Repulic of Iraq

Ministry of Higher Education

and Scientific Research

University of Misan

College of Science

Isolation, identification and infestigation of antibacterial activity of alkaloids compounds from *Capparis spinosa* in Misan/Iraq

A Thesis Submitted to

The Council of the College of Science / University of Misan In Partial Fulfillment of the Requirements for the Master Degree in Biology

By

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(2016)

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2022 A.D 1444 A.H

بسم الله الرحمن الرحيم

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ
فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ
شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لا شَرْقِيَّةٍ وَلا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ
تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ
وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ (٣٥)

صدق الله العلي العظيم

سورة النور – الآيه ٣٥

Supervisor Certification

I certify that this thesis which entitled:

(Isolation, identification and infestigation of antibacterial activity of alkaloids compounds from *Capparis spinosa* in Misan/Iraq)

Presented by (Hawraa Saleh Mahdi) was prepared under my supervision in the Department of Biology, College of Science, University of Misan, as a partial fulfillment of the requirements for the Degree of Master in Biology.

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Acknowledgments

First of all, great thanks for Allah the most merciful the most compassionate, who gave me health, strength, and facilitated the ways for me to accomplish this work, and the prayer and peace of Allah be upon our Master and prophet Mohammed and his divine good family, who guided us to get out of darkness of nescience.

It is a pleasure to express my deep appreciation and thanks to my supervisor Professor **Dr. Waleed M. Ali** for highly inspiring guidance, encouragement and support for completing this thesis.

I offer my thanks and appreciation to all members of the Deanery of College of Science /University of Misan and Biology Department for their kind cooperation.

I would also like to commend the positive efforts and invaluable assistance of Mr. Ahmed and Mr. Hassan to facilitate my work.

Finally, I am sincerely indebted and grateful to my family, who were a great supporter for me during the most difficult circumstances that I faced, and a great tribute with my deep apology to all whom I have not mentioned with my respect.

Hawraa

Dedication

To the candles of my life, to the lights by their inspiration I see my way to the best

my father & my mother

To those who share each moment of happiness and sadness as well, to the soul partners

my brothers & my sisters

To those who have given me their time, patience and knowledge

my supervisors & To everyone I love

Hawraa

Abstract

Collected plant samples *Capparis spinosa* from the center of Misan City from October 1, 2020 to November 1, 2020. It was transferred to the Biology Laboratory/College of Science/ University of Misan, for drying, grinding and extraction process and after completing the process of extracting the crude compounds and alkaloid compounds, they were biologically tested in the laboratory on pathogenic bacteria Gram-positive and Gram-negative isolated from Al-Sadr Hospital in Misan City (identified by VITEK2), and had a strong effect on these pathogenic bacteia to varying degrees according to extract type.

The current study included the preparation of alcoholic (methanol and ethanol) and aqueous extracts of a kind of medicinal plants indigenous in Iraq (leaves, stems and roots) of *Capparis spinosa* and studying the chemical components of those extracts by using appropriate reagents**.**

The extracts have a substantial effect on *Staphylococcus aureus* and *Escherichia coli* bacteria. *Capparis spinosa* has the potential to be a source of natural antibacterial compounds with therapeutic benefits. *Capparis spinosa* is a significant plant that produces a large number of active metabolites naturally through the secondary metabolism pathway.

Escherichia coli, which is already known to be multi-resistant to different antibiotics had inhibited its growth by aqueous and methanolic extracts of *Capparis spinosa* roots at a concentration of 250mg/ml with an inhibition zone of 20.5mm and 20mm respectively. As for *Staphlococcus aureus* bacteria its growth was inhibited by the methanol alcohol extract of the root of *Capparis spinosa* at a concentration of 250mg/ml in the inhibition zone 19mm, as well as the ethanol extract at a concentration of 250mg/ml for the roots and leaves of *Capparis spinosa* in the 11mm and 10mm inhibition zone respectively.Such results are very

interesting because these bacteria were isolated from the hospital environment. It is very difficult to control it by therapeutic means.

The results of the alkaloid extracts showed that the diameter of the inhibition zone for the extracts, the direction of bacterial growth, increased significantly with the increase of the concentration. *Capparis spinosa* roots showed that the maximum inhibition zone diameter at 250 mg/ml concentration was 13mm and 20mm for *Staphylococcus aureus* and *Escherichia coli* respectively.

Bacterial strains were isolated from Inflammation of the urinary tract (UTI) samples and the basic bacteriological procedures were used to identify and diagnose bacterial species, as well as Vitek2 was used to identify the isolates more.

This study also included the separation, purification and identification of the alkaloid compounds from the roots of the *Capparis spinosa* plant. Alkaloids were diagnosed through the use of some physical and chemical tests, which represented the degree of melting point and solubility, as well as gas chromatography-mass spectroscpy, thin layer chromatography, ultraviolet spectrometry (UV) and fourier transform infrared spectrometry (FT-IR).

The results of thin layer chromatography reveals that the appearance of one spot using the solvent system chloroform: methanol was used to confirm the purity of alkaloids (0.5 : 9.5) the relative flow value was 0.25 .

In addition, the safe use of the compound was determined by conducting a cytotoxicity test on human red blood cells. The results of the examination showed that the compound did not have any cytotoxicity at all studied concentrations.

The extract was evaluated using GC-MS gas chromatography and many chemical compounds were identified. Gas chromatography shows the presence of most linolenic acids in abundance and their esters such as n-Hexadecanoic acid and Octadecanoic and other compounds such as Phenol,2,6-dimethoxy-, Diethyl

Phthalate, Pyrrobutamine, 5-Hydroxy-4',7-dimethoxyflavone, Xylometazoline acetate, and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6 6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one.

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List of Abbreviations

Abbreviations	Key
e.g.	exempil gratia
DNA	Deoxyribonucleic acid
G	Gram
PCR	Polymerase Chain Reaction
TLC	Thin - layer chromatography
GC-MS	Gas Chromatography – Mass Spectroscopy
FT-IR	Fourier Transform Infrared Spectrometry
UV	Ultra violet
WHO	Would Health Organization
S.aureus	*Staphylococcus aureus*
E.coli	*Escherichia Coli*
+ve	Gram positive
-ve	Gram negative
µg/gm	Microgram/gram
µl	Microliter
ml	Milliliter
bp	base pair
rpm	rotation per minute
Rf	Relative flow Coefficient
RBCs	Red Blood Cells
MDR	Multi drug resistant
EDTA	Ethylendiemintetraacetate
° C	Celsius degree
NCBI-BLAST	National Center for Biotechnology Information-Basic Local Alignment Search Tool
Vitek	Vitality index of traditional environmental knowledge
UTI	Inflammation of the urinary tract
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant genes
Coh.E	Alcoholic extract ethanol

Coh.M	Alcohol extract methanol
C.A	Cold Aqueous.
NMD	Nuclear magnetic resonance
R	Resistant

Chapter One

Introduction

&

Literatures Review

1:Introduction

1.1:Introduction

Capparis spinosa L., commonly known as (Caper), is a perennial winter deciduous species with rounded, fleshy leaves and flowers white to pinkish blooms that grow in a wide range of climatic conditions, from the desert to cooler elevations of mountains (Azaizeh *et al*., 2003). Caper plant parts associated with drugs prepraration, cosmetics, and food, as well as a seasoning, It has alot of oil and high in vitamin E (Giuffrida *et al*., 2002). Various compounds have been distinguished in *C. spinosa*, including alkaloids, flavonoids, unstable oils and unsaturated fats.

Medical plants therapeutic value are from their biochemical activity in treating a variety of ailments caused by harmful microorganisms like bacteria, fungus, and parasites. (Macedo *et al*., 2021). These medicinal plants have a variety of active chemical compounds with various active functional groups in their chemical structures (AL-Salman *et al.,* 2017). Large number of active chemical compounde were synthesize in the secondary metabolism in the medicinal plants. Essential oils, phenols, glycosides, alkaloids, steroids, saponins, and terpenes are chemical substances within up these natural groups (Mohammed and Al-Maliki, 2014; Tlili *et al*., 2010). As a result, the list of herbal-based medications is growing. According to the World Health Organization (WHO), roughly 74 percent of 119 plant-derived pharmaceutical medications are used in contemporary medicine in methods that are directly related to their traditional applications as plant medicine by indigenous peoples (Heinrich *et at*., 2017).

Medicinal plants knowledge has its origins in ancient civilizations, and cultures, and its main aim is to return the body to a state of natural balance with its active ingredients (Khoshkharam *et al.,* 2021; Shahrajabian *et al.*, 2021). The main medification functions of medicinal herbs in traditional herbal sciences of

countries like Iran, China, India and Japan have been received notable attentions in recent years (Sen and Chakaborty, 2017). Traditional medicine, based largely on herbs, still supports the primary healthcare of more people worldwide than conventional or western medicine (Zhang *et al*., 2015), and they are culturally acceptable and readily available even in modern era (Shahrajabian *et al*., 2021).

Interest in medicinal plants increased after the discovery of modern methods of separation, as scientists began to uncover and search for what these plants hide from therapeutic secrets and buried treasures, and within them lies the secret of humankind's summary of some incurable diseases and saving millions of lives of millions of people, so interest in them returned as sources Essential for the treatment of many diseases at present and their contribution to limiting the spread of epidemics and eliminating some diseases (Plotkin, 1994).

As most of the currently known medicines are of plant compounds and about 25% of modern pharmacopeia are of vegetable origin (O'Hara *et al*., 1998). Phenolic compounds, flavonoids, tannins, alkaloids, glycosides, essential oils, and steroids are active chemical compounds found in diverse portions of medicinal plants, and their presence gives these plants their pharmacological and biochemical effect in treating various disorders (Ullah *et al*., 2011). Clinical microbiologists are interested in the antimicrobial activity of active chemical compounds extracted from medicinal plants for a variety of reasons. The first is the medical importance of photochemical as drugs against the biological action of microorganisms, followed by the use of natural components to treat human diseases. The second reason is that these active compounds isolated from plants have no side effects (Parekh, 2007).

It was suggested that *C. spinosa* contain a rich source of sulfur compounds, as well as phenolic and flavonoid glycosides, which involve the potent cytotoxic effect (Bakr and El Bishbishy, 2016). *C. spinosa* berries are rich in carbohydrates

(5%), dietary (3%), protein (2%), and fats (0.9%), as wall as a moderate amount of vitamin C (4 mg/100 g FW) content (Allaith, 2016). Alkaloids, glycosides, carbohydrates, tannins, phenols, and triterpenoids were found in the ethanolic extract, and alkaloids, steroids, carbohydrates, flavonoids, tannins, phenols, and saponins were found in the aqueous extract of *C. spinosa* (aerial parts) (Fatin, 2012).

Alkaloids are heterocyclic nitrogenous chemicals found throughout the plant kingdom that have powerful antibacterial, antifungal, anti-parasitic, anti-cancer, and anti-tumor activity. Alkaloids are basic chemicals that include one or more nitrogen atoms and are formed from plants (usually in the heterocyclic ring). In addition, an alkaloid molecule must have at least one ring and be bonded to at least two carbon atoms (Shenta and Al-maliki, 2013; Al-maliki., 2011). Among the secondary metabolites that are produced by plants, the alkaloids act as a very prominent class of defence compounds. Over 21,000 alkaloids have been identif ied, hence they constitute the largest group among the nitrogen-containing secondary metabolites (besides 700 nonprotein amino acids, 100 amines, 150 alkylamides, 100 glucosinolates and 60 cyanogenic glycosides). Alkaloids are usually present as a mixture of a few major and several minor alkaloids of a particular biosynthetic unit, which differ in functional groups (Wink *et al*., 2005).

1.1.1: Aim of the study

Despite the important role of medicinal plants in different fields, and their growth in different regions of Iraq, they did not receive sufficient attention from researchers, and studies and research on them were few, so this study focused on several aspects.

1-Preliminary test for the efficacy of aqueous and alcoholic extracts of the roots, leaves, and stems of *Capparis spinosa* against some Gram-positive and Gram-negative bacteria.

2-Isolation, purification and identification of some compounds that have an effect on the bacteria from *Cappars spinosa*.

3-Study of the biological activity of isolated alkaloids against some multi-resistant clinical bacterial isolates.

4-Testing the cytotoxicity of the extract aganst RBC of humans to determine the capacity and using alkaloids compounds as antibiotics alternative.

1.2: Literature Review

1.2.1 :Antibacterial activity of medicinal plants

Human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substance from various sources, medicinal plants are valuable natural resource and regarded as potentially safe drugs, they have been playing an important role in alleviating human sufferings (Zahin et al., 2010; AL-Naimy et al., 2012).

Numerous different examinations were accomplished concerning the biochemical action of dynamic synthetic metabolites as antibacterial, antifungal, antiparasitic, antitumor, and anticancer specialists likewise incredible outcomes were gotten from the impact of these synthetics against the development of various microbes and malignancy cells in this manner the spices and plants were considered as treatments and medications to treat all diseases and irritation brought about by these pathogenic microorganisms (Hudson *et al*., 2000; Padmini *et al* ., 2010).

Several decades ago, it was found that there are strains of pathogenic bacteria that are resistant to antibiotics as they are considered one of the major problems in clinical settings (Frieri *et al*., 2017). Fortunately, these antibiotic-resistant bacterial strains were a great stimulus for the development of new methods that rely on natural sources to eliminate these resistant bacterial strains. Therefore, most of the microbial studies in recent years focused on discovering new anti-microbial compounds from different sources, which confirmed that the

process of systematic screening of natural plants will Lead to the discovery of effective compounds against germs (Banerjee *et al*., 2011).

Many studies indicated that the studied plant extracts possessed an anti-microorganism effect. It was mentioned that the possession of aqueous and alcoholic extracts of all parts of the plant was effective against microbes towards several positive and negative bacterial isolates for the chromium dye (Thakur *et al*., 2011).

A study showed that the crude extracts of all parts of the plant (leaves, stems, roots, and flowers) have efficacy against the microbial direction of bacteria (Goyal *et al*., 2008).

The Based of *C. spinosa* is viewed as antibacterial and its alcoholic concentrates of the husk of natural products, seeds and blossoms were exhibited to be antibacterial (Ramezani *et al*., 2008; Orient and Vaidyaratnam, 1994). An examination by (Steenkamp *et al*., 2004) referenced that *C. spinosa* has antibacterial movement against both G+ve and G-ve *S. aureus, Streptococcus pyogenes, E. coli and Pseudomonas aerugenosa.*

1.2.2: Medicinal plant of present study

1.2.2.1: The caper plant (*Capparis spinosa*)

Many species of *Capparis* have been reported in Iraq, from the northern to southern plateaux of the nation Chakaraty, and there are about 150-200 species of the genus *Capparis* (Capparaceae) (1976). *Capparis spinosa* ., commonly known as (Caper), is a perennial winter deciduous species with rounded, fleshy leaves and flowrers white to pinkish blooms that grow in a wide range of climatic conditions, from the desert to cooler elevations of mountains (Azaizeh *et al*., 2003). *C. spinosa* has a wide bactericidal impact on gram-positive and negative-microscopic

organisms. At the end of the day, it seemed significant impact against biotic and abiotic stresses (Alupului and Lavric, 2008). Interestingly, the bark and fruit aqueous extracts from this species have been reported to act as a diuretic, poultice, expectorant and astringent as well as they possess anti-inflammatory and antifungal activities (Al-Said *et al*., 1988; Ali-Shtayeh and Abu Ghdeib, 1999; Ihsan *et al*., 1999; Eddouks *et* al., 2005; Hussain *et* al., 2007; Uysal *et al*.,2012). The reported medicinal health functions and nutritional attributes of *C. spinosa* can be mainly attributed to the occurrence of alkaloids, glucosides, reducing sugars, essential fatty acid , vitamin C , terpenoids, flavonoids, and resins in the fruit and leaves of this species (Rastogi and Mehrotra, 1990; Joshi *et al*., 2011).Moreover, *C. spinosa* has cancer prevention agent, antimicrobial, anticancer, and hepatoprotective impacts, which brought about its acquiring distinctive pharmacological impacts (Yadav and Agarwala, 2011).

In customary medication, changing pieces of *C. spinosa* have been generally utilized for the treatment of different human diseases (Santhi and Sengottuvel, 2016). like airborne parts and roots that have been utilized for the treatment of ailment, gastrointestinal issues, migraine, kidney and liver illness just as toothache (Banjara *et al*., 2012). The antioxidant, nephroprotective, and hepatoprotective effects of *C. spinosa* methanolic extract are linked to its phytochemical content, and nine compounds, rutin, resveratrol, coumarin, epicatechin, luteolin, catechin, kaempferol, vanillic acid, and gallic acid, are more responsible for the traditional use of *C. spinosa* to treat kidney and liver diseases (Tlili *et al.*, 2017). *C. spinosa*, Rosa canina, Securidaca secure era, Silybum marianum, Urtica dioica, Trigonella foenum-graecum, and Vaccinium Arctostaphylos are used in a traditional Persian medicinal formulation for diabetic Mellitus, with no hepatic, renal, or gastrointestinal side effects (Mehrzadi *et al.,* 2021).

Middle eastern customary medication has been proposed to utilize leaves, roots, and buds of *C. spinosa* in the treatment of spleen infections, stomach issues, skin sicknesses, ear infections, and kidney infections just as hepatic sicknesses notwithstanding the suggestion for the therapy of loss of motion, seizures and gum issues . and alcoholic extract of root possesses significant antibacterial activities was active against *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* (Bouriche *et al*., 2011; Boga *et al*., 2011).

1.2.2.2: Classification

According to the botanical scheme of Engler, the plants is classified as follows:

- Kingdom : Plants
- Division : Phaneerogamae
- Subdivision : Angospermae
- Class : Dicotyledonae
- Subclass : Polypetalae
- Order : Thalamiflorae
- Suborder : Parietales
- Family : Capparidaceae
- Genus : *Capparis*
- Species : *spinosa* (Metcalfe & Chalk, 1950)

1.2.2.3: Phytochemistry

It is well understood that the biological activities of plants and their formulations are due to the presence of a wide variety of phytochemicals such as alkaloids, steroids, terpenoids, polyphenols, and tocopherols among others (Joshi *et al*., 2011).

Capparis spinosa is rich in a wide variety of biologically active compounds including natural antioxidants (phenolic acids , flavonoids, tocopherols), alkaloids, polyphrenols, glucosinolates, and reducing sugars along with several essential minerals, proteins, and lipids. Fruit of *C. spinosa* has been well studied for its phytochemical constituents and found to contain alkaloids (0.74%), glucosides (0.083%), fats (3.75%), ascorbic acid (13.5%), reducing sugars (32.9%), resins (23.75%) and organic acids (14.1%) (Rastogi and Mehrotra, 1995).

Capparis spinosa berries are high in carbohydrate (5%), dietary (3%), protein (2%), and fats (0.9%), and they have a moderate amount of vitamin C (4 mg/100 g FW) content. According to (Allaith, 2016). Alkaloids, glycosides, carbohydrates, tannins, phenols, and triterpenoids were found in the ethanolic extract, and alkaloids, steroids, carbohydrates, flavonoids, tannins, phenols, and saponins were found in the aqueous extract of *Capparis spinosa* (aerial parts) (Fatin, 2012).

Phytochemical studies have revealed the existence of several beneficial substances such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols, and carotenoids, according to (Tlili *et al.*, 2011). The primary component in non-fermented berry extracts is glucocapparin, which is completely destroyed during the fermentation process, whereas epicatechin levels are decreased and free quercetin is seen after fermentation (Jimenez-Lopez *et al.*, 2018).

1.2.2.4 :Biological activity

A few analysts have detailed distinctive natural exercises of *C.spinosa* separates in different in vivo and in vitro test models. Certain pharmacological properties of incredible interest of *C. spinosa* had been recognized and others are being considered (Moufid *et* al., 2015). It is significant that the greater part of the confirmations about natural movement phytochemistry gets from the investigation of wild plant material. *C. spinosa* watery concentrates showed a critical enemy of hyperglycemic movement and against stoutness impacts (Eddouks *et al*., 2004, 2005; Lemhadri *et al*., 2007). Likewise, a study on caper fruit ethanol extracts on type 2 diabetic patients in Iran showed a significant decrease in fasting blood glucose levels and glycosylated hemoglobin and also a significant decrease in triglyceride level, thus assuring previous results on the anti-hyperglycemic and hypolipidemic effects of *C.spinosa* (Huseini *et al*., 2013).

Up to now, there has been much scientific evidence showing that *C. spinosa* possesses different pharmacological effects including antioxidant, antimicrobial, anticancer, and hepatoprotective effects (Mishra *et al*., 2007; Tesoriere *et al*., 2007; Lam and Ng, 2009; Aghel *et al*., 2010; Tlili *et al*., 2010; Gull *et al*., 2015). have been shown to cure asthma, cough, got, ulcer, rheumatism, diuretic, tuberculosis and tumor genesis (Khare, 2008).

