1	5.6
25%	8.4	7.7	8.0	8.01	8.02
50%	9.4	9.8	10.0	10.0	9.8
75%	8.5	8.5	9.6	10.6	3.9
100%	11.2	12.7	10.7	10.7	11.32

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	247.67	4	61.92	10.163	5.85E-05	2.776
Within Groups	146.21	24	6.092			
Total	393.88	28				

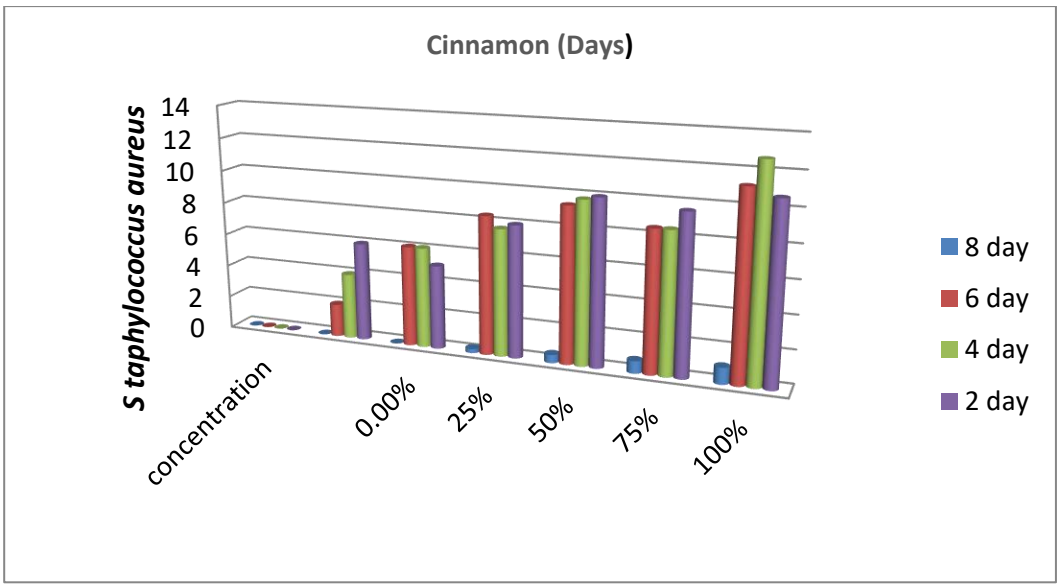

Fig. (4.11) Effects of different concentrations of the Cinnamon on the inhibition zone of *Staphylococcus aureus*

Table (4.12) Effects of different concentrations of the Ceratonia on the inhibition zone of *Staphylococcus aureus*

concentration	Days				Mean
	2	4	6	8	
0.0%	4	4	4	4	4
25%	6.4	6.7	7.0	7.01	1.93
50%	9.4	9.8	10.0	10.0	9.8
75%	10.5	11.5	11.6	11.6	11.3
100%	9.9	9.9	9.9	9.9	9.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	226.72	4	56.68	7.104	0.0006	2.776
Within Groups	191.48	24	7.978			
Total	418.20	28				

