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**Napata Collage
School of Pharmacy**



Batch 1

**Phytochemicals Composition And Antimicrobial activity, Of Allium
Satvium (Clove) [garic]**

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الاية

قال الله تعالى:

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

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Dedication

To who makes us rise when we fall, caregivers, granter of love, hope,
piece, soul mates ... our mothers.

To little big stars shine in our dark sky... our friends

To scandals of our life, happiness of our day, breathe of our souls ...our
sisters

To our back, smiles of our sadness, sun light... our brothers

Glory Mountains, way supporters, true lovers... our fathers

And to those who believe in freedom, peace and justice god bless you

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- I thank God for giving me the courage and the determination, as well as guidance in conducting this research study, despite all difficulties.
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Abstract

Allium sativum L. belongs to family Liliaceae, has been used as a medicine through ancient and modern time to prevent and treat a wide range of conditions, diseases and tumors due to presence of phenolic compounds and volatile oils which contains sulfur atom in their chemical structures, and the major antibiotic ALLICIN (unstable) which proven to has an antimicrobial effect against the pathogenic bacteria, parasites, viruses and fungi. This study was carried out to determine the phytochemical composition of the macerated ethanolic extract of the garlic pulp using GC-MS technique and to evaluate its antimicrobial activity against pathogenic bacterial strains using disc diffusion method. GC-MS revealed a total of (16)... compounds which were identified in the ethanolic extract ranging from terpenes, essential oils, phenolic compounds, esters, and others. The major constituents were; Diallyldisulphide (14.26%) 17.34 %), 1,2,3- thiadiazole, 5-methyl- (4.24%, 5.91%) and Trisulfide, di-2-propnyl (19.84%, 14.36%) respectively.

Then the disc diffusion methods was employed to explore the antimicrobial activity of ethanolic extract against four pathogenic bacterial strains *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *C. Albican*. The zone of inhibition observed was 10.5 – 13.5 mm at higher Concentration for used.

Keywords: *Allium sativum*. Gas Chromatography, activity, Antimicrobial activity.

الخلاصة

ينتمي الثوم الي عائلة تسمى ليليسيا ،وقد تم استخدامه كدواء عبر العصور القديمة و الحديثة لمنع وعلاج مجموعة واسعة من الامراض والاورام بسبب وجود المركبات الفينولية والزيوت المتطايرة التي تحتوي علي زرة الكبريت في المواد الكيميائية والمضادات الحيوية الرئيسية أليسين (غير مستقرة) التي ثبت أن لها تأثير مضاد البكيريا المسببة للأمراض و الطفيليات والفيروسات و الفطريات . أجريت هذه الدراسة لتحديد التركيب الكيميائي النباتي للخلاصة الأيثانولية المتحللة في لب الثوم باستخدام تقنية (جي سي ماس) ولتقييم نشاطها المضاد للميكروبات ضد السلالات البكتيرية المسببة للأمراض باستخدام طريقة انتشار ديك . كشف جي سي عن اجمالي (16) مركبا تم التعرف عليه في المستخلص الأيثانولي من التربينات والزيوت الأساسية والمركبات الفينولية والاسترات وغيرها . المكونات الرئيسية كانت ؛ ثنائي كبريتيد ديليل (14.26 , 17.34 6) % 1،2 ، 3- ثياديازول ، 5-ميثيل (5.91% ، 4.24%) و ثلاثي كبريتيد ، ثنائي 2- بروبنيل (14.36% ، 19.84%) علي التوالي . ثم تم استخدام طرق الانتشار القرصي لاكتشاف النشاط المضاد للمستخلص الأيثانولي ضد أربعة سلالات بكتيرية مسببة للأمراض .

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List of Abbreviation

DAS: Diallyl sulfide.

DATS: Diallyltrisulfide.

DADS: Diallyldisulphide.

GBM; Glioblastomas

E. coli: Escherichia coli.

EMB: Eosin methylene blue.

GC-MC: Gas chromatography- Mass spectrometry

Chapter One

Introduction

Background:

Herbal Medicine is the fulcrum of complementary and alternative medicine ,which is recent time is increasingly gaining widespread popularity all over the world and gradually stream to word integration into the mainstream health care system.

The pharmacological and most toxic effects that have been linked to the activities of secondary metabolites

[PSM]are large group of compounds that are synthesized and concentrated optically in certain plant species and organ . [PSM] are organic compounds and can simply be grouped into three major classes terpenes, volatile ,cardioglycoside carotenoids and steroids ;phenolics; phenolic acids ,coumarin,lignans, stilbenes, flavonoids , tannins , and lignin., nitrogen containing compounds, alkaloids and glucosinolates

Sudan exhibits a wide range of variation in its topography, climate, soil and hydrology. This resulted in different ecological habitats, different vegetation zones and consequently rich flora. Sudanese folk medicine represents a unique blend of indigenous cultures of Islamic, Arabic and African traditions. In recent years antibiotic resistance in pathogenic microorganisms has increased due to disorganized use of antimicrobial drugs which created serious threat to human health.[1] Therefore, re-evaluation of the therapeutic use of plants can be used as alternative strategies to combat microbial resistance.[2] Most of the modern drugs are derived from plant. sources and their extracts dominate in ayurvedic medicines .[3]Identification of particular bioactive compound of plants has become easier due to the development of modern analytical tools such as especially gas chromatography–mass spectrometry (GC-MS).