1.2.2.5: Antibacterial activity

The extracts of various pieces of *Capparis* show organic action against an enormous number of microorganisms. The concentrates of Caper (roots, flwers and stems) have shown better antibacterial action in contrast with anti-microbials as it is demonstrated by essentially high development restraint acquired in different bioassays at an exceptionally low focus (Upadhyay *et al*., 2010). The antibacterial activities of petroleum ether, water, butanol, methanol, and hexane crude extracts

obtained from the aerial parts of *C. spinosa* were examined by the agar well diffusion method. Different fractions exhibited good to moderate degrees of activity against most of the tested bacteria. Extracts were most active against *Staphylococcus epidermidis* and *Streptococcus faecalis* (Al-shayeb, 2012). Ethanolic and petroleum ether extracts were used to study the antimicrobial activity of *C. spinosa* against gram- positive and gram- negative micro-organisms by the disc diffusion method. Both extracts showed significant antimicrobial activity against gram-positive organisms, *Bacillus cereus*, and *Staphylococus aureus*, and gram- negative organisms, *Pseudomonas aeruginosa*, and *Escherichia coli* compared with standard antibiotics (Reid *et al*., 2005).

1.2.3: Resistance of pathogenic bacteria to antibiotics

Antibiotics are compound substances considered auxiliary metabolites delivering by microorganisms during fixed stages. They can inhibit the growth of microorganisms or kill them, are widely used for the treatment of bacterial infections in humans and animals, as well as in nonmedical applications. The global annual production of antibiotics is estimated to be as high as 100–200 thousand tons, with more than one billion tons having been produced since 1940 (Wang *et al*., 2010;Czekalski *et al*., 2014).

The last decade has seen increasing interest in the spread of bacterial resistance in the natural environment, and this interest has stemmed from growing concern among the medical and scientific community related to the rapid escalation of antibiotic-resistant bacteria (ARB), including resistance to a new generation of antibiotics and pharmaceuticals of last resort. Resistant bacteria are responsible for infections that are more difficult to treat, requiring the use of drugs that are more toxic and more expensive. In some cases, bacteria have become resistant to all known antibiotics (Nureye, 2020).

The phenomenon of drug resistance is exacerbated by the fact that a wide variety of antibiotics are not only used for medical and veterinary purposes but also to promote the growth of livestock (Davies *et al*., 2010).

The massive scale of antibiotic use and antibiotic misuse accelerates the evolution of ARB (antibiotic resistant bacteria) and ARG (antibiotic resistance genes) in the environment, thus increasing the risk of transmission of the environmental resistome to humans (Manaia, 2017).

There are four main mechanisms by which bacteria become resistant to antimicrobial agents, Destruction/Inactivation of the antibiotic, Blockage of transport of the agent into the cell, providing the cell with a replacement for the metabolic step inhibited by the drug, , and protection of the target site by a bacterial protein. Due to these setbacks, it is therefore imperative to seek other sources of therapeutic agents (Lambert, 2005; Elliott *et al*., 2007). The antibiotic resistance could be transferred from one strains to another by transposable gene (Transposons), which could be transposed from the Chromosome to the proper plasmid (Mansouri *et al*., 2001). Some specific examples of extracellular and intracellular microbial species that have developed significant resistance over the years are as follows, *Staphylococcus aureus, Streptococcus pneumonia, Streptococcus viridans, Enterobacter aerogenes, Pseudomonas aeruginosa and Pseudomonas stutzeri*

1.2.3.1: *Staphylococcus aureus*

Staphylococcus aureus is aerobic and facultative anaerobic, oxidase-negative, catalase-positive, nonmotile, sugar fermenter, and non-spore forming bacteria. On a solid culture medium, it forms glistening, smooth, raised, and circular colonies. The single colony can reach up to the size of about 4-6 mm in diameter when grown on non-selective media (Abinet, 2015). The basis of differentiation of *S.*

aureus from other *Staphylococcus* species is its ability to ferment mannitol sugar and production of a yellow zone around the colony on Mannitol Salt Agar (*MSA) medium. S. aureus has the capacity to metabolize othercarbohydrates such as glucose, lactose, sucrose, and maltose, *S. aureus* is capable of growing at a wide pH range (4.8-9.4). It can survive at high temperatures such as 60°C for 30 min and are resistant to dehydration (Crossley *et al.,* 2009). Moreover, these bacteria can tolerate 7.5-10% of salt concentration due to the production of compatible solutes (osmoprotectants) (Graham and Wilkinson, 1992). If conditions for growth are not favorable, *S. aureus* can enter into a dormant stage and can survive for years until conditions become favorable. The ability to survive under extreme environmental conditions is an advantage of enabling the bacteria to become a life-threatening pathogen. In addition, the very thick cell wall of this bacterium helps to exist with the highest internal pressure compared to any type of bacteria (Freeman-Cook and Freeman, 2005). Infections causes by *S. aureus* used to respond to β-lactam antibiotics. However, development of methicillin resistance amongst *S .aureus* isolates (MRSA) left very little choices for treatment. In 2007, resistant profile of Ethopian strains of S. aureus strains revealed 80% resistance against tetracycline and 53% cotrimoxazole and chloramphenicol (Tessema *et al*., 2007).

In a survey 51% *S. aureus* were found to be MRSA, as well as the bacteria cause food poisoning, toxic shock syndrome, scalded skin syndrome. Most of these strains produce β-lactamase is enzyme that cleaves the β-lactam ring and inactivates the antibiotic, such organisms can be treated with β-lactamaseresistant penicillins, e.g., nafcillin or cloxacillin (Cole *et al*., 2001; Mamza *et al*., 2010; Essack.,2002).

1.2.3.2: *Escherichia coli*

Escherichia coli is a member of the Enterobacteriaceae family that is gram-negative, bacillary, motile or non-motile, aerobic or facultatively anaerobic, fermented to lactose sugar, most of which is fermented to same ramenose and sorbetole, producing an β-glucuronidase enzyme. For their growth optimum temperature (36-37) (Wanger *et al*., 2017; Jawetz *et al*., 2016). positive for catalase and negative for oxidase, produced for indole and not consumed for citrate, positive for methyl red ,and negative for Fox Proscor (Vogase-Proskauer) (Hemraj *et al*., 2013) . It lives naturally in the intestines of humans and animals and is at the same time opportunistic pathogens causing many diseases such as diarrhea, meningitis, sepsis, bacteremia and is one of the most common bacterial types that cause urinary tract infections, as they cause About (90%) of the urinary tract infections in the world, and they are more common in childhood (Hadi *et al*., 2014). siderophores, colistin cytotoxic necrotizing factor their possession of surface structures such as flagella, capsule, and lipopolysaccharides (LPS), which give bacteria antigen properties by producing flagellar antigen H (H), somatic antigen 0 (0 somatic antigens) ,and capsular antigen K (K). They also have cilia (fimbriae or pills) that help them attach to the host's tissues, giving them the ability to form a biofilm (Terlizzi *et al*., 2017; Zowawi *et al*., 2015)

E. coli bacteria are characterized by having multiple antibiotic resistance (Multidrug) Resistance (MDR) (Larid, 2016). It is characterized by its high resistance to antibiotics due to its possession of resistance enzymes such as β-lactamases that confer resistance to β-lactams, and enzymes that confer resistance to aminoglycosides and quinolones. These bacteria also possess other mechanisms that give them antibiotic resistance, such as changing cell membrane permeability, altering the target site, inhibiting protein synthesis, and bacterial possession of

efflux pumps, which confer antibiotic resistance such as macrolides, novobiocin and Rifamicn (Kapoor, 2017).

1.2.4: Alkaloids

Alkaloids, one of the largest groups of natural products, represent a highly diverse group of chemical entities. Alkaloids encompass an enormous class of approximately 12 000 natural products. The principal requirement for classification as an alkaloid is the presence of a basic nitrogen atom at any position in the molecule, which does not include nitrogen in an amide or peptide bond. As implied by this exceptionally broad definition, the alkaloids from a group of structurally diverse and biogenically unrelated molecules. Many of these compounds possess potent pharmacological effects. For example, the well-known plant alkaloids include the narcotic analgesics, morphine and codeine, apomorphine (a derivative of morphine) used in Parkinson's disease, the muscle relaxant papaverine, and antimicrobial agents sanguinarine and berberine. Also, several potent anti-cancer drugs have been developed from plant compounds (Salminen *et al*., 2011;Bribi , 2018). Other alkaloids, including cocaine, caffeine, and nicotine, possess restorative or stimulating activities and have been used in energy drinks or as recreational drugs. Some alkaloids can be toxic to human beings such as atropine and solanine (Noureddine *et al*., 2013 ;Bribi *et al*., 2015). The seeking of new bioactive alkaloids from plant extracts appeared to be an attractive source for drug discovery (Bribi *et al*., 2016).

Alkaloids also occur in the animal kingdom and microorganisms, some examples being the *Aspergillus spp* alkaloids (Yamamoto and Arai, 1986) and Pyocyanine from *Pseudomonas aeruginosa* (Cordell, 1983) and many alkaloids have been recently isolated from marine environment, especially from the sponges (Gallimore *et al*., 2005). It is worth mentioning

that *C. spinosa* is a rich source of different classes of alkaloids which include spermidine, indole, and pyrrole alkaloids along with indol-aldehyde and indol-nitrile type derivatives. Several new alkaloids and their glycosides have also been identified in *C. spinosa*. For example, three new spermidine alkaloids including capparispine, 26-O-β-D-glucoside, and cadabicine 26-O-β-D-glucoside hydrochloride were separated from the roots of *C. spinosa* and their structures were confirmed by NMR spectroscopy (Fu *et al*., 2008).

1.2.4.1: Antibacterial properties

Alkaloids have been accounted for as having a wide scope of natural properties, Vittatine alkaloid has antibacterial action against the *S. aureus* and *E. coli* (Dubouzet *et al*., 2005), and the china mine alkaloid shows solid action against B. subtilis and *S. aureus* (Adesanya *et al*., 1992). There is data about paracrin, which shows antibacterial movement against *S. aureus and P. aeroginosa*, just as biscarbazole alkaloids showed solid antibacterial action against the Gram-negative microscopic organisms *E. coli* and *P. Vulgaris* (Loeffler and Zenk, 1990). Antibacterial properties are referenced on account of Campangine as an aftereffect of in vitro examines (Kluza *et al*., 2005). A Study by (Pick *et al*., 2006) affirmed that bromotyrosine alkaloids have viable antibacterial action against Gram-positive microscopic organisms. The indole alkaloids from marine conditions are a promising and dynamic gathering of atoms against G+ ve and G-ve microscopic organisms (Gul and Hamann, 2005).

1.2.5: Parameters of Identification of alkaloids compounds

1.2.5.1: Melting Point (MP)

Melting point (MP) is a simple and fast method used in many (many different kinds of people or things) areas of chemistry to get a first impression of the purity of a substance. This is because even small amounts of dirt change the melting point, or at least enlarge its melting range. Melting point strong desire about somethings is more than just a classroom exercise in the organic chemistry lab. The test is still an important way of doing things for measuring the purity of organic and drug-based compounds. The strong desire for something of melting points is one of the oldest identification and test methods for organic substances (Strouse, 2003)

1.2.5.2 : Thin Layer Chromatography (TLC)

Since the early 1960s, thin layer chromatography (TLC) has been widely used for the separation and identification of both known and undiscovered chemicals in biological materials (Langman and Kapur , 2006). TLC has aseveral well-known features, including its simplicity and ease of use, high sample throughput, and ease of sample preparation, Low analysis costs, varied visual detection, and equal application to pure substances, medicinal formulations, and illicit drug preparations (Fodor *et al.*,2006; McClean, 2004). Several writers have evaluated a wide range of TLC applications in the literature, spanning from toxicological drug screening to food and agriculture analysis, biological sample analysis, systematic toxicological analysis (STA) in clinical and forensic toxicology, pharmaceutical purity analysis, and pesticide analysis are all examples of STA (Ojanpera, 1992; Maurer, 1994; Sherma, 2000; Sherma and Fried , 2005; ;Olech et al.,2012; Shmera, 2015).

1.2.5.3: Ultraviolet Sectroscopy (UV)

Absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region is referred to as ultraviolet–visible spectroscopy or ultraviolet–visible spectrophotometry. This implies it works with light in the visible and nearby (near-UV and near-infrared (NIR)) wavelength ranges. The color of the substances involved is directly affected by their absorption or reflectance in the visual spectrum. Molecules undergo electronic changes in this part of the electromagnetic spectrum. This technique is similar to fluorescence spectroscopy in that it measures transitions from the excited state to the ground state, whereas fluorescence spectroscopy measures transitions from the ground state to the excited state (AL-Araji and Ali , 2012).

1.2.5.4: Fourier Transform Infrared Spectrometry (FT-IR)

Based on the differences in spectra, FT-IR research will reveal the composition of chemical substances. In addition, the principal chemical compound can be revealed in the greatest amount by understanding the characteristic peaks of chemical function groups. This is why we choose to use FT-IR and then focus on examining metabolite features to gain a sense of the chemical components in distinct tissues (Sun *et al*., 2010; Lu *et al.,* 2008). Later in the metabolite profile study, the FT-IR results can be used to connect it to its chemical composition. A faster and less expensive validation approach can be used to complete part of the aforementioned validation procedure for raw materials (leaves, stems, and roots). As a result, we've taken on the challenge of developing a quick quality assurance method that incorporates statistical modeling for extracting meaningful information from infrared spectroscopic data. This is critical for the production process in the future.

1.2.5.5: Gas Chromatograph Mass Spectrometry (GC-MS)

It has a very wide range of applications, but the first and most important field of use is the separation, and analysis of mixtures of various components such as base oils, hydrocarbons and solvents (Kadhim *et al*., 2016 ; Mohammed *et al*., 2016). And the identification of various organic compounds such as acids, alcohols, aldehydes, esters, short-chain fatty acids, fat oxides, terpenes, and phenolics (Roze *et al*., 2012).

Gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Kanthal et al., 2014).

Chapter Two

Materials & Methods

2: Materials and methods:

2.1 :Mterials

2.1.1: Table (2.1) shows all the equipments and instruments, which used in the current tudy.

Table (2.1): Equipments and Instruments used in this study.

No.	Equipments &Instruments	The Manufacture company (Origin)
1.	Microwave	Shownic (Korea)
2.	Test tubes	ALS (Canada)
3.	Filter papers	Whatman No.1 (UK)
4.	Visible spectroscop UV	Shimadzu (Japan)
5.	Distallwato	GFR (Germnay)
6.	Magnatic stirrers	Heidolph (Germnay)
7.	Flask	Iso Lab (Germnay)
8.	Slides and cover slides	Superestar (India)
9.	Oven	Memmert (Germnay)
10.	TLC	Macherey-nagel(MN) (England)
11.	Fourier transform infrared (FT-IR 8400s) Spectrophotometer	Shimadzu (Japan)
12.	Electrothermal (Melting point apparatus)	Engineering LTD (Japan)
13.	Test tube	Samco (India)

14.	Water bath	Memmert (Germany)
15.	Micropipette	Watson (Japan)
16.	Shaker incubator	Lsi_3016R (Korea)
17.	Burner	Amal (Turkey)
18.	Hot plate	Gallen Kamp (England)
19.	Syringe	ISO9001 (Germany)
20.	Refrigerator	Ishtar (Switezeland)
21.	Electeic mill	KRAVE (Koeea)
22.	Cotton swabs with transport media	MDIC (India)
23.	Centrifuge	Memmert (Germany)
24.	Appendroff tube	Gallen (Kamp)
25.	Vortex mixer	Heidolph (Germany)
26.	Digital balance	Denver (Germany)
27.	McFarland meter	Biomerieux (France)

2.1.2: Culture Media and Biological Materials

Culture media were prepared according to the manufacture's instructions, sterilized by autoclaving at 121° C for 15 minutes, and adjusted pH adjustment. The culture media and biological materials are mentioned in table (2.2).

Table (2.2): The Culture media used in this study.

No.	Culture media	The Manufacture company (Origin)
1.	Nutrient agar	Himedia (India)
2.	Muller-Hinton agar	Himedia (India)
3.	Nutrient broth	Himedia (India)
4.	Blood agar	Himedia (India)

2.1.3: Chemical Materials

Table (2.3) shows the chemicals , which used in the current study.

Table (2.3): The Chemical materials used in this study.

No.	Chamical Material	The Manufacture company (Origin)
1.	Absolute ethanol	RBL (Spain)
2.	Absolute mathanol	RBL (Spain)
3.	Acetone	Sigma (USA)
4.	Chloroform	RBL (Spain)
5.	H_2SO_4	GFR (Germnay)

2.1.4: Reagents

2.1.4.1: Preparation of chemical reagents

1-Dragendroff′s reagent

The reagent was prepared according to the protocol described by (Harborne,1984).

- Solution (A) was set up by adding 0.6 g of Bismuth Subnitrate and 2 ml of Hydrochloric corrosive to 10 ml of refined water.
- Solution (B) was set up by adding 6 g of Pottasium iodide to 10 ml of refined water.
- Solution (A) and Solution (B) were mixed, and afterward, 7 ml of HCL was added to the mixure, and water to atotal volume of 200 ml.

2-Mayer's reagent

Mayer's reagent was set up by:

- Dissolving 1.35 g of Mercuric chloride in 60 ml of refined water.
- Dissolving 5 g of Potassium iodide in 10 ml of refined water.
- Both arrangements were refined water to final volume of 100ml (Contreras *et al*., 1999).

3-Marqu's reagent

It was prepared based on the protocol of (Tyler *et al*., 1988) one ml of for (40%) were mixed with concenteated sulfuric corrosive.

2.1.5: Antibiotic Discs

The antibiotic discs were purchased from acomercial supplier (Bioanalyse, Turkey).

Table (2.4): List of the antibiotic disks used in this study.

No.	Antibiotic class	Antibiotic name	Symbol	Disk concentration µg
1.	Antimycobacterials	Rifampin	RA	5 µg
2.	Macrolides	Erythromycin	E	10 µg
3.	Tetracyclines	Oxytetracyclin	OXT	30 µg
4.	Fluoroquinolones	Ciprofloxacin	CIP	10 µg

2.1.6: Kits

The kits used in this study were listed in the table (2.5).

Table (2-5): Kits used in this study.

No.	Kit type	Supplier	Origin
1.	Gram stain Kit	Titan Biotech . LTD	India
2.	Vitek2 Kit Gram positive ID . Kit Vitek2 Kit Gram Negative ID .Kit	Biomerieux	France

2.2: Methods: The general steps for research are shown in figure (2-1)

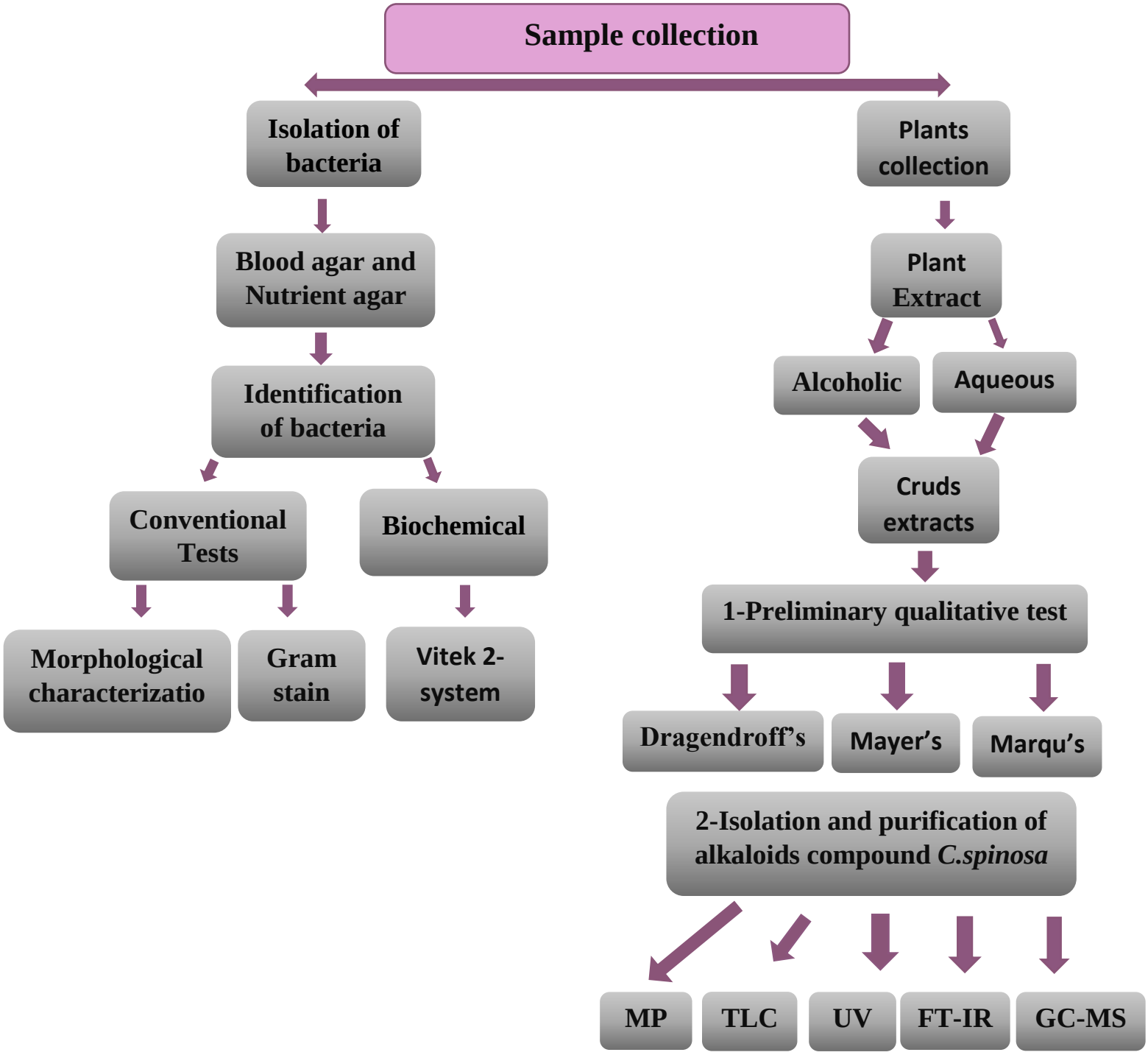


Figure (2-1) : Higlighted the flow work staps of the present study.