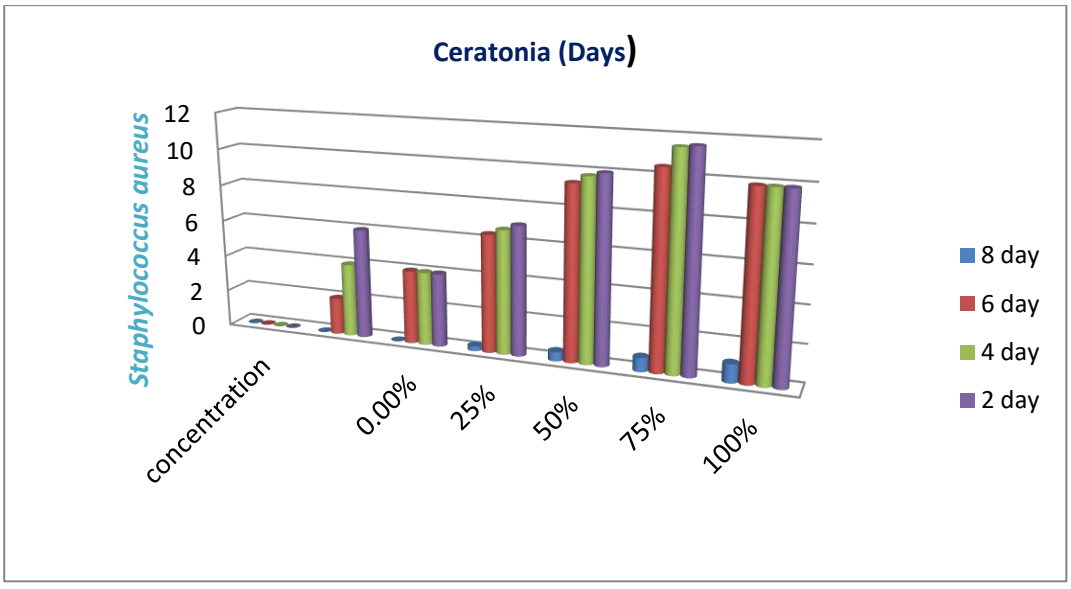

Fig. (4.12) Effects of different concentrations of the Ceratonia on the inhibition zone of *Staphylococcus aureus*

Table (4.13) Proximate analysis (%) of Fenugreek, Cinnamon and Ceratonia

Chemical analysis	Fenugreek	Cinnamon	Ceratonia
Moisture	76.01	6.45	10.51
Ash	3.47	16.81	19.11
Fat	2.21	9.46	1.83
Fiber	6.3	5.08	6.00
Protein	1.63	6.64	1.19
Carbohydrate	10.38	55.56	61.36

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3851.19	5	770.23	5.948	0.0083	3.325
Columns	543.15	2	271.57	2.097	0.1735	4.102
Error	1294.80	10	129.48			
Total	5689.15	17				

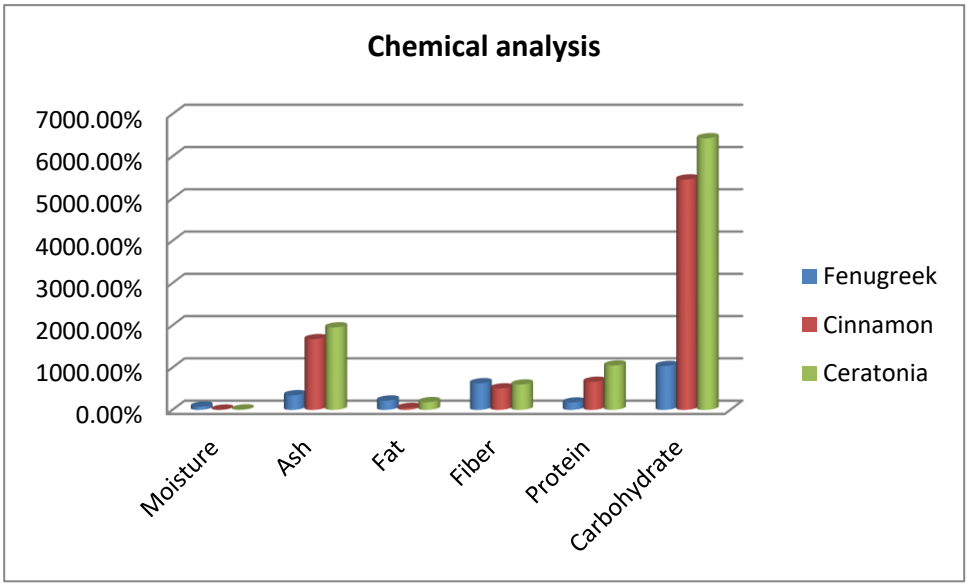

Fig. (4.13) Proximate analysis of Fenugreek, Cinnamon and Ceratonia

The proximate analysis of the three plants (Fenukreek, Cinnamon and Ceratonia) are showed on the Table (4.13). Cinnamon and Ceratonia have more proteins 10.50% compared to only 1.74 in Fenureek similar effects are also found by the carbohydrates the Cinnamon and Ceratonia were found to carbohydrate 54.53 and 64.27, respectively compared to only 10.38% in the Fenukreek extracts. The Fenukreek on the other hand contain more fats 2.2% compared to 0.46 and 1.83 by the Cinnamon and Ceratnia Fenukreek contain only high moisture contain 76.01% compared to only 6.39 and 10.51 in Cinnamon and Ceratonia, respectively.