2.2.1 : Plants collection and authentication

The plants samples of *C. spinosa* were collected from the center of Misan City in October 2020 and identified by Dr.Abdullah.H.AL-Tamimi, Biology Department, Faculty of Sciences/ Basrah University Figure (2.2)

Figure (2.2): Shows *C. spinosa*

2.2.2 : Preparation of Plants Samples

According to (Cremades *et a*l., 2018), each piece of plant was cleaned by faucet water, dried at room temperature ,and ground into powder by an electrical processor. The powdered plant parts was kept in aplastic cylinders tabl in the refrigerator at 4 ° C for 1h untituse.

2.2.3 : Preparation of Plant Extracts

The plant was extracted with three types of solvents, in cluding distilled water , ethanol and methanol alcohol. In both cases, the extracted parts of plants were *C. spinosa* leaves, stems and roots (Harborne, 1998).

2.2.3.1 : Aqueous extracts

The extracts were prepared according to (Ahmad *et al*., 1998; Al-Jboriy *et al*., 2010), 28 grams of powdered plant and 500 ml of distilled water were shaked in 500 ml flask on Magnatic stirrer plate for 24 hours at room temperature , Then the extract was filtered by using multi-layer of medical gauze and by filter paper type Whattman, No. 1 and dried at room temperature at 25 °C. following the dried extrac was scratched by sterilized clean knife. After that it has been weighted and kept in refrigerator for furtuer analysis.

2.2.3.2: Alcoholic extracts

The extracts were prepared according to steps in 2.2.3.1 with replacing the distilled water by ethanol and methanol 96% (Ahmad *et al*., 1998; Al-Jboriy *et al*., 2010).

2.2.3.3: Concentration of plants extracts

Different cncentraction of the crude extract were prepared for each 500mg of dried crude extract was dissolved in to 1ml of D.W to prepare 500mg/ml , Similary other concentration could be prepared(Gezahegn *et al*., 2015).

2.2.3.4: Detection of extracts product

The detection of yield was obtained using the following equation (ATememy, 2013).

$$\left[\frac{\text{resultant quantity after extraction}}{\text{Total quantity before extraction}} \right] \times 100$$

2.2.3.5:Chemical detection of the active components in plant extracts

The following reagent were prepared to detect the prescence of active compounds within the crude extract.

2.2.3.5.1 : Preliminary qualitative test

The identification of alkaloids compounds was implemented using several tests such as

- Dragendroff's Reagent
- Marqu's Reagent
- Mayer's Reagent

Alkaloids

Detection by numerous reagents as indicated by (Harborne, 1998).

1-Dragendroff's reagents

About 1 ml of each crude extract was treated with 1 ml of Dragendroff reagents, the improvement of orange residue shows the presence of alkaloids.

2-Mayer's reagent

Five ml of the which concentration of each plant part were treated with 1 ml of Mayer's reagent .The advancement of turbidity and white dregs referes the presence of alkaloids.

3-Marqu's reagent

The positive result indicated by the turbidity of the solution.

2.2.4 : Extraction of distilled water acidified with sulfuric acid

The method of (Therese and Evans, 2003) was followed by placing 50 grams of the root, stem, and leaves of the *Capparis spinosa* plant in a 1000 ml conical flask and adding 400 ml of distilled water acidified with 4% sulfuric acid. The mixture was left with continuous stirring on the magnetic stirrer device for 24 hours at room temperature. The solution was filtered with filter paper type (Whattman, No.1), then transfer the filtrate to a separating funnel, and add to every 10 ml of the solution 30 ml of chloroform and shake the solution gently and leave it to settle for a some time until the solution separates into two layers, then the bottom layer was collected and placed in a Petri dish and left until dry at room temperature Then, the dry substance was washed with acetone to discarded unwanted materials, and then tests were conducted for the dry substance to confirm its purity using the thin layer chromatography technique, to know its relative flow values (Rf Values) and some other confirmatory disclosures such as Melting point, Ultraviolet Spectra (UV), Fourier Transform Infrared Spectrometry (FT-IR) and Gas Chromatography – Mass Spectroscopy (GC-MS) as was conducted It has bioactivity tests to know its effect on some standard bacteria and other pathogens with multiple resistance (MDR).

2.2.5: Identification of alkaloids compounds

2.2.5.1: Thin - Layer Chromatography (TLC)

The glass plates (2 x 10 cm) coated with silica gel was used in the identification of isolated alkaloids compounds. TLC plates are used in glass tanks, using ascending chromatography. The sample was spotted or lined by capillary tube, the spot was allowed to dry and then the plate was placed in the glass chromatography tank with the solvent previously placed in the bottom of the tank to a depth of 10 mm. It was often necessary to equilibrate the vapor in the tank by placing filter paper around the sides of the tank. The solvent is allowed to rise by capillary flow to the top of the plate. However, the mobile phase reaches to two centimeters from the top of the plate then the plate was removed and allowed to dry. The spots are then developed with appropriate reagents for the types of compounds being separated and assessed (visualized by spraying with Dragendroff reagent solution). (Harborne, 1998; Cseke *et al.,* 2016). Relative flow (Rf) calculated from below equation:

Rf = distance a compound moves / distance solvent front (Harborne, 1998).

2.2.5.2: The determination of Melting Point (MP)

The melting points of chemical compounds are regularly reported, and they can serve as a useful indicator of substance identity and purity. However, the meaning of these reported data is not always clear. Organic chemistry textbooks and laboratory manuals commonly report that (complete, with nothing else mixed in) substances melt over narrow temperature ranges ,and reagent bottles are often labeled with "m. p." ranges of (more than two, but not a lot of) degrees (McCullagh and Daggett, 2007). Electrothermal melting point apparatus was used for the

determination of melting point of the isolated compounds. Melting points of single compounds alkaloids were determined in open capillary tubes (Harborne, 1998).

2.2.5.3: Ultraviolet Spectra (UV)

Ultraviolet spectra of purified compounds were obtained on UV-1601PC UV-Visible spectrophotometer (Shimadzu) using alcohol methanol as solvent. Ultraviolet spectra of the isolated compounds were carried out in the Department of Chemistry / Faculty of Sciences/University of Misan .

2.2.5.4: Fourier Transform Infrared Spectrometry (FT-IR)

Spectra of the isolated compounds were measured with (FT-IR) to determine functional groups of purified compounds, and were recorded in KBr disc using a FT-IR-8400S Fourier transforminfrared spectrophotometer (Shimadzu), in the Chemistry Department / Faculty of Sciences/University of Misan.

2.2.5.5: Gas Chromatography – Mass Spectroscopy (GC-MS)

The alkaloids isolated from *Capparis spinosa* extracts were separated and identified by using the gas chromatography-mass spectrum (GC – Mass) technique by using GC-Mass instrument type (Agilent Technoloies GC 7890A GC system) in Iran. The alkaloids samples were injected by the concentration of in gas chromatography instrument with standard optimizations. Different peaks were separated and recorded at different retention times. Then separated chemical compounds were identified by using mass spectrum.

2.2.6: Identification of bacterial isolation

2.2.6.1 : Conventional Tests

Primative identification of bacteria was carried out by applying two conventional methods ,morphological and staining methods as folloing :

2.2.6.1.1 : Morphological characterization of bacterial isolation

A single colony was taken from each primary positive culture on blood agar, MacConckey agar and mannitol salt agar and repeat growth for gain pure culture, then it was identified depending on its morphological and cultural characteristics (blood hemolysis, lactose fermentation, mannitole fermentation, colony shape, size, colour, borders, and texture) then it was examined under the microscope after making smear from pure colony on clean slid and stain with Gram's stain for observation arrangement and reaction bacteria with stain (Habib et al., 2015).

2.2.6.1.2 : Gram Staining

Bacteial isolates were stained with Gram staining to differentiate batween G – ve and G +ve bacteria (Beveridge, 2001).

2.2.6.2 : Identification of bacteria by Vitek2 system

The Vitek2 method was utilized to confirm the diagnosis of UTI bacterial isolates in the current study. The system requires a bacterial suspension from the suspected germs, which is deposited in an infected tube and then transferred to a card, which is then incubated under temperature control. Colors change in the card as a result of the bacteria's metabolic activity, and light intensity were measured in an interrupted form every 15 minutes. Finally, the data is automatically saved, processed, and printed (Pincus, 2006).

The steps are described in more detail as follows

I- Preparation of Bacterial Suspension

An adequate number of bacterial colonies are transferred using a sterile disposable loop from pure culture produced on MacConkey agar, blood agar, or nutrient agar, and suspended in sterile saline solution 0.45% (3 ml) in a clear plastic test tube. After adjusting the turbidity with a turbidity meter (densities) at the range, the density of the suspension was tested (0.5- 0.63).

II- Inoculation of Identification Card

The identification card was inoculated with bacterial suspension using an integrated vacuum apparatus and the cassette (special rack) was placed in a test tube containing the bacterial suspension in the slot next to the identification card. As soon as it's placed into the appropriate suspension tube (transfer tube). Up to 10 or up to 15 tests can be stored on the cassette. After that, the filled cassette is delivered into a vacuum chamber station, either automatically or manually. Following the reintroduction of air and the application of vacuum to the station, the bacterial suspension was injected and drove down the transfer tube into microchannels, filling all of the test wells.

III- Card Sealing and Incubation

An inoculated card was passed via a mechanism that shut off the transfer tube and then locked the card before loading it into the carousel incubator. All card types were nurtured on-line at (35.5 + 1.0° C) in the carousel incubator, which can store up to 30 or 60 cards. Each card is transferred from the carousel incubator once every (15 minutes) and carried to the optical system for reaction reading. The carousel incubator was then returned until the following reading time. Data were collected at 15-minute intervals throughout the incubation phase.

2.2.6.3: Preservation of bacterial isolates

1-Short time Preservation

On the nutritional agar culture plates and nutrient agar slants, a single pure colony of the bacterial isolate was streaked. Incubated for 18 hours at 37° C , sealed well, and stored at 4 ° C in the refrigerator for one month for plates and three months for slants (Alabi, 2008).

2-long time Preservation

The bacterial isolate was inoculated into the nutrient broth and incubated at 37° C for 18 hours, after which the broth culture was maintained by adding glycerol to a final concentration of 20% and storing it at -20° C for 12-18 months (Terajima *et al.,* 2000).

2.2.6.4: Antibiotic Sensitivity Test

Antibiotic sensitivity of bacteria was investigated, according to the procedare described by (Harley and Prescott *et al.,* 1996).

1. The ends of 4-5 isolated colonies were plucked from the original culture using a sterile wire loop and placed into a test tube containing 10 ml Mueller Hinton agar, then incubated at 37 ° C for 2 to 5 hours to form a moderately turbid bacterial solution. McFarland tube No. 0.5 was used to compare turbidity.
2. A sterile cotton swab was dipped into the standardized bacterial suspension 15 minutes after altering the density of the inoculums. By spinning the swab hard against the inside of the tube above the fluid level, the surplus fluid was evacuated. To achieve uniform distribution, the swab was swiped in two separate planes onto the dried surface of a Mueller-Hinton plate.

3. The plate lids were replaced, and the inoculated plates were set aside for 3-5 minutes on a flat, level surface to allow excess moisture to evaporate.
4. The selected discs were placed on the inoculated plate and gently pressed into the agar using sterile forceps. The inoculation plates were incubated inverted at 37° C for 18-24 hours after being inoculated in 15 minutes.
5. Using reflected light and a ruler, the diameters of the complete inhibition zone were noted and measured after incubation. The area with no observable growth was chosen as the endpoint, which was measured to the nearest millimeter.
6. The diameter of the inhibition zone for each antimicrobial agent was converted to sensitive and resistant categories using an interpretive chart from the National Committee for Clinical Laboratory Standards of Antimicrobial Susceptibility Testing (Atlas *et al.,* 1995),

2.2.6.5: Antibacterial activity of extract

The agar diffusion method was used to determine the antibacterial activity of the extracts, replacing the antibiotic disc with filter paper discs with a diameter of 6 mm. The aqueous extracts are dissolved in water with the dosages: 1mg/ml, 250 mg/ml , 125 mg/ml,62.5 and 30 mg/ml. The methanolic extracts have been dissolved in methanol alcohol with: 250 mg/ml, 125 mg/ml , 62.5 mg/ml and 30mg/ml. The Ethanolic extracts have been dissolved in ethanol alcohol with: 250 mg/ml, 125 mg/ml , 62.5 mg/ml and 30mg/ml. The bacterial strains are subcultured by the streaking method, then incubation at 37° C for 18 to 24 h in order to obtain a young culture and isolated colonies. A colony well isolated has been mixed with 5 ml of sterile distilled water in a test tube in order to have an initial cell density or a turbidity close to 0.5 Mc Farland (Abs: 0.08-0.1 at 625 nm). Inoculation is carried out by using a

swab dipped in the inoculum, and spread over the entire surface of the Mueller-Hinton agar. The operation is repeated two more times by turning the box 60 °C each time to ensure a homogeneous distribution of the inoculum. Finally, the swab is passed around the edge of the agar surface. Then 6 mm diameters of Whatman filter paper No3 discs were prepared and impregnated with 30 µl of the different concentrations of extracts of *Capparis spinosa*. The impregnated discs are then gently deposited by sterile forceps on the surface of the inoculated agar. A disk impregnated with Alcohol / distilled water is also deposited as a negative control, as well as a ready-to-use disk serving as a positive control. The petri dishes are incubated in an oven at 37 ° C between 18 and 24 hours. The diameter of inhibition zone has been measured by transparent ruler to nearest mm.

2.2.6.6: Alkaloids' cytotoxicity

Human red blood cells were employed for the toxicity test, which was carried out according to (He *et al.*, 1994) .

- Two ml of RBC were transfered in the tube containing the anticoagulant EDTA substance.
- Using normal saline, a concentration of 500 mg/ml of compounds alkaloids was created.
- A negative control tube (containing only normal saline) and a positive control tube (containing tap water) were employed.
- For each alkaloid compound, a series of dilutes were made with normal saline solution (1: 1 and 1: 10 and 1: 100 and 1: 1000), with 0.8 ml of each dilute deposited in a tube.
- A pipe sterilizer was used to add 0.2 ml of RBCs to each tube, resulting in a final volume of 1 ml.

- Tubes were incubated in an incubator at 37°C for 3 hours, and the tubes were inspected after each hour of snuggling up to the note assessments of red blood cells.

Chapter Three

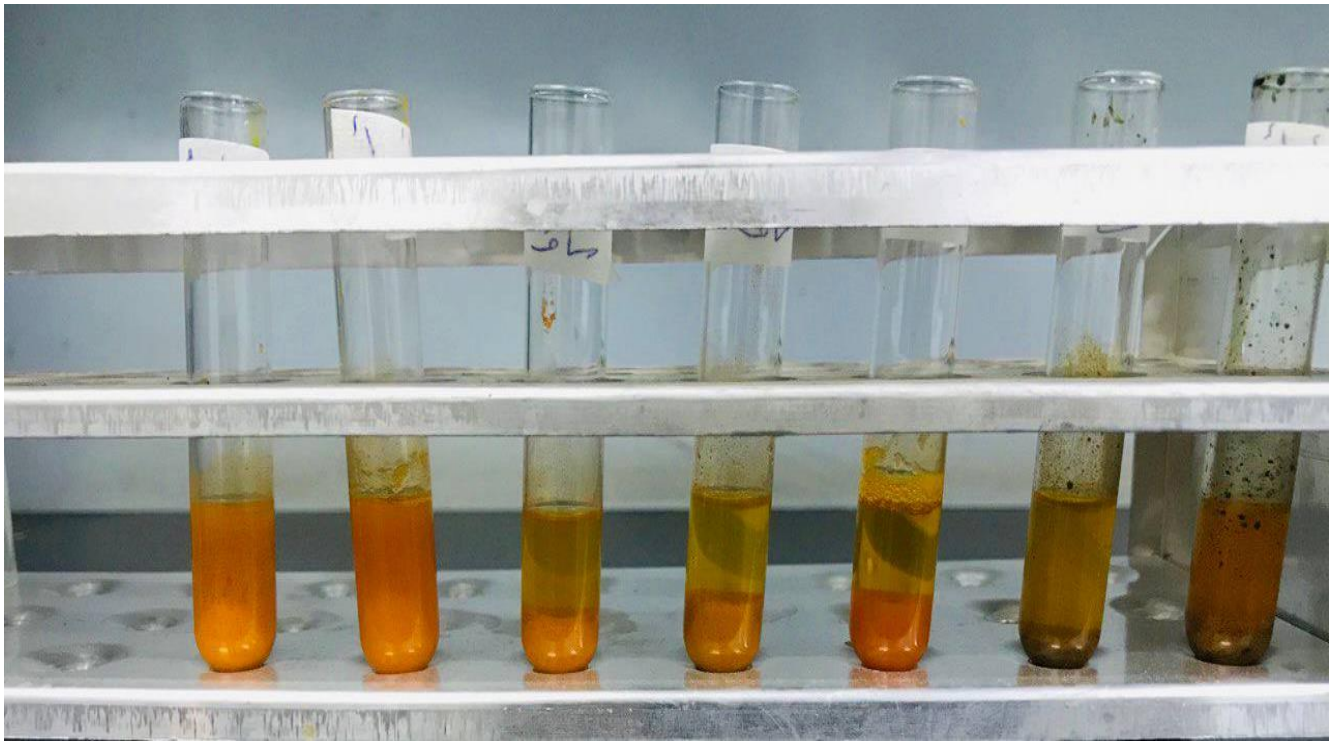
Results

3: The Results

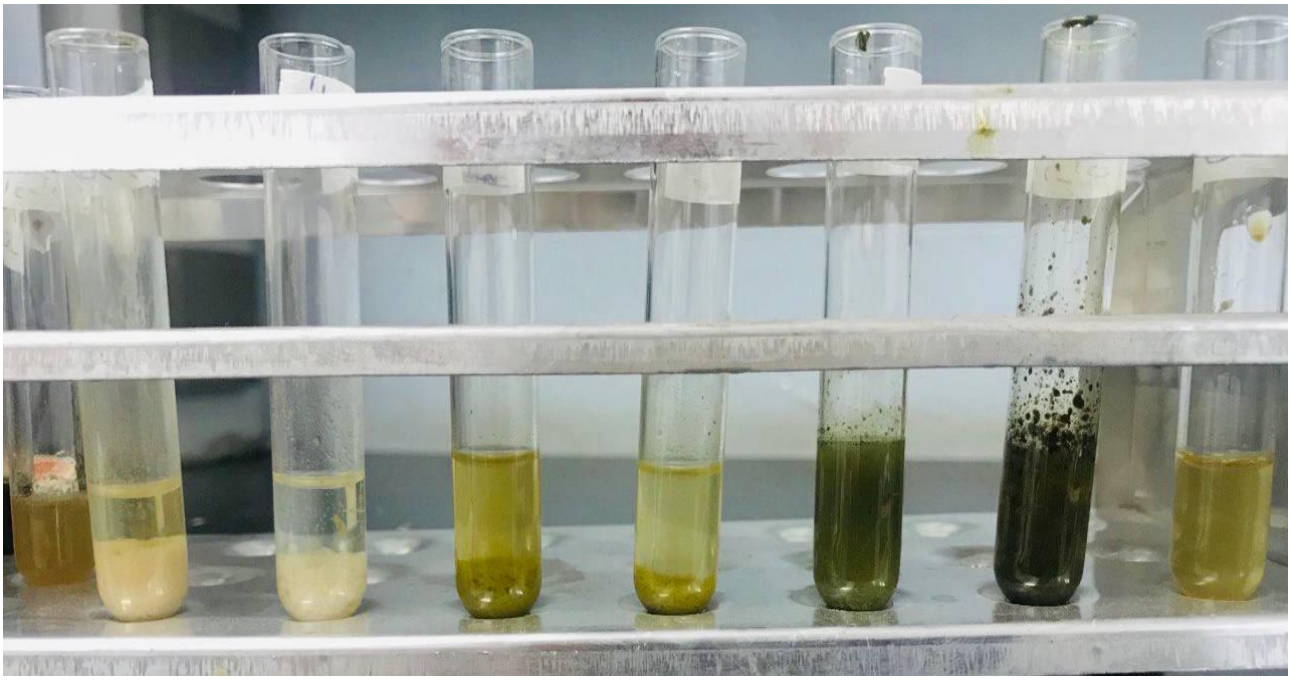
3.1: Phytochemical screening of *C.spinosa*

The results of phytochemical screening of alcohol methanol - ethanol and cold aqueous extracts of *C. spinose* leaves, stems and roots.

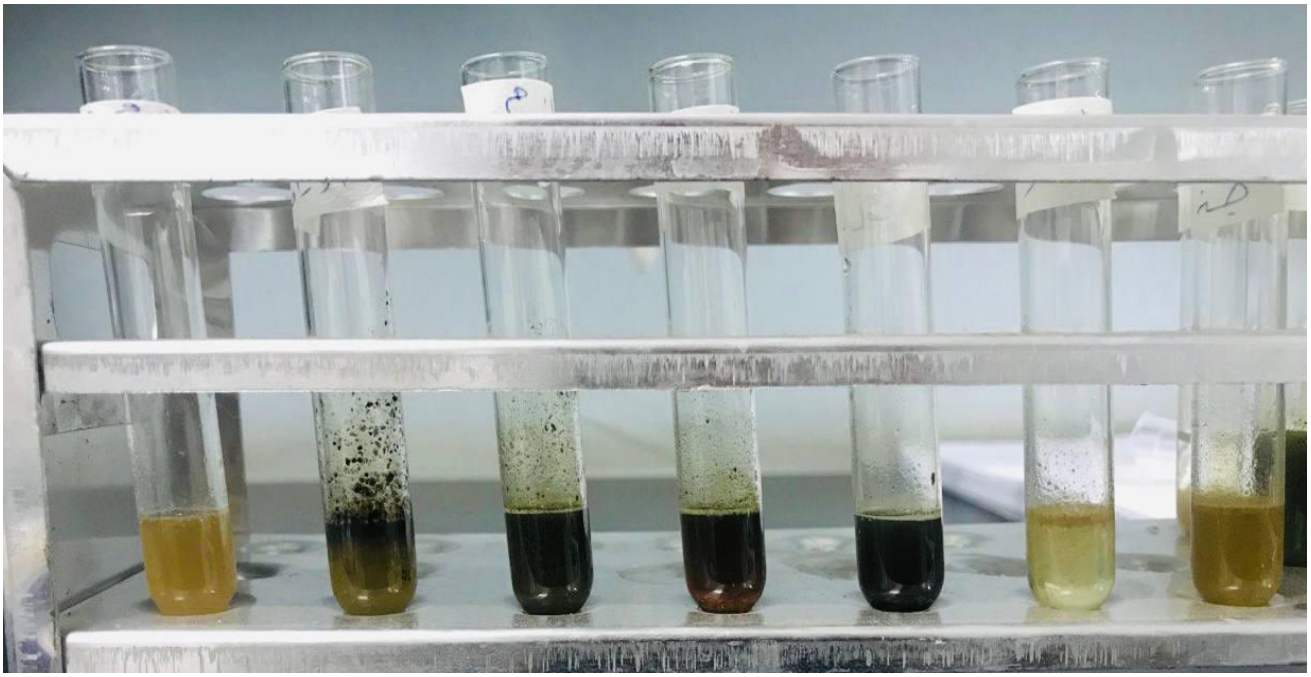
The orange andred preciptate highlighted the presence of alkaloid compounds in *C.spinosa* extracts when Dragndroff's and Marqu's reagents applied while no preciptate appeared with Mayer's reagents .This results confirmed the prescance of alkaloid in the exteact in Figure (3.1) and Table (3.1).



A- Dragndroff's reagent



B- Mayer's reagent



C-Marqu's reagent

Figure (3.1): (A, B, C) Phytochemical screening of *C.spinosa*

Table:(3.1): Preliminary phytochemical screening of *C. spinosa* extracts.

Plants parts	Type of extracts	Reagents		
		Dragendroff's reagents	Mayer's reagent	Marqu's reagent
Leaves of *C. spinosa*	Coh. E.	+	-	-
	Coh.M.	-	+	-
	C.A.	+	+	-
Stems of *C. spinosa*	Coh. E.	+	-	-
	Coh.M.	+	-	-
	C.A.	+	+	-
Roots of *C.spinosa*	Coh. E.	+	+	-
	Coh.M.	+	+	-
	C.A.	+	+	-

+ : The extract contains the alkaloid compound.

- : The extract doesn't contain the alkaloid compound.

3.2:The total product of all extracts

The product of extraction were revealed in Table (3.2) which show that different products of recovered studied plants extracts were observed by treating the same quantity of sample , the total products of extracts of *C. spinosa* using distilled water recorded the highest value 12.5% of the root while methanol extract showed the least product 1.58% of the stem.

Table(3.2): Product of the crude extracts using different solvent.

No.	Species	Type of extract	Part of plant	Product of extraction %
1.	*C. spinosa*	Coh.E	Leaf	4.44
			Stem	1.58
			Root	1.68
		Coh.M	Leaf	9.38
			Stem	6.00
			Root	5.58
		C.A	Leaf	12.5
			Stem	5.58
			Root	5.38

3.3: Effect of crude extracts of *C.spinosa* plant against bacteria (*E.coli* and *S.aureus*)

Two bacterial isolates, *E.coli* and *S.aureus* was used to investigate the antibacteria activity of the plnt extract.There were different concentraction were used (250, 125, 62, 5, 30) mg/ml .The growth inhibition of bacteria increased as the concentration of extract increased, the susceptibility pattern to the extracts on *E.coli* of aqueous crude extract of *C. spinosa* roots expressed maximum inhibitory zone at concentration 250 mg/ml which was 20.5 mm and the aqueous extracts showed no zone of inhibition on *S.aureus* . The methanolic crude extract of the roots of *C. spinosa* showed the inhibition zone at the concentration of (250, 125, 62, 5, 30) mg/ml respectively (20, 13, 12, 11) mm on *E.coli* . While methanolic

crude extracts of the stems *C.spinosa* showed the inhibition zone of *S.aureus* was 19 mm at concentration 250 mg/ml in the figure (3.2) . As for ethanolic extracts, they have moderate inhibition on both types of bacteria (*E.coli and S.aureus*). Inhibition zone with diameter was less than 12 mm. were considered as having no antibacterial activity, diameter between 12 and 16 mm. were considered moderately active, and these with > 16 mm. were considered highly active (Egharevba et al., 2010) as in the two tables (3.3) (3.4).

Table (3.3): The inhibition zone of crude extracts from (leaves ,Stems and roots) of *C. spinosa* on the growth of bacteria *E. coli* .

Plants parts	Type of extracts	Concentration mg/ml				
		30	62.5	125	250	Control
Leaves of *C. spinosa*	Coh.E	R	R	R	12	R
	Coh.M	R	7	8	9	R
	C.A.	R	8	9	11	R
Stems of *C. spinosa*	Coh.E	R	7	8	11	R
	Coh.M	R	8	8	9	R
	C.A.	R	R	10	13	R
Roots of *C.spinosa*	Coh.E	R	R	R	7	R
	Coh.M	11	12	13	20	R
	C.A.	R	R	R	20.5	R

L.S.D.$_{(0.05)}$ =2.341 for interaction

Table (3.4): The inhibition zone of crude extracts from (leaves ,stems and roots) of *C. spinosa* on the growth of bacteria *S. aureus* .

Plants parts	Type of extracts	Concentration mg/ml				
		30	62.5	125	250	Control
Leaves of *C. spinosa*	Coh.E	R	R	R	10	R
	Coh.M	R	R	R	R	R
	C.A.	R	R	7	8	R
Stems of *C. spinosa*	Coh.E	R	R	R	9	R
	Coh.M	R	R	7	19	R
	C.A.	R	R	R	R	R
Roots of *C.spinosa*	Coh.E	R	R	9	11	R
	Coh.M	R	R	R	7	R
	C.A.	R	R	R	7	R

L.S.D.$_{(0.05)}$ =1.823 for interaction

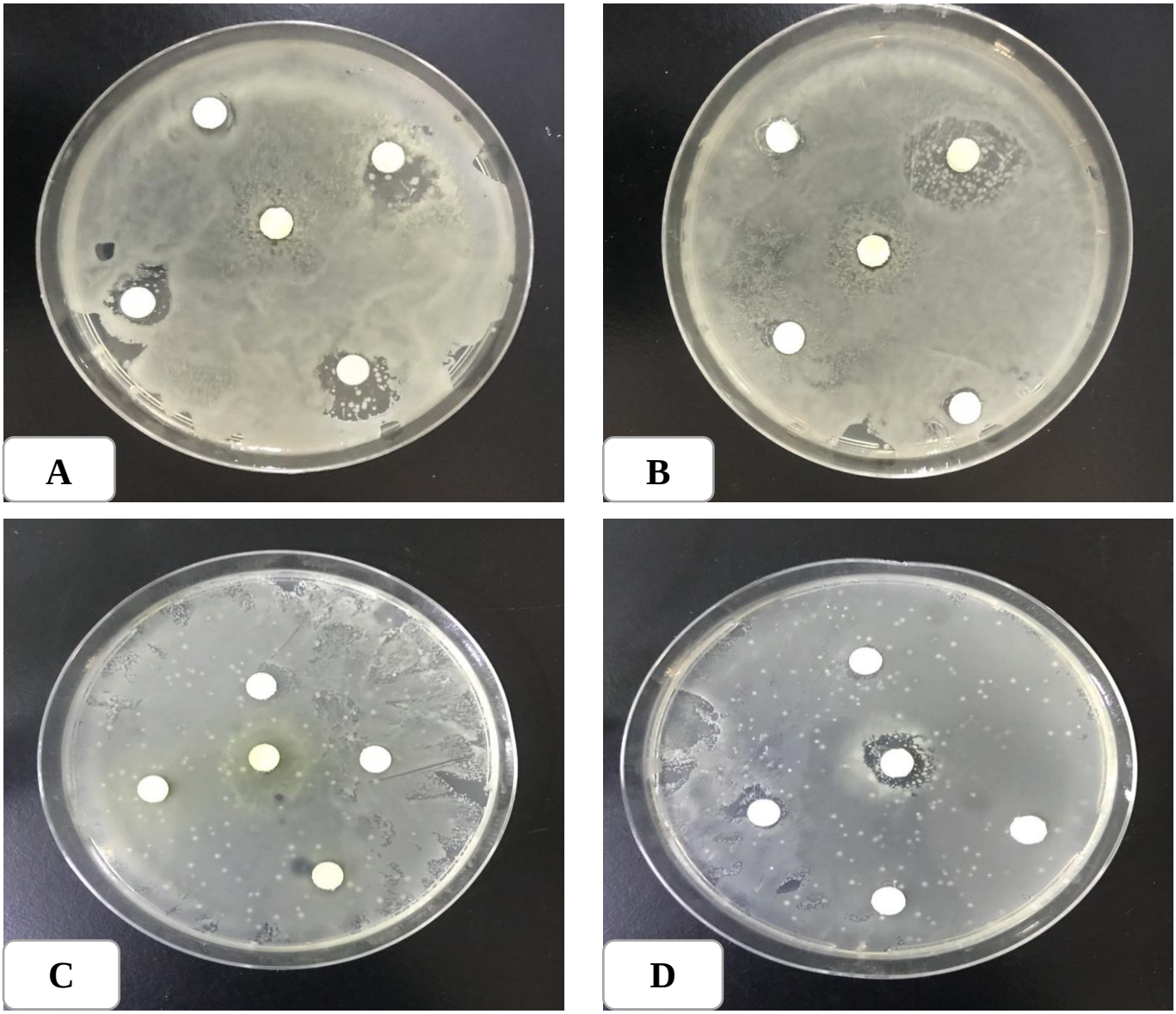


Figure (3.2):The effect of crude extracts on (A,B) *E.coli* and (C,D) *S.aureus* .

3.4 : Identification of alkaloids compounds

3.4.1 :Physical Properties

Table (3.5) and Figure (3.3) illustrate the physical properties of extracted alkaloids compounds including Phenol,2,6 dimethoxy-, Diethyl Phthalate, n-Hexadecanoic acid, Octadecanoic acid, Pyrrobutamine, 5-Hydroxy-4',7-

dimethoxyflavone, Xylometazoline acetate, and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one are fairly stable A beige gelatinous substance with a brownish color and higher melting points the previous compounds were characterized as.

At room temperature, we found that the isolated compounds dissolve easily in methanol alcohol and water howere they slightly soluble in ethanol, acetone and chloroform.This character encourages us to study the biological activity of these alkaloids. The structures of the isolated compounds are assigned on the basis of their TLC, UV, IR spectra ,and Gas Chromatography-Mass Spectroscopy(GC-MS).

Table(3.5):Physical properties of Alkaloids compounds.

Biological name	**Alkaloids**
Molecular formula	$C_8 H_{10} O_3$, $C_{12} H_{14} O_4$, $C_{16} H_{32} O$, $C_{18} H_{36} O$, $C_2 H_{22} CIN$, $C_{17} H_{14} O_5$, $C_{16} H_{24} N_2$ and $C_{39} H_{56} O_2$.
M.P.	221 ° C
Weight before isolation (gm)	40
Weight after isolation (gm)	9.8
Shape and Status of isolated compounds	A beige gelatinous substance with a browish color

M.P. = Melting point

1- Steps of alkaloids purificion using acetone from *C.spinosa* spp. **2- Alkaloid of *C.spinosa* spp.**

Figure (3.3): (1 and 2) Isolate the alkaloid using the method Therese & Evans

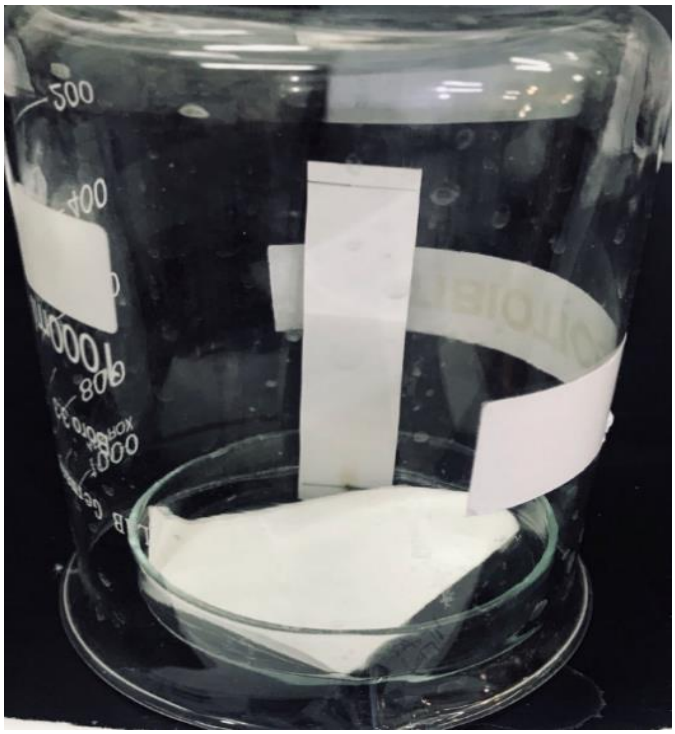
3.4.2: Chemical properties

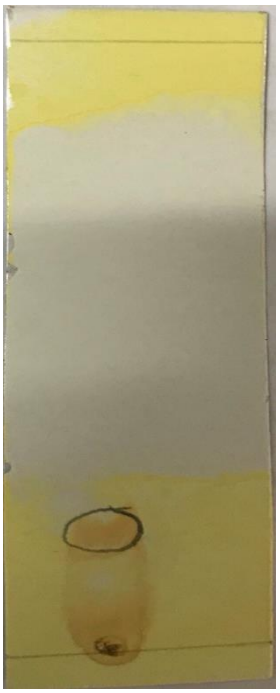
3.4.2.1: Thin Layer Chromatography Technique (TLC)

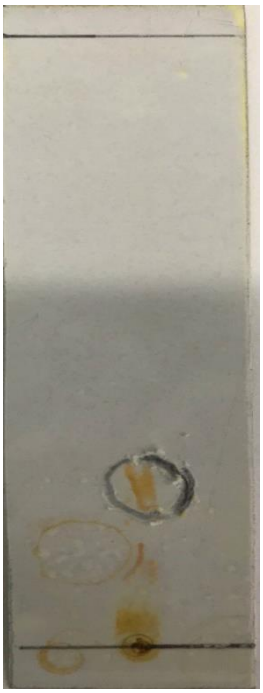
Table (3.6) and Figure(3.4) show the TLC results which noted, that the behavior of our isolated compounds was similar to the behavior of alkaloids (orang, after spraying with Dragendroff reagents) and compounds which isolated from the roots of *C. spinosa* (Alkaloids) Rf = 0.25 and Rf=0.23 . The difference in the value of Rf of alkaloids is ascribed to the degree of polarity and function groups of the each compounds and may be to the mobile phase.

Table(3.6): TLC results of alkaloids compounds.

Properties	Alkiloids
Rf	*0.23 ** 0.25
Color in visible light	Yellow
Color in dragenddroff reagent	Orange
Mobile phase	*NH3OH: CHCl3 (19.5: 0.5) **NH3OH:C3H6O: CHCl3 (9:0.5:0.5)
Time of posting	28 : 34 minute.







***NH3OH:CHCL3,(19.5:0.5), Rf= 23, 28m**

****NH3OH:C3H6O:CHCL3 (9:0.5:0.5), Rf=25, 34m**

Figure(3.4): TLC For the alkaloid extract of a plant *C.spinosa.*

Rf = distance a compound moves / distance solvent front (Harborne, 1998).

3.4.2.2: Ultraviolet Spectra (UV)

Ultraviolet spectra by methanol alcohol of alkaloids compounds were determined. The qualitative UV spectra profile of Alkaloids that isolated from root of *C. spinosa* of were selected at wavelength from 200-600 nm due to sharpness of the peaks and proper baseline. The electronic absorption data of the investigated compounds are displayed in Table (3.7) and the spectra of this compound are shown in Figure (3.5).

Table(3.7): UV spectral data of compounds by using methanol as solvent, λ max (nm).

No.	0.05 mg/ml			
	Band I		Band II	
	λ max	Abs.	λ max	Abs.
Compounds	283.00	0.2	255.00	0.2

Abs. = Absorption.