Table (4.14) Proximate analysis (%) of Fenugreek and Cinnamon

Chemical analysis	Fenugreek	Cinnamon
Moisture	30.54	2.33
Ash	9.49	66.25
Fat	10.70	0.03
Fiber	17.1	15.40
Protein	19.90	12.58
Carbohydrate	12.27	3.41
Total	**100**	**100**

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1816.54	5	363.30	0.816	0.585	5.050
Columns	98.15	1	98.15	0.220	0.658	6.607
Error	2225.98	5	445.19			
Total	4140.69	11				

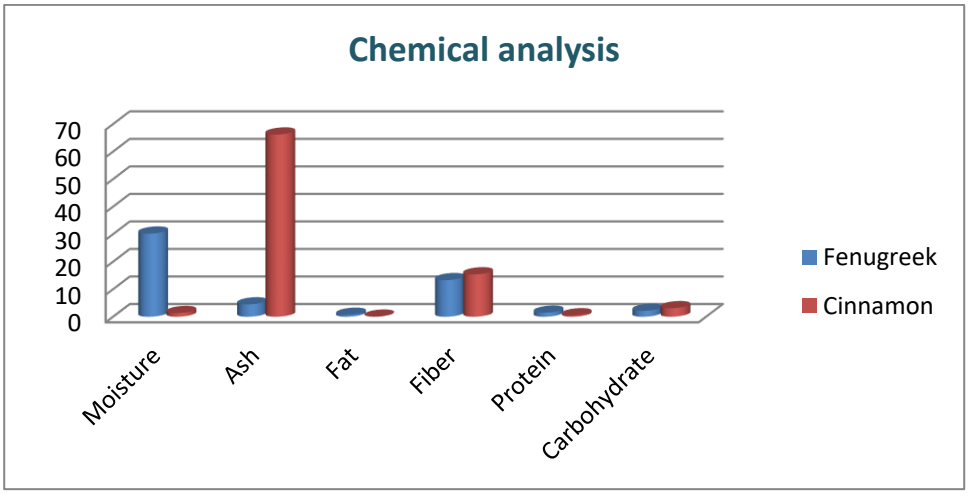

Fig. (4.14) Proximate analysis of Fenugreek and Cinnamon

Table (4.14) is showing the proximate analysis of both Fenugreek and Cinnamon. The Fenugreek have very high moisture contain 30.02% compared to only 1.33 of the Cinnamon. On the other hand the Cinnamon contain high contend of ash (66.20), compared to only 4.49 of the Fenugreek

The phytochemical screening of the Fenugreek, Cinnamon and Ceratonia on Table (4.15). However the table also contend photochemical screening of the Fenugreek, Cinnamon and Ceratonia .The Fenugreek contains tannins, flavonids, alkaloid and saponnin but not contains glycoside and sterols. The Cinnamon contains tannins, saponnins and Steroids but not contains alkaloid, flavonoids and glycosides whereas Ceratonia contains tannins and saponnins, but not contains alkaloid, flavonoids, glycoside and Steroids.

Table (4.15) Phytochemical screening of the Fenugreek, Cinnammon and Ceratonia

Item	Presence (+) or absence (-)		
	Fenugreek	**Cinnamon**	Ceratonia
Tannins	+	+	+
Saponins	++	++	+
Alkaloids	+	_	_
Flavonoids	++	_	_
Glycosides	_	_	_
Steroids	_	+	_

++ indicated the presence of the class (in relatively high concentration.

+ indicated the presence of the class.

- indicated the absence the class.