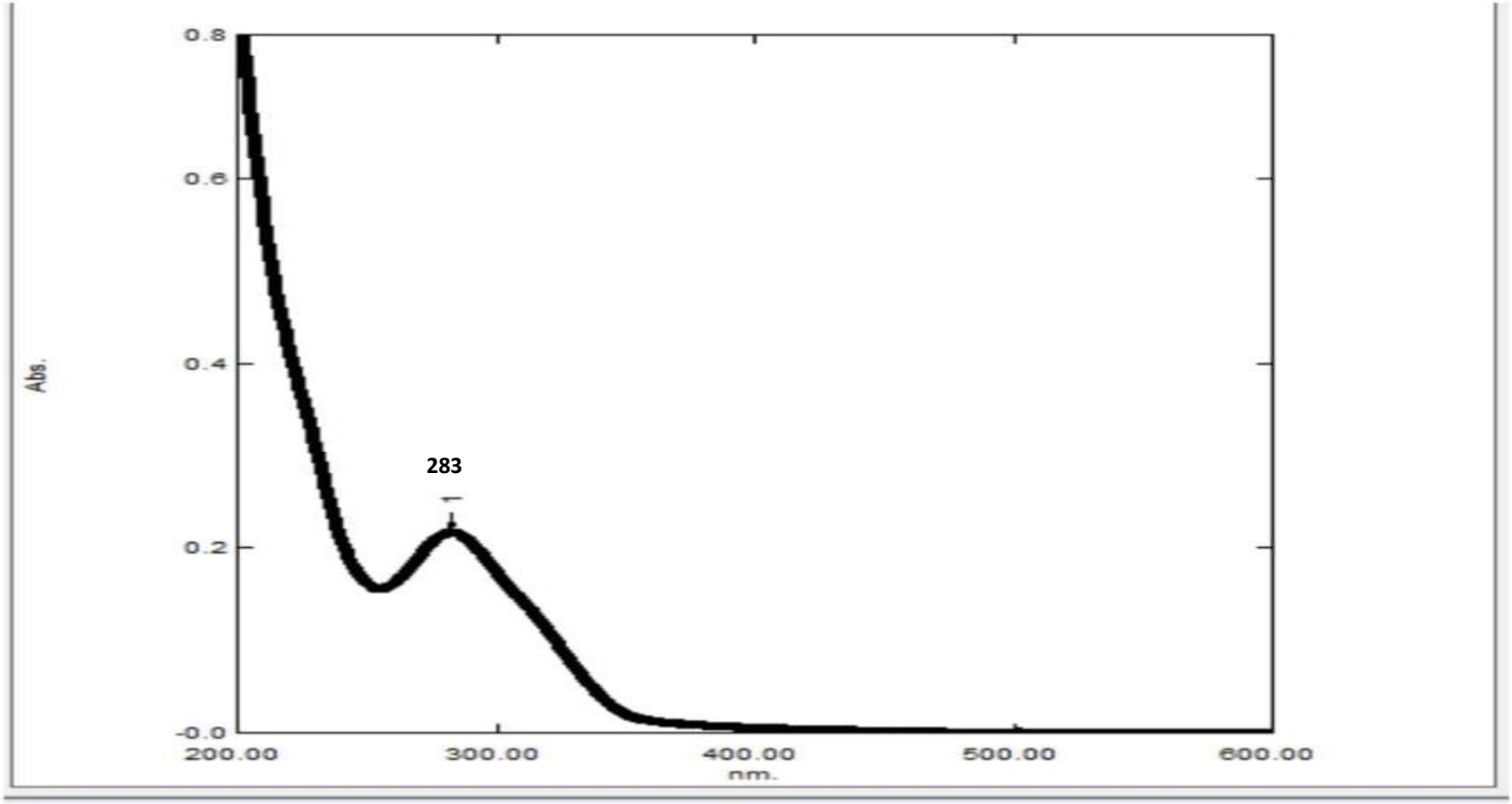


Figure (3.5): UV Spectrum of compounds *C.spinosa*.

3.4.2.3: Fourier Transform Infrared Spectrometry (FT-IR)

The FT-IR spectrum was used to determine the functional group of the active components based on the peak value in the infrared region. IR spectra of the studied alkaloids as KBr discs and representative spectra are shown in Table (3.8) and Figure (3.6).

The spectrum of *C.spinosa* is characterized by five bands corresponding to the stretching vibrations of the aromatic NH, OH, and aliphatic CH groups, C = C and C = O, which occur at (3100-3400), (3200-3500), (1500-1650), (1500- 1650) and (1650-1800) cm-1 respectively.

Table (3.8): FT-IR spectral data of compounds recorded as KBr discs(cm-1).

Functional groups	Grequency cm-1
NH	3100-3400
OH St	3200-3500
CH St	1500-1650
C=O St	1650-1800

St=Stretching

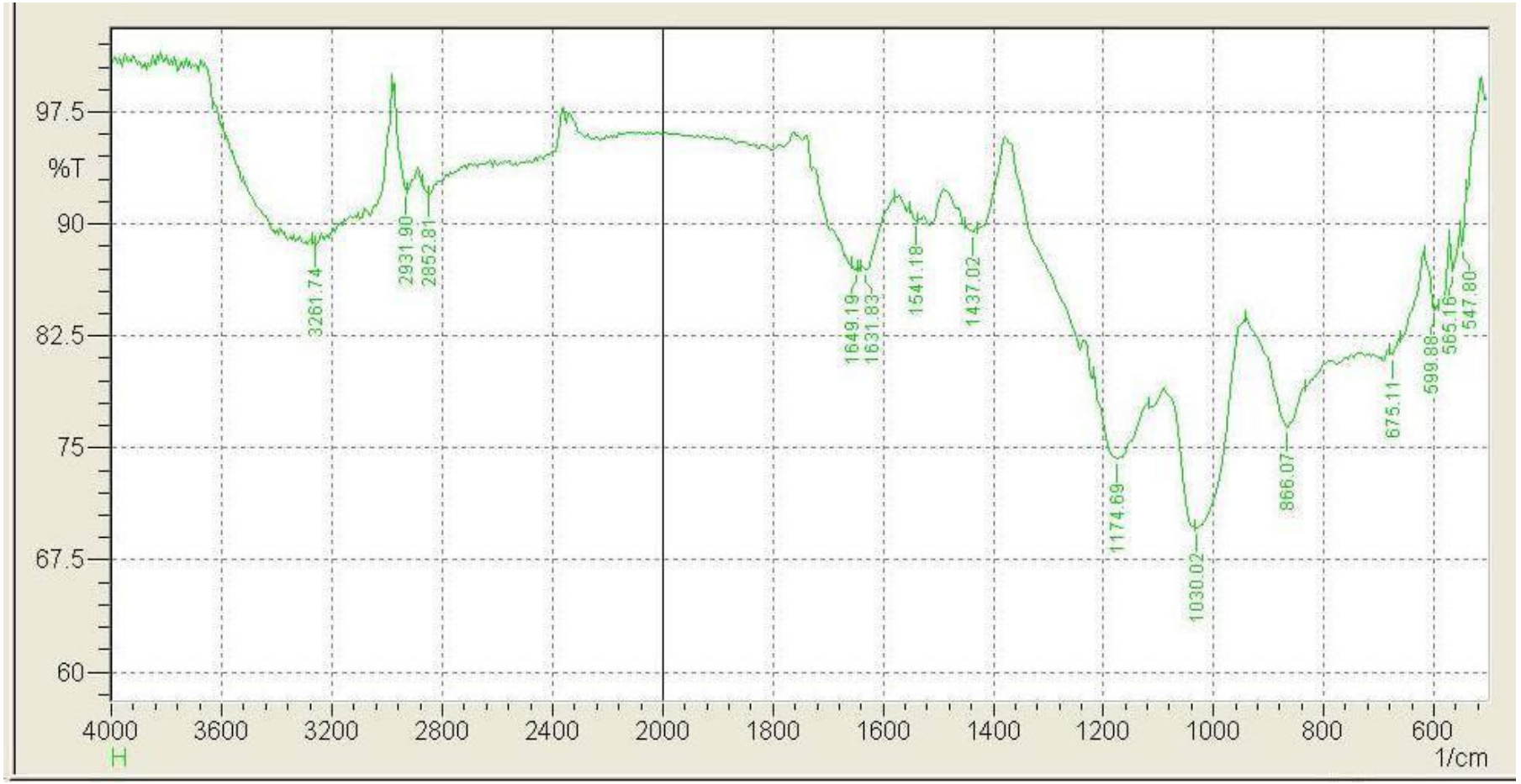


Figure. (3.6): FT-IR Spectrum of compounds *C.spinosa*.

3.4.2.4: Gas Chromatography-Mass Spectroscopy(GC-MS)

The aqueous extract acidified with sulfuric acid of the *C.spinosa* roots are Phenol,2,6 dimethoxy-**,** Diethyl Phthalate, n-Hexadecanoic acid, Octadecanoic acid, Pyrrobutamine, 5-Hydroxy-4',7-dimethoxyflavone, Xylometazoline acetate, and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,1 4,14a,14b-octadecahydro-2H-picen-3-one.This finding suggested that caper roots contain a significant amount of bioactive chemicals. The majority of compounds form a branch of phytotherapy that is mostly utilized to treat ailments. These results are shown in figure (3.7) and Table(3.9) and (3.10) .

Table (3.9): GC-MS Analysis of *C.spinosa*.

No.	RT	Name of the compound	Molecular formular	Molecular weight	Area%
1.	15.042	Phenol,2,6 dimethoxy-	C8 H10 O3	154.1632	0.57
2.	20.065	Diethyl Phthalate	C12 H14 O4	222.24	0.36
3.	26.468	n-Hexadecanoic acid	C16 H32 O	256.4241	0.35
4.	29.565	Octadecanoic acid	C18 H36 O	284.4772	0.16
5.	37.68	Pyrrobutamine	C2 H22 CIN	311.8491	0.17
6.	39.595	5-Hydroxy-4',7-dimethoxyflavone	C17 H14 O5	298.29	0.16
7.	42.672	Xylometazoline acetate	C16 H24 N2	244.3752	0.98
8.	44.784	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,1 4,14a,14b-octadecahydro-2H-picen-3-one	C39 H56 O2	5569	3.01

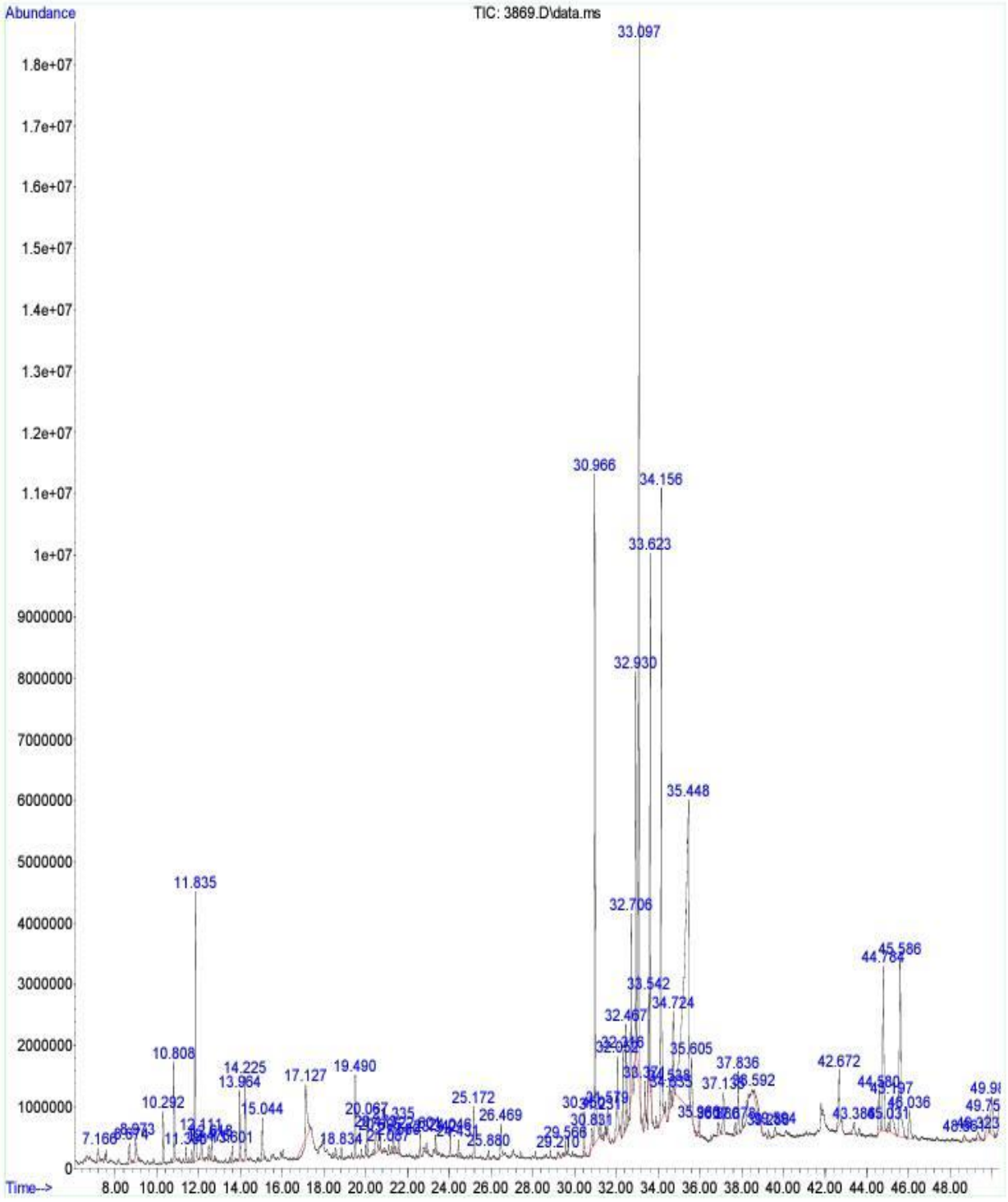


Figure (3.7): Chromotogram of *C.spinosa* roots extract by GC-MS.

Table (3.10): Bioactive compound and their biological activity.

No.	RT	Name of the compound	Activity of the compound
1.	15.042	Phenol,2,6 dimethoxy-	Antioxidant, antibacterial, anti-inflamamatory and anthelmintic.
2.	20.065	Diethyl Phthalate	Antimicrobial activity
3.	26.468	n-Hexadecanoic acid	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5- Alpha reductase inhibitor, potent mosquito larvicide.
4.	29.565	Octadecanoic acid	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
5.	37.68	Pyrrobutamine	antihistamine
6.	39.595	5-Hydroxy-4',7-dimethoxyflavone	antifungal
7.	42.672	Xylometazoline acetate	Nasal decongestant
8.	44.784	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	antisickling

3.5: Toxicity of alkaloids compounds

To determine the safety of using the alkaloids compounds as atheraeutic agent in the figure (3.8). Compounds have been evaluated for their hemolytic activity against RBC.The results showed that there is non-hemolytic activity against RBCs. The results indicated that the compounds are safe and non-toxic.

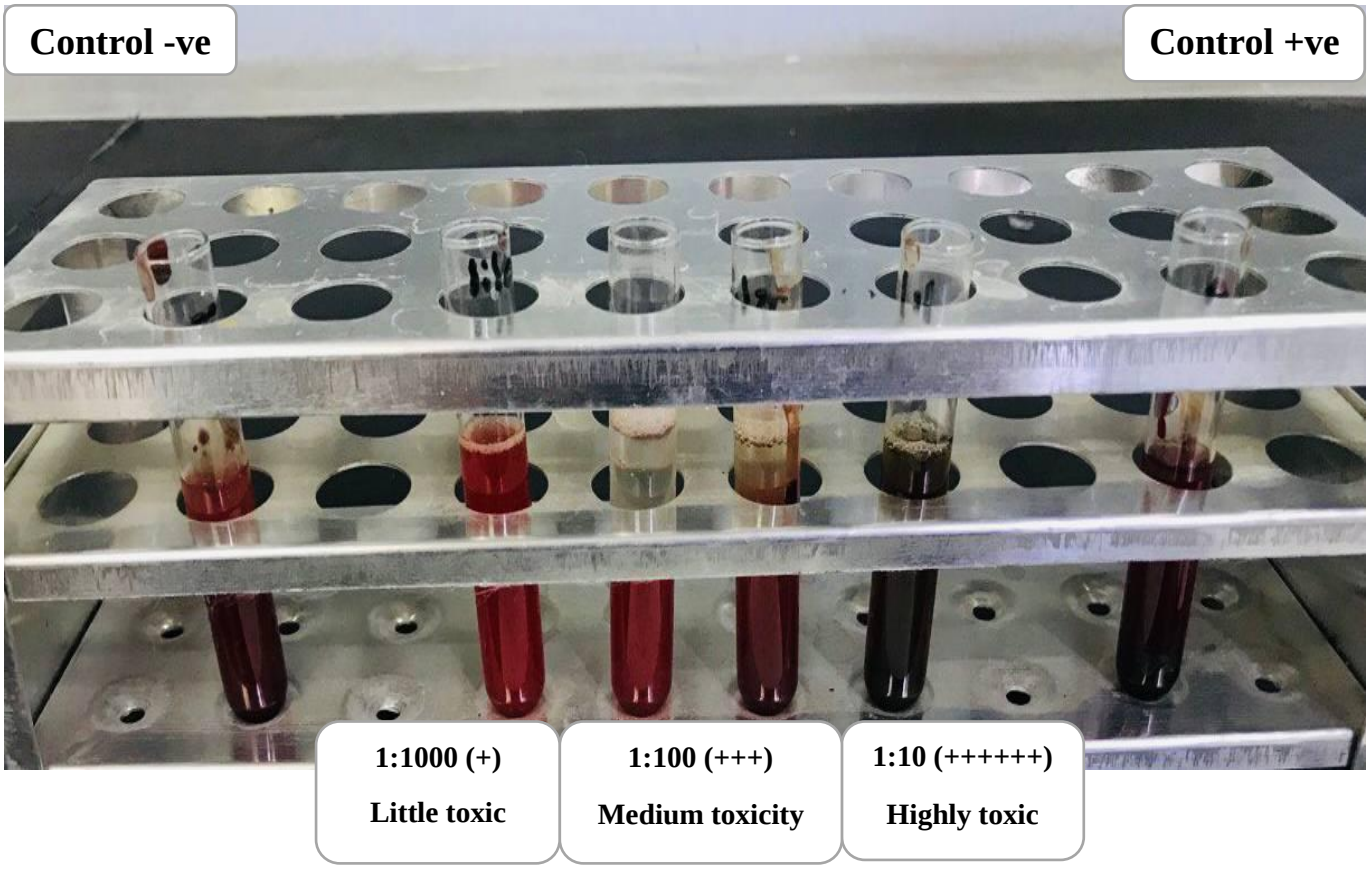


Figure (3.8) :Cytotoxicity of alkaloids for *C.spinosa*.

3.6: Isolation and identification of bacteria

S. aureus and *E.coli* were isolated from patients attending Al-Sadder hospital in Misan City. These bacteria were identified by biochemical tests and VITEK-2 compact technique.

3.7: Antibacterial activity of alkaloids compounds bacteria (*S.aureus* and *E.coli*)

The antibacterial activity of alkaloid extract of *C. spinosa* roots was further investigated by extracting alkaloids and testing it against a bacterial strain.The ability of the alkaloids compounds to prevent the growth of typical bacteria *E. coli* and *S. aureus* was tested using an agar-disk diffusion inhibition test in comparison to Ciprofloxacin (10 µg), Rifampin (5 µg), Erythromycin (10 µg), Oxytetracycline (30 µg) which is regarded as standard antibiotic as preliminary test, no references for the inhibitory effect of our alkaloids compounds were found in the literature, the results were explained in Table (3.11) shows the effect of different concentrations (30 , 62.5, 125 and 250 mg/ml) of alkaloids compounds . Therefore these results are shown in figures (3.9).

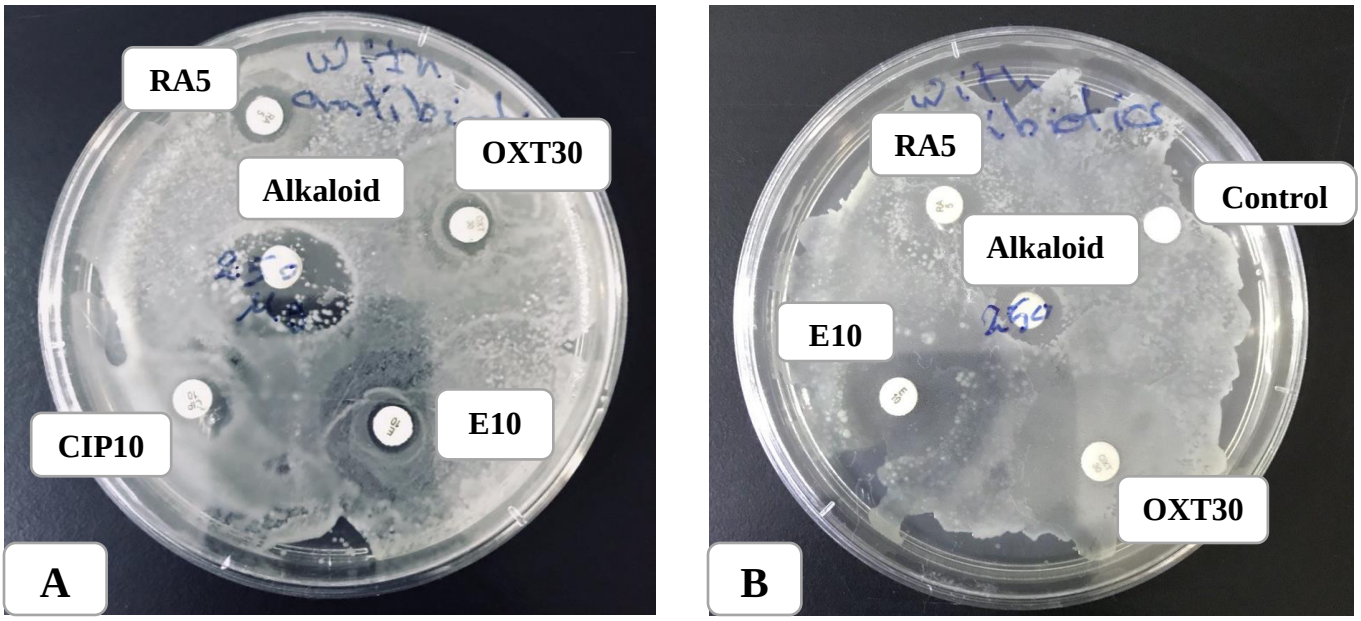


Figure (3.9):The effect of alkaloid compounds with antibiotic on (A) *E.coli* and (B) *S.aureus* at 250 mg/ml con.

Table (3.11): The inhibition zone of Alkaloids on the growth of bacteria *E. coli* and *S. aureus* .

No.	Con. mg/ml	*E. coli*	*S. aureus*
		Alkaloids	**Alkaloids**
1.	**30**	R	R
2.	**62.5**	7	8
3.	**125**	8	10
4.	**250**	20	13
5.	**Control**	R	R
6.	**Oxytetracyclin(30 µg)**	8	R
7.	**Erythromycin(10 µg)**	7	20
8.	**Ciprofloxacin(10 µg)**	R	R
9.	**Rifampin(5 µg)**	9	8

For interaction LSD $_{(0.05)}$ = 1.712 **1.712**

Chapter Four

Discussion

4.1: Phytochemical screening of *C.spinosa*

Previous studies showed an abundance of alkaloids as active chemical compounds, in leaves, stems, roots and fruits of the *Capparis spinosa* Plant (Tlili *et al*., 2010; Satyanarayana *et al*., 2009; Karnouf *et al*., 2011). Also, the roots of *Capparis spinosa* (caper) were used as an ethanolic extract to investigate the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in CCl4-intoxicated mice(Aghel *et al*., 2007).

The biochemical advantage of the abundance of alkaloids in medicinal plants including *C. spinosa* is the elimination of toxic materials from plant, storage of some essential elements such as nitrogen, regulators of growth and protection of plants from the attack of fungi and insects (Dey and Harborne, 1997). The high solubility of our alkaloids compounds in water is ascribed to the presence of polarized groups in the structures of these compoun (Majd *et al*., 2020)

4.2:The total product of all extracts

The average alkaloid extract efficiency of the caper extract was 24.5%, which was similar to results obtained by (Hosseini *et al*.,2013) (24%) who used water and ethanol solvents.The extractions by using solvents are the most commonly to prepare extracts from plant materials due to their ease of use , and wide .

It is generally known that the product of chemical extraction depends on the type of solvents with different polarities, extraction time and temperature, sample-to-solvent ratio as well as on the (percentages of different chemicals within a substance) and physical (features/ qualities/ traits) of the samples, this result agrees with that provious work (Nwachukwu and Uzoeto, 2010).

The products of chemical compounds alkaloids, glycosides, more boring pens, flavonoids, phenols saponins, tannins, coumarins and amino acids were varied (having to do with figuring out the quality of things without measuring them with numbers) in different parts of plants due to the solvent of extraction and these differences can be attributed to the fact that the extracts contain different voters a lot in their relative concentrations the result is supported by (Matthhaus and Ozcan, 2005). From this high quality of natural products of *C. spinosa* roots , leaves and stems.

4.3: Identification of alkaloids compounds

4.3.1: Thin layer chromatography

Thin layer chromatography, which reveals the appearance of a single spot using a chloroform:methanol solvent system, was used to confirm the purity of the alkaloid (0.5 : 9.5) the peak value of Rf was 0.25. As the appearance of one spot is evidence of the purity of the alkaloid. Small Rf value indicated low dissolvability of compound in mobile phase therefore the compound slowly move to up. Big Rf value indicated high dissolvability of compound in mobile phase therefore the compound readily move to up **(**Motar and AL-Hadad, 2018).

4.3.2: Ultraviolet Spectra (UV)

The Alkaloids are characterized by two bands, the short band at 255 nm of Alkaloids . The appearance of the short bands in the electronic absorption spectra of isolated alkaloids compounds is ascribed to the locally excited by $n \longrightarrow \pi^*$ transition of the compounds. The spectra of long band at 283 nm of Alkaloids , is ascribed to the $n \longrightarrow \pi^*$ transitions (Hairin, 2016).

4.3.3: Fourier Transform Infrared Spectrometry (FT-IR)

A section of the *C.spinosa* plant was subjected to FT-IR analysis. The findings demonstrated the ability to identify effective functional groups in the chemical components of each part of the caper plant, as well as the ability to distinguish between aromatic and non-aromatic compounds, alkenes, alkanes, esters, ethers, carboxylic acids, and unknown compounds, as each compound has its fingerprint. Also, knowing the chemical bonds such as CH, CO, OH, CF stretching, or others, and measuring the intensities (Altameme, 2015) of each peak of the peak curve and determining the group frequency to confirm the biological activity of each compound made it possible to understand the chemical and physical properties of each compound.

4.3.4: Gas Chromatography-Mass Spectroscopy(GC-MS)

The extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to identify secondary metabolites in this investigation. The concentration of eluted chemicals is plotted as a function of retention time in the gas chromatogram (RT). The identified chemical components are shown by the chromatogram peaks. The concentrations of eluted chemical ingredients were reflected by the height of the peaks. A compound's mass spectrum is a graphical representation of ion distribution based on its mass and charge ratio (m/z), which is critical for identifying the chemical structure and its characteristics. The peak area (concentration) of the chemical ingredients discovered is n-hexadecanoic acid, which has a concentration of 0.35%. Linolenic acid, often known as palmitic acid, is a naturally occurring chemical. It is, nevertheless, found in most natural sources and is responsible for its therapeutic properties. n-hexadecanoic acid has been shown to have anti-inflammatory, antioxidant, anti-androgenic, and hypocholesterolemic properties in previous research (Chinnadurai *et al.*,2019).

The inhibiting impact of n-hexadecanoic acid on the phospholipase A2 enzyme (Nkadimeng *et al.,* 2020). inhibits the inflammatory process. Palmitic acid inhibits macrophage invasion, which may diminish macrophage buildup in the synovial fluid of arthritic joints (Lohdip *et al.,* 2019). Furthermore, in-silico cytotoxicity tests revealed that n-hexadecanoic acid interacts with the DNA topoisomerase-1 enzyme, causing cytotoxic effects that are responsible for its anti-cancer action (Ravi and Krishnan, 2017). These secondary metabolites, on the other hand, are responsible for their pharmacological effects, such as anti-inflammatory, antioxidant, and anti-cancer properties (Chinnadurai *et al.,* 2019; Ravi and Krishnan, 2017; Lohdip *et al.,* 2019) . Antibacterial and antifungal properties are also present in octadecanoic acid (Abubakar and Majinda, 2016).

On the other hand, one of the things to consider is the type of solution that can be used in the extraction and thus affects the chemical compounds in analysis, because another study by Altameme, 2016, revealed the existence of fifty- three compounds such as Heptadecanoic acid, ethyl ester, 1,2Benzenedicarboxylic acid, monoacid (2-Ethylhexyl ester, 9,12-octadecadie . These findings revealed that caper roots have a significant role and a big number of chemical components that, when compared to other portions, support the use of caper in traditional medicine (Nabavi *et al*.,2016). Caper has also been found to have certain medicinal and antioxidant characteristics (Yadav and Malpathak, 2016), as well as aromatic plants in Mediterranean cooking, based on the flavor profile (Rosa *et al*., 2022). However, these findings are still preliminary, and more experimental and clinical research is required.

4.3: Effect of crude extracts of *C.spinosa* plant against bacteria (*E.coli* and *S.aureus*)

In this study, agar well diffusion method was used to determine the antibacterial effect of the crude extracts of leaves,stems and roots of *C. spinosa*.

E.coli, which is already known to be multi-resistant to different antibiotics, had inhibited its growth by aqueous, and methanolic extracts of *C.spinosa* roots at a concentration of 250mg/ml with an inhibition zone of 20,5mm and 20mm, respectively. As for *S.aureus* bacteria, its growth was inhibited by the methanol alcohol extract of the root of *C. spinosa* at a concentration of 250mg/ml in the inhibition zone 19mm, as well as the ethanol extract at a concentration of 250mg/ml for the roots and leaves of *C. spinosa* in the 11mm and 10mm inhibition zone, respectively.Such results are very interesting because these bacteria were isolated from the hospital environment. It is very difficult to control it by therapeutic means. Studies must be conducted on the way these compounds act in the bacteria cell.

These results could be confirmed by different studies such as, (Ramani *et al.,* 2020) who demonstrated that, different part extracts of *Capparis* species show biological activity against large numbers of pathogens bacterial isolates. Studied the antimicrobial activity of methanol, ethanol, ethyl acetate, and aqueous extracts for both roots and fruits of *C. spinosa* (separately) against a various of micro organisms and they found that ethanolic Caper fruits extract had the most activity against Gram- negative bacteria and *Streaptococcus* sp (Mahboubi and Mahboubi, 2014). Showed that methanolic extracts were more effective than ethanolic extracts and acetone against *Staphylococcus aureus*, *Esherichia coli*, *Bacillus subtilis* and *Pasteurella multocida* (Gull *et al*., 2015). Showed that *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Esherichia coli* and

Salmonella typhi are very sensitive to extracts of *C. spinosa* (Hashim et al., 2017). Abu-Shama (2019) studied the antimicrobial effect of silver nano-particles which were produced using the extract of *C. spinosa* leaves (NPs) against some selected pathogenic bacteria strains such as *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*, They found that, the synthesized silver nanoparticles in *C. spinosa* leaves extract, showed an excellent antibacterial property.

The results showed that the aqueous extract had a strong bactericidal effect on the G-ve bacteria, and it did not show any activity on the G+ ve bacteria, while the alcoholic extracts had moderate effects on both types. The alcoholic extract of *C. spinosa* roots bark has antimicrobial and antihelminthic activity, according to (Akkari *et al*., 2016; Mishra *et al*., 2007) made a similar observation, stating that *C. spinosa* had strong antibacterial activity against both G+ ve and G- ve bacteria.

The extracts have a substantial effect on *S. aureus* and *E.coli* bacteria. *C. spinosa* has the potential to be a source of natural antibacterial compounds with therapeutic benefits. *C. spinosa* is a significant plant that produces a large number of active metabolites naturally through the secondary metabolism pathway. Because alkaloids substances have a variety of physiological effects, they are capable of possessing biochemical activities that destroy harmful microorganisms such as bacteria and fungi. Alkaloids activity extracted from medicinal plants has been shown in certain research because these chemical compounds have physiological impacts and spectacular therapeutic properties for destroying harmful microorganisms such as bacteria (Sen *et al*., 2010; Singh *et al.,* 2008).

4.4: Antibacterial activity of alkaloids compounds against s bacteria (*S.aureus* and *E.coli*)

The Clinical and Laboratory Standards Institute's disc diffusion method was used to measure susceptibility of harmful bacteria to four different antibiotics (CLSI, 2017).

Bacterial resistance to antibiotics remains a serious public health concern because widespread nosocomial pathogens with immune systems weakness by disease or genetic disposition, *S. aureus* is a problematic nosocomial pathogen and this is complicated further by the fact that humans are a natural reservoir for this organism (Revelas, 2012) and infections caused by multi-drug resistance bacterial species are among the most difficult to treat with conventional antibiotics, in the present study the growth of *S. aureus* and *E.coli* that were found to be resistant towards many standard antibiotics, was remarkably inhibited by the alkaloids compounds, this shows the potential of these plant components to control the growth of drug resistance microbes.

Most bacteria tested showed resistance to commonly used antibiotics such as Oxytetreacyclion, Erythromycin, Ciprofloxacin, and Rifampin except *S. aureus*, which was susceptible to Erythromycine, (Sosa and colleagues, 2010) The region of inhibition for the alkaloid extract was 13mm against *S.aureus* and 20 against *E.coli* at a concentration of 250 mg/ml. These results summarized that the alkaloid extract has a greater effect on the anti-bacterial properties, which can be attributed to its ability to penetrate the cell wall by alkaloid compounds and this agrees with (Weissenberg, 2001) explained how the alkaloid Solasodine inhibits the development of normal bacteria. The results of this test prompted researchers to conduct an activity test of alkaloids compounds against pathogenic microorganisms.

Because most bacteria were resistant to several antibiotics, Oxytetreacyclion, Erythromycin, Ciprofloxacin, and Rifampin were used in the synergistic assays because resistance to at least one of these antibiotics was common in all bacteria tested.

4.5: Toxicity of alkaloids compounds

Because mammalian cells are more sensitive to chemical action than microbial cells, many chemicals may be antibacterial, but only a few will be viable therapeutic agents (Gaddala and Nataru, 2015). To adhere to the prescribed status, medications derived from natural products must undergo thorough pharmacological, toxicological, and clinical testing. Toxicity tests on human RBCs at four concentrations (62.5, 125, 250, and 500 mg/ml) revealed that the plants in this study were non-toxic because of the absence of any substantial hemolytic activity on human RBCs rules out the presence of direct membrane toxicity. These findings were discussed with (Angeline *et al.*, 1991), who stated that no reports on the toxic manifestations of *C. spinosa* after acute, sub-acute, or chronic treatment are available in the scientific literature. Furthermore, insensitive people, allergic responses to any natural product can develop (Gardiner *et al.*, 2000).

Conclusions
&
Recommendation

Conclusions

1-The parts (roots, stems, and leaves) of *C.spinosa* contain active compounds: alkaloids while some of these compounds were not detected in other parts of plants.

2-The alcoholic extracts showed anti-bacterial activity, both G +ve and G -ve, and it was more effective than the aqueous extracts.

3-GC-MS It is one of the standard analytical methods for describing the botanical components present in herbal plants.

4-The results of this study showed that the active compounds present in the extract were responsible for its pharmacological effects. It is worth mentioning that the discovered compounds have anti-inflammatory, anti-microbial, anti-fungal, and anti-oxidant activities that may be responsible for their medicinal uses.

5-GC-MS of the aqueous extract acidified with sulfuric acid of the roots of *C. spinosa* showed about eight compounds, respectively, and it is a good source of useful chemical and plant substances. These substances are important in biological activity.

Recommendations

1- Isolation and purification of the more active components of the investigated plant sections, to employ them as antibacterial medications against other pathogenic bacteria.

2- Research the antifungal, antiviral, and antiparasitic properties of crude extracts.

3- Traditional medical knowledge and biological studies must find ways to expand the benefits and capabilities of this natural resource of plants.

4- More clinical trials can be recommended to evaluate its clinical efficacy and safety in modern pharmaceutical sciences by examining its chemical components.

5-Using nanoparticles and other modern strategies to increase the bioavailability of most bioactive constituents of *C. spinosa*.

6- Study of the LD50 of isolated alkaloids compounds to determine appropriate concentrations for future use as medicines.

7- Determine the most effective dose for future clinical trials on *C. spinosa* beneficial effects.

8-There is a need for more investigations to evaluate the safety and efficacy of this traditional herbal plant in various diseases.

Chapter Five

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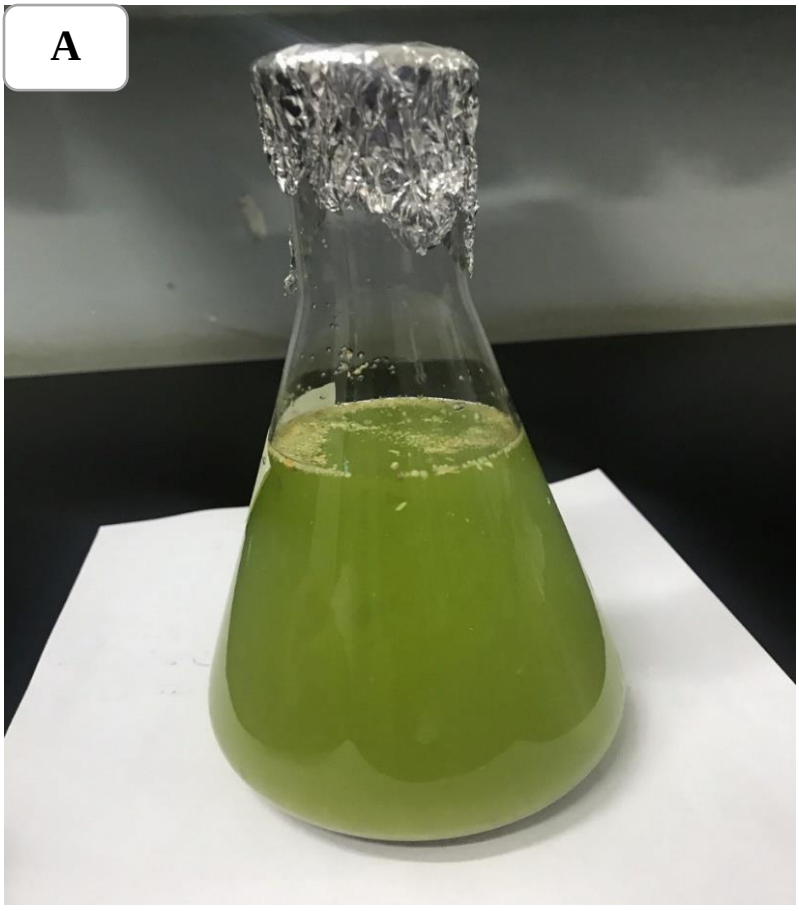
Appendixes

Appendix (1): Common names of capers used in around Mediterranean Sea region.

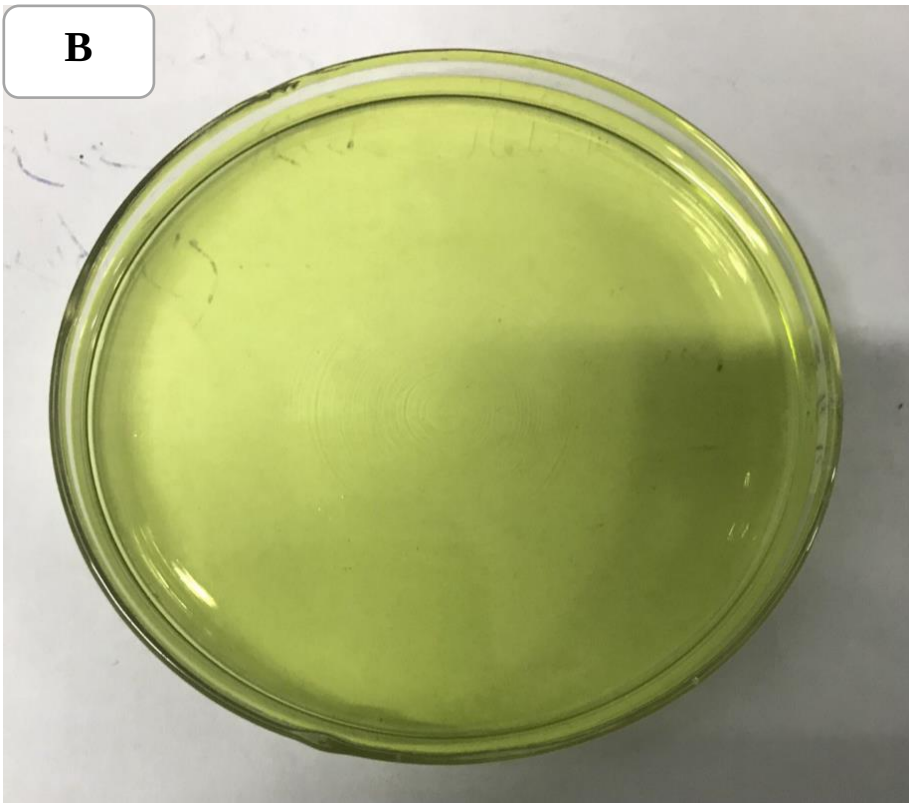
- Arabic : Kabar, Kabbar, Kabur, Azuf.
- English : Caperplant, Caperberry, Caperbush.
- Cyprus : Kapar, Kapara.
- German: Kaper, Capern, Kapernstrauch, Kapernbaum.
- Greece : Kappari.
- Egypt : Lussef.
- Turkey: Kapari, kebere, gebere
- Iran : Cebir, Curak
- Kannada : Chippuri, Karira, Nispatigay.