Qualitative Analysis Report

Table (4.16) Peak Report TLC (Fenugreek)

Peak	R. Time	Area%	Name	Mol Weight	Mol Formula
1	9.540	0.85	Heptanoic acid, 6-oxo-	144	$C_7H_{12}O_3$
2	11.284	0.31	Cyclohexanone, 3-hydroxy-	114	$C_6H_{10}O_2$
3	11.555	0.72	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	144	$C_6H_8O_4$
4	11.914	0.17	5-Hepten-1-ol, 2-ethenyl-6-methyl-	254	$C_{16}H_{30}O_2$
5	12.524	0.29	5-Hepten-1-ol, 2-ethenyl-6-methyl-	142	$C_8H_{14}O_2$
6	13.942	0.43	Benzofuran, 2,3-dihydro-	120	C_8H_8O
7	14.301	1.90	5-Hydroxymethylfurfural	126	$C_6H_6O_3$
8	15.307	0.08	1-Gala-1-ido-octose	240	$C_8H_{16}O_8$
9	16.151	1.00	1,2-Epoxynonane	142	$C_9H_{18}O$
10	16.634	0.14	3-Nonyn-2-ol	140	$C_9H_{16}O$
11	18.261	0.40	N-(1-Cyclopenten-1-yl)-morpholine	153	$C_9H_{15}NO$
12	19.503	0.19	Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	154	$C_{10}H_{18}O$

13	20.849	0.26	2-Nonen-1-ol	142	$C_9H_{18}O$
14	20.955	0.20	2-Nonen-1-ol	142	$C_9H_{18}O$
15	21.355	2.84	1,2-Dioxaspiro[4.5]decan-3-one, 4-methylen	168	$C_9H_{12}O_3$
16	21.488	1.81	(6-Methyl-tetrahydropyran-2-yl)-acetonitrile	139	$C_8H_{13}NO$
17	22.256	0.70	8-Azabicycio[3.2.1]octan-3-ol, 2-bromo-8-m	219	$C_8H_{14}BrNO$
18	23.722	14.71	Ethyl .alpha.-d-glucopyranoside	208	$C_8H_{16}O_6$
19	25.455	15.02	4-o-Methylmannose	194	$C_7H_{14}O_6$
20	27.583	8.38	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
21	27.914	0.33	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$
22	28.777	0.17	Eicosanoic acid	280	$C_{18}H_{32}O_2$
23	29.243	0.65	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcy	280	$C_{18}H_{32}O_2$
24	29.462	0.16	Undecanal	184	$C_{12}H_{24}O$
25	29.869	31.30	9,12-Octadecadienoic acid(ZZ)-	196	$C_{11}H_{20}N_2O$
26	30.064	1.26	9,12-Octadecadienoic acid(ZZ)-	313	$C_{17}H_{31}NO_4$
27	31.418	0.61	Undecanal, 2-methyl	330	$C_{19}H_{38}O_4$
28	31.853	4.26	2-Pyrrolidinone, 1-[2-(4-piperidinyl]-	334	$C_{22}H_{38}O_2$
29	33.315	2.24	Fumaric acid, 2-dimethylaminoethyl nonly ester	170	$C_{11}H_{22}O$
30	33.953	8.62	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme)	312	$C_{20}H_{40}O_2$
		100.00			

Table (4.17) Peak Report TLC (Cinnamon)

Peak	R. Time	Area%	Name	Mol Weight	Mol Formula
1	3.476	0.59	Silanediol, ddimethyl-	114	$C_5H_{10}N_2O$
2	6.103	0.70	Urea, 1-methylcyclopropyl-	98	$C_5H_6O_2$
3	6.210	0.79	6-Oxa-bicyclo[3.1.0]hexan-3one	92	$C_2H_8O_{25}$
4	8.521	0.10	Glycerin	144	$C_6H_8O_4$
5	11.665	0.26	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	110	$C_6H_6O_2$
6	13.391	1.31	Catechol	126	$C_6H_6O_3$