Gupta and Shama, (2007).

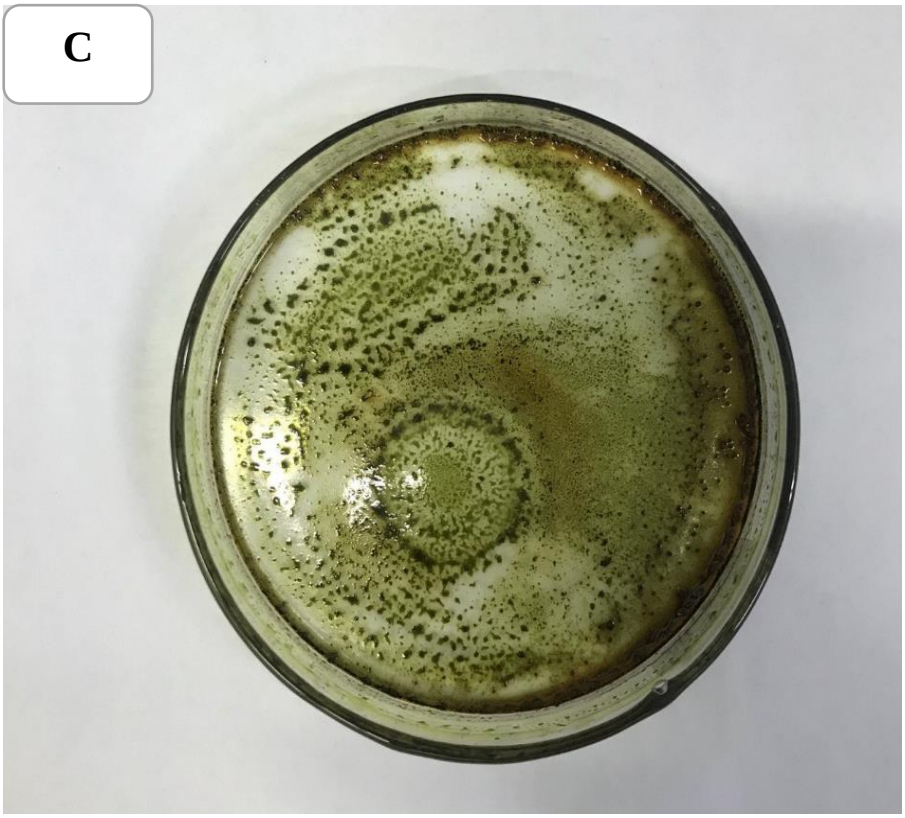
Appendix(2): A,B,C The isolation stages of crude extracts for *C.spinosa*.



B



C



Appendix (3): Statistics

Analysis of variance

Variate: E coli

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
plant_parts	2	25.627	12.814	2.14	0.133
extract	2	31.033	15.516	2.59	0.089
concentration	4	237.771	59.443	9.92	001.>
Residual	36	215.775	5.994		
Total	44	510.205			

Tables of means

Variate: E coli

Grand mean 8.11

plant_parts	Leaves of C.Spinosa	Roots of C.Spinosa	Stalks of C.Spinosa
	7.47	9.17	7.69

extract	C.A	Coh.E	Coh.M
	8.32	7.00	9.00

concentration	0.0	30.0	62.5	125.0	250.0
	6.00	6.56	7.33	8.18	12.46

Least significant differences of means (5% level)

Table	plant_parts	extract	concentration
rep.	15	15	9
d.f.	36	36	36
l.s.d.	1.813	1.813	2.341

```
43  "General Analysis of Variance."
44  BLOCK "No Blocking"
45  TREATMENTS plant_parts+extract+concentration
46  COVARIATE "No Covariate"
47  ANOVA [PRINT=aovtable,information,means; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
48   PSE=lsd; LSDLEVEL=5] st_aurus
```

Analysis of variance

Variate: st_aurus

Source of variation	d.f.	s.s.	m.s.	v.r.	p- value
plant_parts	2	3.511	1.756	0.48	0.621
extract	2	5.378	2.689	0.74	0.484
concentration	4	70.533	17.633	4.85	0.003
Residual	36	130.889	3.636		
Total	44	210.311			

Tables of means

Variate: st_aurus

Grand mean 6.76

plant_parts	Leaves of C.Spinosa	Roots of C.Spinosa	Stalks of C.Spinosa
	6.47	6.67	7.13

extract	C.A	Coh.E	Coh.M
	6.27	7.00	7.00

concentration	0.0	30.0	62.5	125.0	250.0
	6.00	6.00	6.00	6.56	9.22

Least significant differences of means (5% level)

Table	plant parts	extract	concentration
rep.	15	15	9
d.f.	36	36	36
l.s.d.	1.412	1.412	1.823

Analysis of variance

Variate: Alkaloids_E_coli

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Con_mg_ml	8	755.834	94.479	89.13	<.001
Residual	20	21.200	1.060		
Total	28	777.034			

Information summary

All terms orthogonal, none aliased.

Tables of means

Grand mean 9.4138

Con_mg_ml	125	250	30	62.5	Ciprofloxacin
mean	8.0000	20.4000	6.0000	7.0000	6.0000
rep.	3	5	3	3	3
Con_mg_ml	Control	Erthromycin	Oxytetreacin	Rifampin	
mean	6.0000	7.0000	8.0000	9.0000	
rep.	3	3	3	3	

least significant difference 1.712

Analysis of variance

Variate: Alkaloids_S_aureus

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Con_mg_ml	8	539.834	67.479	63.66	<.001
Residual	20	21.200	1.060		
Total	28	561.034			

Tables of means

Grand mean 9.4138

Con_mg_ml	125	250	30	62.5	Ciprofloxacin
mean	10.0000	12.6000	6.0000	8.0000	6.0000
rep.	3	5	3	3	3
Con_mg_ml	Control	Erthromycin	Oxytetreacin	Rifampin	
mean	6.0000	20.0000	6.0000	8.0000	
rep.	3	3	3	3	

least significant difference 1.712

Appendix (4): Vitek

Isolate: 177-1 (Qualified)

Printed Dec 29, 2020 08:49 CST
Printed by: Labadmin

Card Type: AST-P580 Bar Code: 3601232203633095 Testing Instrument: 00001B1B3611 (19482)
Setup Technologist: Laboratory Administrator(Labadmin)

Patient ID:

Organism Quantity:

Selected Organism: Staphylococcus aureus

Comments:	

Identification Information					
Organism Origin	Technologist				
Selected Organism	Staphylococcus aureus				
	Entered: Dec 28, 2020 08:30 CST	By: Labadmin			
Analysis Messages: Low Level Resistance - an MIC of 2, 4, 32, 64 for mupirocin represents the entire intermediate range (2-256).					

Susceptibility Information	Card: AST-P580		Lot Number: 3601232203	Expires: Apr 11, 2021 13:00 CDT	
	Completed: Dec 28, 2020 00:57 CST		Status: Final	Analysis Time: 15.08 hours	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Teicoplanin	2	S
Benzylpenicillin	>= 0.5	R	Vancomycin	2	S
Oxacillin	>= 4	R	Tetracycline	2	S
Gentamicin	4	S	Tigecycline	<= 0.12	S
Tobramycin	8	I	Fosfomycin		
Levofloxacin	2	I	Nitrofurantoin	<= 16	S
Moxifloxacin	2	R	Fusidic Acid	8	R
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	16	R
Clindamycin	2	I	Trimethoprim/Sulfamethoxazole	80	R
Linezolid	2	S			

+= Deduced drug *= AES modified **= User modified

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Natural Resistance

Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Last Modified: Jul 20, 2019 13:46 CDT

Page 1 of 2

System #:

Laboratory Report

Patient Name:
Isolate: 157-1 (Qualified)

Printed Dec 23, 2020 08:51 CST
Printed by: Labadmin

Patient ID:

Card Type: AST-N222 Bar Code: 6221143103101278 Testing Instrument: 00001B1B3611 (19482)
Setup Technologist: Laboratory Administrator(Labadmin)
Organism Quantity:

Selected Organism: Escherichia coli

Comments:	

Identification Information	
Organism Origin	Technologist
Selected Organism	Escherichia coli
	Entered: Dec 23, 2020 08:47 CST By: Labadmin

Analysis Messages:
The following antibiotic(s) are not claimed:
Rifampicin,

Susceptibility Information	Card:	AST-N222	Lot Number:	6221143103	Expires:	Jan 12, 2021 12:00 CST
	Completed:	Dec 21, 2020 21:36 CST	Status:	Final	Analysis Time:	12.17 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ticarcillin	>= 128	R	Amikacin	<= 2	S
Ticarcillin/Clavulanic Acid	>= 128	R	Gentamicin	>= 16	R
Piperacillin	>= 128	R	Tobramycin	>= 16	R
Piperacillin/Tazobactam	64	I	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Minocycline	>= 16	R
Aztreonam	>= 64	R	Colistin		
Imipenem	<= 0.25	S	Rifampicin		
Meropenem	<= 0.25	S	Trimethoprim/Sulfamethoxazole	>= 320	R

+= Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Jul 20, 2019 13:46 CDT	Parameter Set: Global CLSI-based+Natural Resistance
Confidence Level:	Consistent	

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Natural Resistance

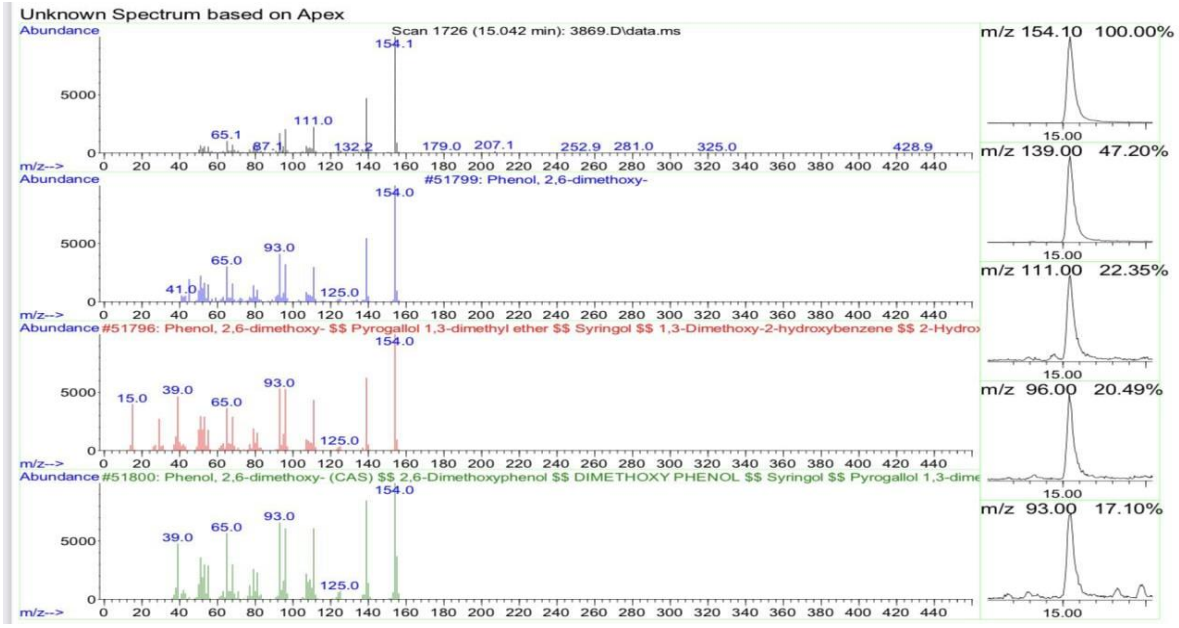
Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Last Modified: Jul 20, 2019 13:46 CDT

Appendix (5): GC-Mass compounds for roots of *C.spinosa*.

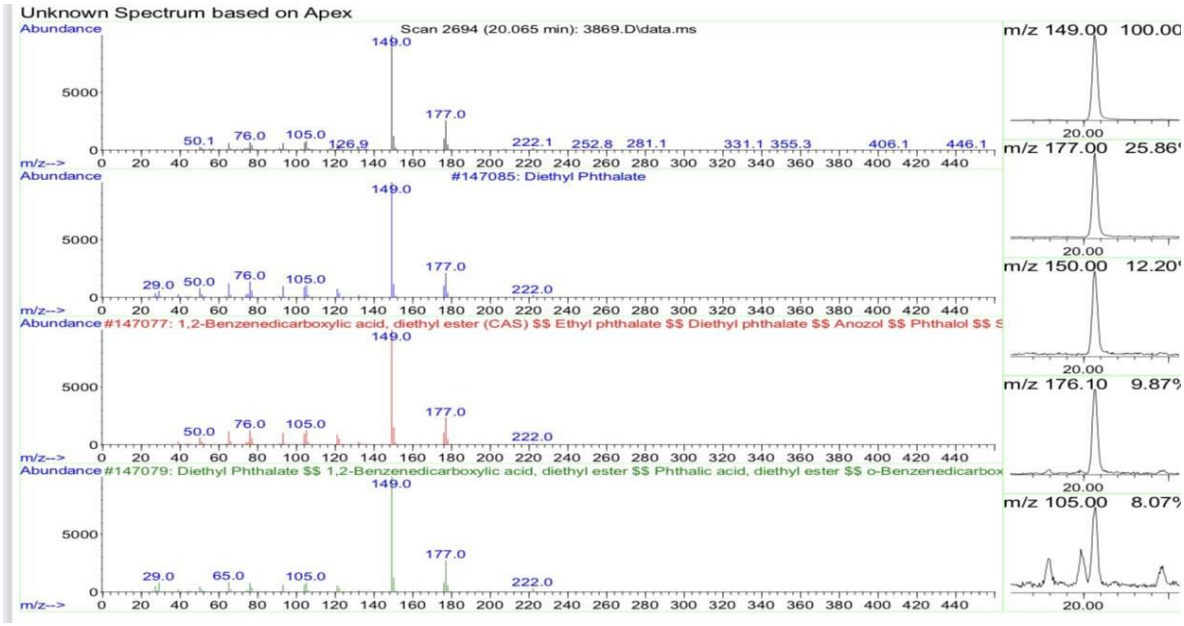
ck

No	RT (min)	Area%	Name	Quality	CAS Number
1	7.166	0.20	Glycol methacrylate	64	000868-77-9
2	8.676	0.33	Thymine	50	000065-71-4
3	8.972	0.51	Guaiacol	94	000090-05-1
4	10.295	0.55	Camphor	98	000076-22-2
5	10.808	1.13	BORNEOL L	97	000464-45-9
6	11.384	0.16	(+)-2-CARENE	92	000000-00-0
7	11.836	2.83	(S)-(-)-Verbenone	96	001196-01-6
8	12.111	0.50	2,3-DIHYDRO-BENZOFURAN	87	000000-00-0
9	12.474	0.17	Camphene	60	000079-92-5
10	12.619	0.22	Bicyclo[2.2.1]hept-2-ene, 2,7,7-trimethyl-	64	000514-14-7
11	13.6	0.16	Bornyl acetate	97	005655-61-8
12	13.963	0.74	Carvacrol	97	000499-75-2
13	14.228	0.99	4-Vinylguaiacol	96	007786-61-0
14	15.042	0.57	Phenol, 2,6-dimethoxy-	94	000091-10-1
15	17.128	0.73	2,1,3-Benzothiadiazole	59	000273-13-2
16	18.835	0.16	exo-Tricyclo[5.3.2.0(1,7)]dodecan-2-ol	53	094250-29-0
17	19.489	0.87	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	90	040113-58-4
18	20.065	0.36	Diethyl Phthalate	97	000084-66-2
19	20.475	0.58	D(-)-Quininic acid	64	000077-95-2
20	20.693	0.16	MEGASTIGMATRIENONE 4	99	000000-00-0
21	21.087	0.13	(4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone	81	000122-48-5
22	21.336	0.28	Isoaromadendrene epoxide	78	000000-00-0
23	21.58	0.18	Longifolenaldehyde	70	019890-84-7
24	22.602	0.24	(+,-)-Jasmine ketolactone	93	068931-43-1
25	23.344	0.23	1-Butanone, 1-(2,4,6-trihydroxy-3-methylphenyl)-	74	001509-06-4
26	24.045	0.23	Furan, 2-heptyl-	60	003777-71-7
27	24.429	0.15	NEOPHYTADIENE	99	000000-00-0
28	25.171	0.58	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	99	000000-00-0
29	25.881	0.15	Methyl palmitate	98	000112-39-0
30	26.468	0.35	n-Hexadecanoic acid	99	000057-10-3
31	29.213	0.18	2,5-Furandione, 3-(2-dodecenyl)dihydro-	56	019780-11-1
32	29.565	0.16	Octadecanoic acid	93	000057-11-4
33	30.453	0.45	6H-1,3-Oxazin-6-one, 4-(acetyloxy)-2-(2-phenylethenyl)-, (E)-	43	138744-82-8
34	30.831	0.33	4-Acetyl-2,2'-spiro-(s-hydrindacene)	83	115171-70-5
35	30.966	8.40	yl-6(S)-(3-methyl-2-butenyl)tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepin	93	000000-00-0
36	31.231	0.33	[(Bis(trimethylsilyl)methylene]-4-methyl-5-phenyl-4-cyclopentene-1,3-dior	83	000000-00-0
37	31.579	0.44	(10.beta.H)-Des-a-urs-12-en-5-one	93	000000-00-0
38	32.051	1.47	13-Isopropylpodocarpen-12-ol-20-al	95	000000-00-0
39	32.315	0.89	2-Dicyano-1-(1,2-dimethyl-3-indolyl)-2-(1,2,4,5-tetramethyl-3-pyrrolyl)ethe	90	000000-00-0
40	32.466	1.11	Androst-4-en-17-one, 3,6-dihydroxy-, (3.alpha.,6.beta.)- (CAS)	78	070005-44-6
41	32.704	1.80	3-Methylseleno-2-(1,3-dioxolan-2-yl)benzo[B]thiophene	96	071740-02-8
42	32.928	3.32	Brasilin	86	000474-07-7
43	33.099	14.66	zofuran-2(3H),1'-[2,5]cyclohexadiene]-3,4'-dione, 2'-hydroxy-6-methoxy-4,6	52	026891-80-5
44	33.369	0.56	8-methyl-9-hydroxy-9,10,11,12-tetrahydro-BbF	86	095741-64-3
45	33.545	0.34	4'-tert-Butyl-2,5-dimethoxybenzophenone	64	000000-00-0
46	33.623	5.70	3,3-dimethyl-2,4-diphenyl-endo-tricyclo[3.3.0.0(2,4)]oct-6-ene	78	039781-96-9
47	34.157	7.88	Benzo[c]coumarine, 3,4,8-trimethoxy-	90	088038-06-6
48	34.536	0.31	Morphinan-2,6-diol, 4,5-epoxy-N-methyl-, (2.beta., 5.alpha., 6.alpha.)-	46	084230-07-9
49	34.635	0.15	Retinoyl fluoride (all-trans)	55	083802-77-1
50	34.723	1.02	Morphinan-6-one, 4,5-epoxy-2-hydroxy-, (5.alpha.)-	44	079700-24-6
51	35.449	18.98	Methyl 3-(1-formyl-3,4-methylenedioxy)benzoate	93	000000-00-0
52	35.605	1.15	Diethyl 6-trimethylsilyl-3,6-dihydro-2H-pyran-2,2-dicarboxylate	83	061518-46-5
53	35.968	0.18	Dibenz[c,E]cycloheptanone, 3,4,7-trimethoxy-	83	000000-00-0
54	36.881	0.33	3-[N-[4-Bromophenyl]sulfonylamino]benzamide	95	000000-00-0
55	37.136	0.70	1-Phenyl-3,4,5,8-methyl-7-oxo-4,5,6,7(8H)-hydropyrazolo(3,4-b)(1,4)-diaze	91	064899-20-3
56	37.68	0.17	Pyrrobutamine	44	000091-82-7
57	37.836	0.75	4H-1-Benzopyran-4-one, 3,5,7,8-tetrahydroxy-6-methyl-2-phenyl-	89	114567-39-4
58	38.594	0.48	3-Methylseleno-2-(1,3-dioxolan-2-yl)benzo[B]thiophene	95	071740-02-8
59	39.284	0.15	-methyl-2-oxo-5,6,7,8,9,11,12,13,14,15,16,17-dodecahydro-2H-cyclopenta[	45	000000-00-0
60	39.595	0.16	5-Hydroxy-4',7-dimethoxyflavone	89	005128-44-9
61	42.672	0.98	XYLOMETAZOLINE ACETATE	93	000000-00-0
62	43.388	0.31	1,2,3,4-tetrahydro-2,3-bis(methylene)-1,4-epoxynapthacene-6,11-dione	46	104157-27-9
63	44.581	0.78	Cholest-5-en-3-ol, 23-ethyl-, (3.beta.,23S)- (CAS)	99	113845-28-6
64	44.784	3.01	b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecah	87	000000-00-0
65	45.033	0.14	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	58	000000-00-0
66	45.199	1.04	.beta.-Amyrin	95	000559-70-6
67	45.588	4.17	.alpha.-Amyrin	98	000638-95-9
68	46.034	0.65	3-KETO-URS-12-ENE	92	000000-00-0
69	48.66	0.14	1,3-dimethyl-4-azaphenanthrene	43	000000-00-0
70	49.324	0.26	1,1,1,3,5,5,5-Heptamethyltrisiloxane	50	001873-88-7
71	49.754	0.53	Methyl 3.beta.-hydroxyolean-12-en-28-oate	46	035933-00-7
72	49.983	0.99	Methyl betulinate	91	002259-06-5

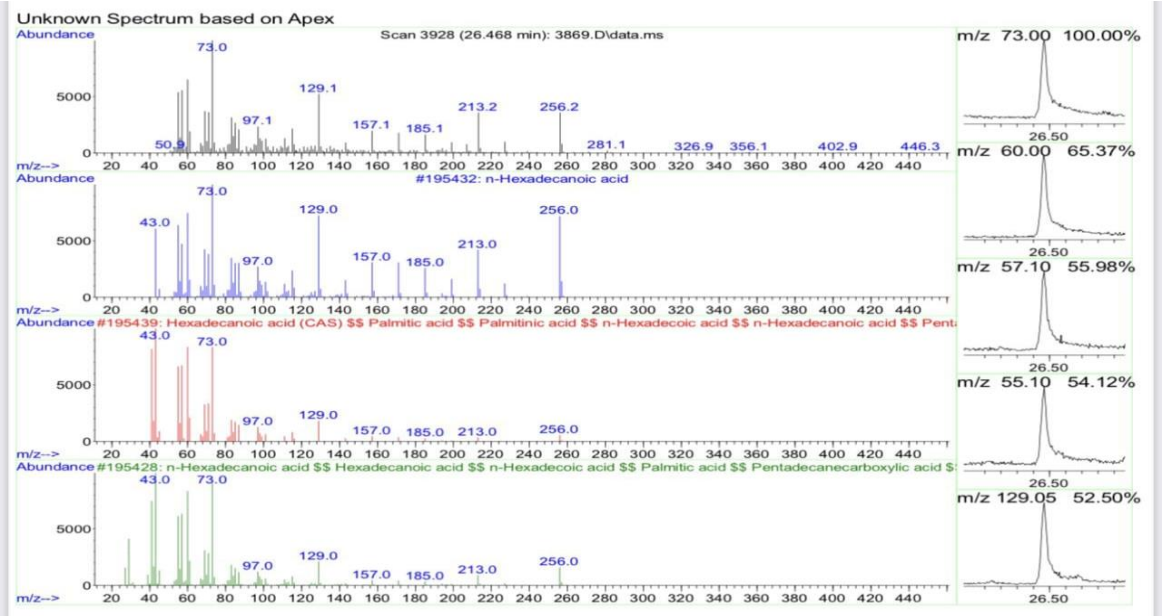
Appendix (6): Phenol,2,6 dimethoxy- structure in the roots extract of *C.spinosa*.



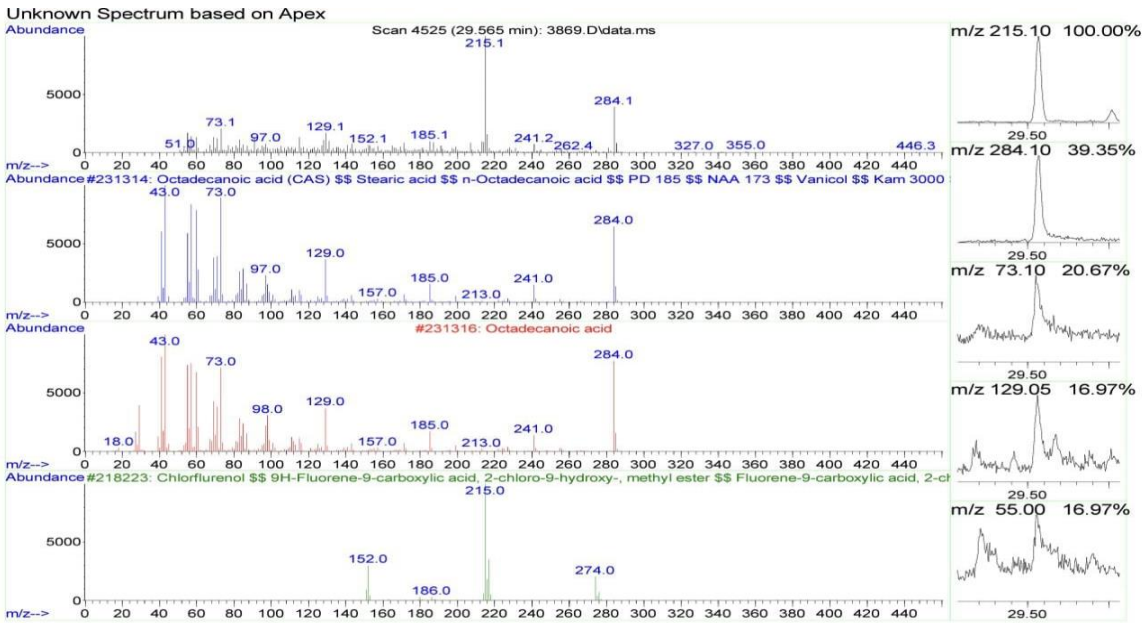
Appendix (7): Diethyl Phthalate structure in the roots extract of *C.spinosa*.



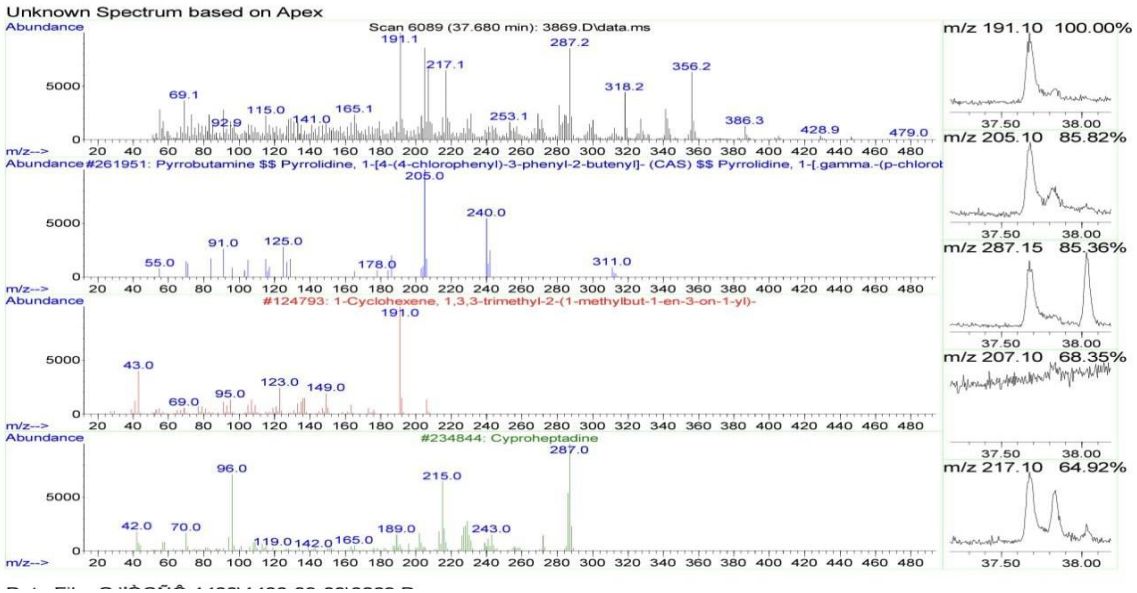
Appendix (8): n-Hexadecanoic acid structure in the roots extract of *C.spinosa*.



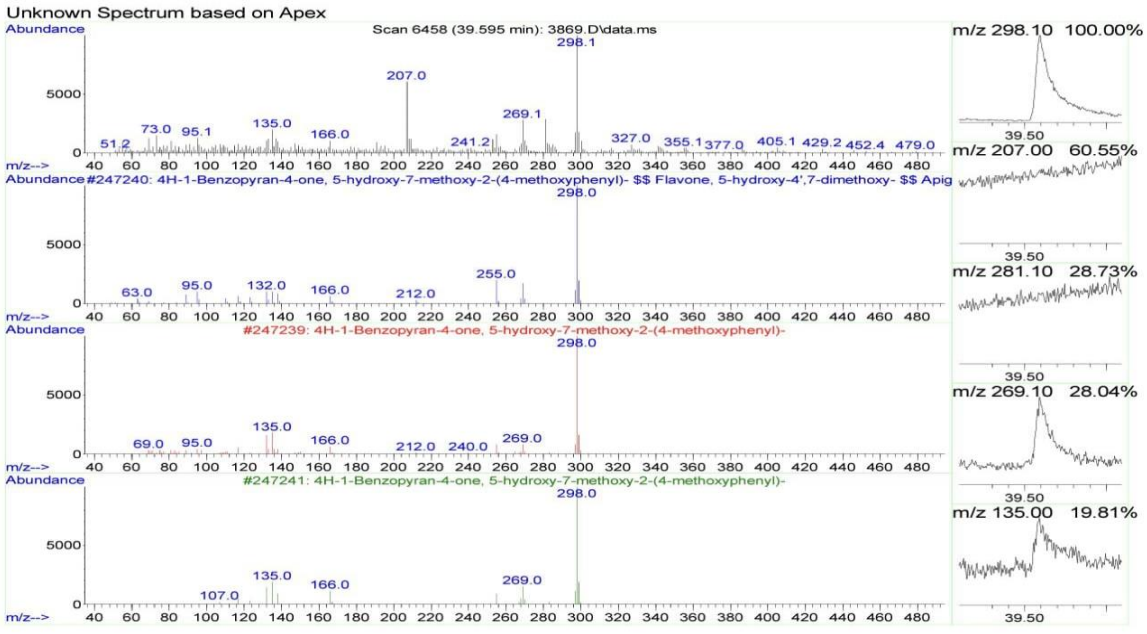
Appendix (9): Octadecanoic acid structure in the roots extract of *C.spinosa*.



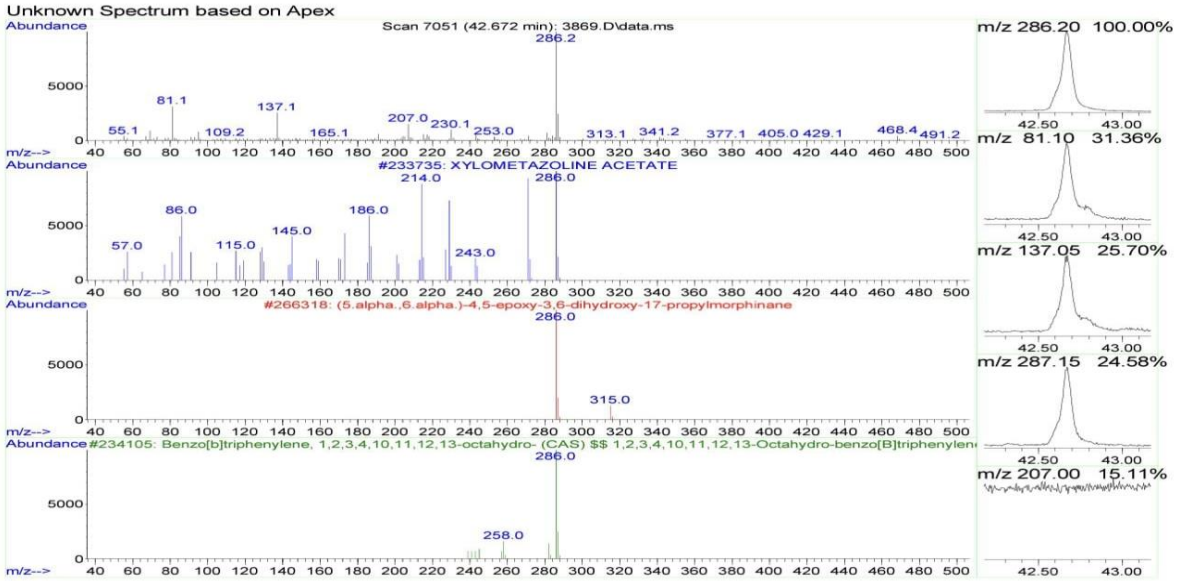
Appendix (10): Pyrrobutamine structure in the roots extract of *C.spinosa*.



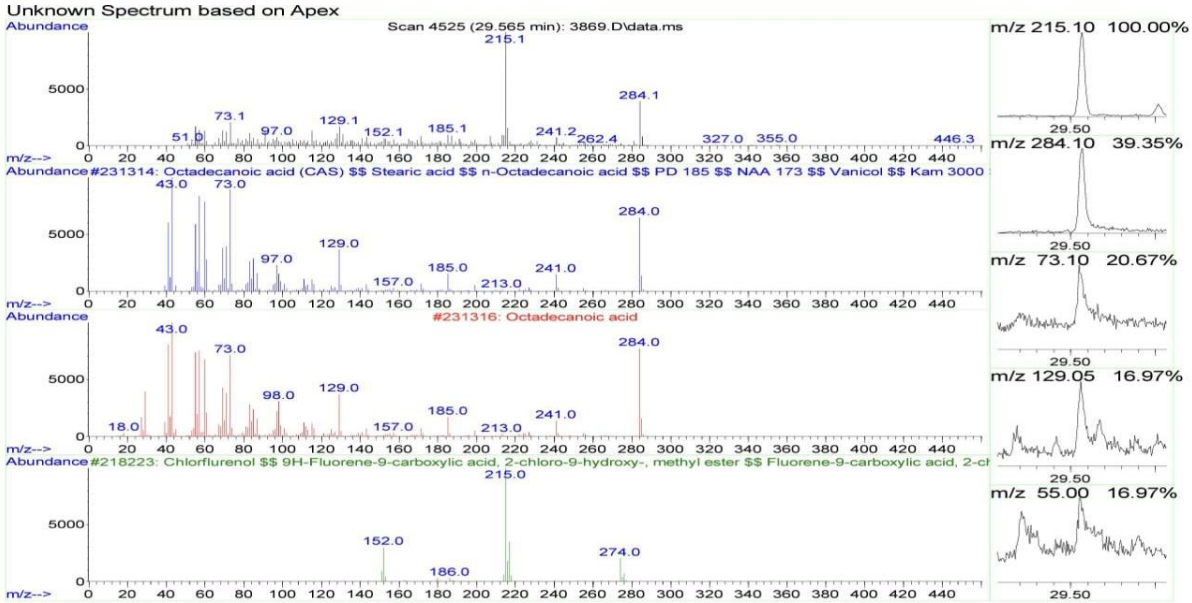
Appendix (11): 5-Hydroxy-4',7-dimethoxyflavone structure in the roots extract of *C.spinosa*.



Appendix (12): Xylometazoline acetate structure in the roots extract of *C.spinosa*.



Appendix (13): 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one structure in the roots extract of *C.spinosa*.



الخلاصة

جمعت عينات النبات *Capparis spinosa* من مركز محافظة ميسان من الفترة (2020/10/1 ولغاية 2020/11/1). ونقلت الى مختبر علوم الحياة جامعة ميسان لتجفيفها وطحنها وإجراء عملية الاستخلاص وبعد الانتهاء من عملية استخلاص المركبات الخام والمركبات القلويدية تم اختبارها بيولوجياً في المختبرعلى البكتيريا المرضية الموجبة والسالبة لصبغة كرام المعزول من مستشفى الصدر التعليمي بمحافظة ميسان وكان له تأثير قوي على هذه البكتريا بدرجات متفاوتة حسب نوع المستخلص.

اشتملت الدراسة الحالية على تحضير المستخلصات الكحولية (الميثانول والإيثانول) والمستخلصات المائية لنوع من النباتات الطبية في العراق (أوراق وسيقان وجذور) لنبات الشفلح *Capparis spinosa* ودراسة المكونات الكيميائية لتلك المستخلصات باستخدام الكواشف المناسبة.

المستخلصات النباتية للشفلح لها تأثير كبير على بكتريا *Staphlococcus uareus* و *Escherichia coli* يمكن أن يكون الشفلح مصدرًا للمركبات الطبيعية المضادة للبكتيريا ذات الفوائد العلاجية. الشفلح نبات هام ينتج عددًا كبيرًا من المستقبلات النشطة بشكل طبيعي من خلال مسار التمثيل الغذائي الثانوي.

Escherichia coli معروفة بكونها متعدد المقاومة للمضادات الحيوية المختلفة قد ثبط نموها بواسطة المستخلصات المائية والميثانولية لجذورالشفلح *Capparis spinosa* بتركيز 250mg/ml مع منطقة تثبيط 20.5mm و 20mm على التوالي. أما بالنسبة لبكتريا *Staphlococcus aureus* فقد تم تثبيط نموها بواسطة المستخلص الميثانولي لجذور الشفلح *Capparis spinosa* بتركيز 250mg/ml في منطقة التثبيط 19 وكذلك المستخلص الإيثانول بتركيز 250mg/ml لجذور وأوراق الشفلح *Capparis spinosa* في منطقتي التثبيط 11mm و 10mm على التوالي. هذه النتائج مثيرة للغاية لأن هذه البكتيريا تم عزلها من بيئة المستشفى من الصعب للغاية السيطرة عليه بالوسائل العلاجية.

أظهرت نتائج المستخلصات القلويدية أن قطر منطقة التثبيط واتجاه نمو البكتيريا يزداد مع زيادة تركيزالمستخلص. أظهرت جذور نبات الشفلح *Capparis spinosa* أقصى قطر تثبيط عند تركيز 250mg/ml والذي كان 13mm, 20mm ، *Eschericia coli* و*Staphlococcus uareus* على التوالي.

تم عزل السلالات البكتيرية من عينات التهاب المسالك البولية واستخدمت الإجراءات البكتريولوجية الاساسية لتحديد وتشخيص الأنواع البكتيرية وكذلك تم استخدام جهاز Vitek2 للتعرف على العزلات بشكل اكبر.

تضمنت هذه الدراسة أيضًا فصل وتنقية وتحديد المركبات القلويدية من جذور نبات *Capparis spinosa* تم تشخيص القلويدات من خلال استخدام بعض الاختبارات الفيزيائية والكيميائية والتي تمثل درجة الانصهار والقابلية للذوبان وكذلك التحليل الطيفي الكتلي للكتلة الغازية (GC-MS) وتقنية كروماتوغرافيا الطبقة الرقيقة (TLC) ومطيافية الاشعه فوق البنفسجيه (UV) ومطيافية الأشعة تحت الحمراء (FT –IR).

كما اجري تحليل كروماتوغرافيا الطبقة الرقيقة والتي تكشف عن ظهور بقعة واحدة باستخدام نظام المذيبات (الكلوروفورم: الميثانول) تم استخدامه لتأكيد نقاوة القلويدات(0.5 : 9.5) كانت قيمة التدفق النسبي (0.25).

بالإضافة إلى ذلك تم تحديد الاستخدام الآمن للمركب عن طريق إجراء اختبار السمية الخلوية على خلايا الدم الحمراء البشرية وأظهرت نتائج الفحص عدم وجود أي سمية خلوية للمركب في جميع التراكيز المدروسة.

تم تقييم المستخلص باستخدام كروماتوجرافيا الغاز GC-MC وتم تحديد العديد من المركبات الكيميائية. يُظهر كروماتوغرافيا الغاز وجود معظم أحماض اللينولينيك مثل حمض n-Hexadecanoic و Octadecanoic ومركبات أخرى مثل n-Hexadecanoic acid and Phenol,2,6-dimethoxy-, Diethyl Phthalate, Pyrrobutamine, 5-Hydroxy-4',7-dimethoxyflavone, Xylometazoline acetate, and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6 .6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم

عزل وتشخيص ودراسة الفاعلية الضد بكتيرية للمركبات القلويدية لنبات *Capparis spinosa* في ميسان/ العراق

رسالة مقدمة الى
مجلس كلية العلوم/ جامعة ميسان وهي جزء من
متطلبات نيل شهادة الماجستير في علوم الحياة
من قبل

حوراء صالح مهدي
بكالوريوس علوم حياة / جامعة ميسان

(2016)

بأشراف

أ.م.د. وليد محسن علي

1444هـ 2022 م