7	14.311	2.68	5-Hydroxymethylfurfural	132	C_9H_8O
8	15.585	77.89	Cinnamaldehyde (E)-	148	$C_9H_8O_2$
9	19.323	2.22	Trans-Cinnamic acid	164	$C_9H_8O_3$
10	19.466	0.84	2-Propenoic acid, 3-(2-hydroxyphenyl)-,(E)-	162	$C_{10}H_{10}O_2$
11	21.216	8.07	2-Propenal, 3-(2-meth0xphenyl)-	148	$C_{10}H_{12}O$
12	21.831	1.83	Benzenemethanol, .alpha.-2-propenyl-	270	$C_{17}H_{34}O_2$
13	25.598	0.76	Isopropyl myristate	256	C17H36O
14	26.407	0.48	n-Heptadecanol-1	256	$C_{16}H_{32}O_2$
15	27474	0.79	n-Hexadecanoic acid	270	$C_{18}H_{38}O$
16	29058	0.70	1-Octadecanol	92	$C_3H_8O_3$
		100.00			

Table (4.18) Peak Report TLC (Ceratonia)

Peak	**R. Time**	**Area %**	**Name**	**Mol Weight**	**Mol Formula**
1	3.508	0.51	2-propanon,1- hydroxy-	74	$C_3H_6O_2$
2	4.139	0.52	2-propanon,1- hydroxy-	74	$C_3H_6O_2$
3	5.106	0.20	2-Furanmethanol	98	$C_5H_6O_2$
4	5.560	0.37	Propanoic acid, 2-methayl-, methyl ester	98	$C_5H_6O_2$
5	6.313	0.29	6-Oxa-bicyclo[3.1.0]hexan-3-one	144	$C_6H_8O_4$
6	7.375	0.54	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-0	128	$C_7H_{12}O_2$
7	10.063	0.54	6,7-Dioxabicyclo[3.2.2]nonane	102	$C_5H_{10}O_2$
8	12.544	5.00	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	144	$C_6H_8O_4$
9	16.008	31.78	5-Hydroxymethylfurfural	126	$C_6H_6O_3$
10	17.713	1.13	N-Nitroso-2,4,4-trimethyloxazolidine	168	$C_8H_8O_4$
11	19.426	0.33	5-Acetoxymethyl1-2-furaldehyde	165	$C_7H_7N_3O_2$
12	19.840	0.16	3-Bromo-5,5dimethyl-cyclohex- enol	144	$C_6H_8O_4$
13	29.060	58.64	4-o-Mythlmannose	194	$C_7H14_4O_6$
		100.00			

CHAPTER FIVE

5. DISCUSSION

Fenugreek is a herb native to southern Europe. It was originally used by Greek farmers as livestock fodder. This herb offers medicinal benefits. The leaves and seeds help lower the level of glucose in the blood, which reduces fatigue, confusion, dizziness, and headaches associate with diabetes (Pearson, 2011). Fenugreek seeds are commonly used for seasoning proposes and are used ingredients in curry powder and sauces. Fenugreek works to increase appetite and many chest disease. It also contains antioxidants and anti-inflammatory compounds. Maintains blood sugar levels (Abuzied, 1986; Rosengraten, 1969).Cinnamon also has a long history as a spice and medicine. The presence of cinnamon extracts has been observed to increase blood glucose level and contain anti-bacterial, anti- inflammatory and anti- fungal properties. Sage contains antioxidant compounds that treat abdominal pain and cold (Talpur, *et al.,* 2005). Carob grows as a hard, evergreen shrub or tree that reaches a height of 10 meters, and the leaves are partially renewed in the spring (Diamantoglou and Mitrakos, 1981).

The carob is a dioecious species with some hermaphroditic forms; thus male, female and hermaphrodite flowers are generally borne on different trees. Unisexual and bisexual flowers are rare in the inflorescence. The flowers are initially bisexual, but usually one sex is suppressed during late development of functionally male or female flowers (Tucker, 1992a). To be able to benetfit from the treatment of some diseases. This plant is used to gradually lower blood sugar levels in diabetics and has antioxidant and antibacterial properties. It containts active ingredients such as alkaloids, flavonoids, steroids, saponins, etc and is a medical plant (Allen, K .and Ethe, I. (1981) .While the effectivenees of cinnamon is attributed to its distinctive components such has cinnamly alcohol, cinnamaldehyde oil, cinnamic acide, and coumarin, this herb has anti-allergic, antiviral, antimicrobial, and antioxidant properties. Cinnamon has been shown to be effective in removing accumulated sugar Farnworth, N. R.(1985). Interest in the carob plant has increased rapidly in the last two decades according to published publications. (Batlle, I. and J. Tous. 1994). This research aimed to study the plant chemistry and soft chromatography of the components separated from some natural products and their antibacterial activity against *E.coli* and S. aureus Three samples (fenugreek. Cinnamon and carob) were obtained from the local market in Wd madani. They were then shade-dried and swabbed from laboratory tests. This study was conductedin the food Analysis laboratory. Faculty of technology and Microbiology laboratory University of Gezira.The bacterial growth. Colony count, and germination of both bacteria were tested using aculture medium containing NA and EMB. This because cinnamon contains tanins, saponins, alkaloids and flavonoids, while it does not contain glycosides and steroids.Cinnamon,on the other hand, contains tanins, saponins and steroids while it dose not contain alkaloids, flavonoids, glycoside and steroids Huffman, C. and Evans, A. C. (1911).The result of the study showed that the liquid weight with aconcentrations of 100% of the integrated exteracts between 7.707mg and 70156mg for fenugreek, cinnamon and carob on the toilet on E.coli and S. aureus and the weight before drying with aconcentration of 0.0% of the integrated exteracts between 7.712mg 7.335 and 7.88mg for fengreek, cinnamon and carob on the toilet on *E.coli* and *S. aureus*. Therefore the RF values of the extracts were as 0.22mg, 0.55mg and 0.91mg for fenugreek and the RF values for cinnamon were 0.50% and 0.35% and the RF values for carob were 0.40% for the extracts and cinnamon and the original weight was 1g. The solution also contains a high percentage of moisture containing 30.2% compared to only 1.33% in cinnamon. On the other hand, cinnamon contains a high percentage of cheese (66.20%, 4.49%, only for the detection of the approximate analysis of fenugreek, moisture mic, caffeine, fiber and carbohydrate.) 76.0, 3.43, 2,22, 6.3, 1.63 and 10.30. The approximate analysis of cinnamon 10.51. 19.11, 1.83. 6.00. 1.19 and 61.63 and approximate analysis of fenugreek 30.54, 12.27, 19.9017.1, 10.70 and 9.49 and approximate analysis of cinnamon stem 15.49, 66.25, 0.03, 2.33, 15.58 and 4.3 and the GC result of investment plant, cinnamon and yogurt on 15 / 6 / 2022. University of Gezira. The study recommended conducting further studies and research on these plant extracts for other sources that contribute to industries, nutrition cosmetics and pharmaceuticals.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. The physical characteristics showed that the local fenugreek in comparison with Indian sample was relatively similar in characteristics of weight and density but differ in color.
2. Medicinal properties of Carob have to be additionally studied, especially treatment of male infertility.
3. The nutritional and economic potential of Carob are huge. In order to maximize them, more research should be invested in Carob's health and nutrition benefits.
4. Further studies are suggested for diabetic patients' treatment with more or less amount of the fenugreek – cinnamon mixture, and for periods more than two weeks.
5. In the world, there is an increase in some nutrition originated diseases. For preventing this, nutritional habits should include unrefined energy sources. Carob is a suitable option for taking unrefined sugar, minerals and phenolic compounds at the same time.
6. In addition, carob tree can prevent soil degradation. For poor soils, carob tree is valuable from agricultural point of view.

6.2. Recommendations

1. More studies are needed to determine the antibacterial activity of the some natural products (Carob, Fenugreek and Cinnamon) on other microorganisms.
2. Natural products (including carob, fenugreek and cinnamon) need special care in treating patients, other than drugs.
3. More photochemical characterizations need to be done.
4. More studies are needed to determine the antimicrobial activity of the plant extract (Fenugreek, Cinnamon and Carob) on other organisms.
5. Plant Extract (Fenugreek, Cinnamon and Carob) can be used as antibacterial agents' commercial state.

Plate (1) Colonies of *S.aureus* in concentration 100% in Fenukreek extracts

Plate (2) Colonies of *E.coli* in concentration 100% in Cinnamon extracts

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