[3] The traditional medical practitioners in Sudan have no formal training or pharmacopoeia and their knowledge is based on acquired folklore and indigenous traditions.

Garlic (*Allium sativum L.*) belongs to the family Liliaceae, a common vegetable used widely in almost all parts of the world, has been famous for its flavor and aroma as well as for its medicinal uses for centuries. [3] Today, exploitation and understanding of all of the advantages of garlic in nutrition and medicine is still in progress. It is generally accepted that the volatile compounds of garlic are the main reason for its properties. [4] It was in 1892, when a German chemist, by the name of W. Semmler, reported that garlic cloves could be steam distilled to produce strong smelling essential oil. The characteristic odor of this essential oil is mainly caused by the presence of sulphur containing volatiles. [5] More detailed analysis of garlic volatiles has thereafter determined the existence of mono to hexadiallysulfides and vinyl dithiin isomers as the major constituents of garlic oil. Anti microbial is the Collective term for all active principles [agents] that inhibit the growth of bacteria , prevent the formation of microbial colonies , and may destroy microorganisms.

1.2. Justification:

Because of available antimicrobials failure to treat infectious diseases, many researchers have focused on the investigation of natural products as source of new bioactive molecules . A variety of methods are found for this purpose and since not all of them are based on same principles, results obtained will also be profoundly influenced not only by the method selected, but also by the microorganisms used to carry out the test, and by the degree of solubility of each test-compound . The test systems should ideally be simple, rapid, reproducible, and inexpensive and maximize high sample throughput in order to cope with a varied number of extracts and fractions. The complexity of the bioassay must be defined by laboratory facilities and quality available personnel .

1.3 Objectives :

1.3.1 General objectives:

To investigate phytochemical constituents and ,anti microbial activity of A.sativum Clove [garlic]

1.3.2 Specific objectives:

1-To macerate Garlic clove used ethanol extract as solvent.

2-To carry out primary screening for the presence of the active constituents using Gas CHR coupled with mass spectrometry [GC.MS]

3-To evaluate the clove extract for the antimicrobial activity use disc diffusion method

Chapter Two

Literature Review

2. Literature review:

2.1.Natural Products from Plants

Plants have been well documented for their medicinal uses for thousands of years. According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care. . [6] The knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals. [7] The use of natural products as medicines has been described throughout history in the form of traditional traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases.

2.2. Family Liliaceae:

Annual herbs, rarely branched, usually with bulbs and contractile roots. - Flowers: often large, bisexual and actinomorphic; 6 distinct tepals, 3 carpels in a superior ovary, 6 stamens; nectaries at base of petals; spots on petal; dextrose anthers. - Fruit: a loculicidal capsule, sometimes a berry.

2.2.1.GenusAllium

[8]The name “Allium sativum” is derived from the Celtic word “all”, meaning burning or stinging, and the Latin “sativum” meaning planted or cultivated. This medicinal plant is mainly used as condiments and for stopping in different cooking The use of higher plants and their extracts to treat infections is an ancient practice in traditional medicine. Many plants have been used because of their antimicrobial treats, which are chiefly synthesis during secondary metabolism of the plants. The herbal

medicine may be in the form of powders, liquid or mixtures which may be row or boiled, ointments linings and incision .

2.2.2 Taxonomy:

- Kingdom: Plantae
- Clade: Angiosperms
- Phylum: Magnoliophyta
- Class: Lilopsdia
- Order: Amaryllidaceae
- Family: Liliaceae
- Subfamily: Allioideae
- Genus: *Allium*
- Species: *sativum*

2.2.3 Botanical description

[9] The garlic plant is made up of fleshy edible cloves that are encased in a white or pink, thin coat. It has leaves, stem, and flowers located on the head that are also edible. It is easy to grow and can be grown all year round. The leaves are long, narrow and flattened. Garlic plant grows well in well-drained soil and requires a cool and moist period during growth and a relatively dry period as it matures. The transverse section of bulb of *Allium sativum* has cuticle, epidermis, cortex, endodermis and scattered vascular bundles. Epidermis consists of narrow thin walled continuous single layered with rectangular cells, surrounded by cuticle. The cortex region have parenchymatous and homogenous cell having large prismatic crystals of calcium oxalate and vascular bundles.

2.2.4. Geographical distribution:

Garlic cultivated practically throughout the world, it appears to have originated in Central Asia and then spread to China, the Near East, and the Mediterranean region before moving west to the Central and Southern Europe, Northern Africa(Egypt) and Mexico. [10] According to Wayne and his colleagues, the growth of garlic requires an even, consistent supply of water. Garlic grows best on friable (crumbly), loamy soil that are fertile and have some organic matters. This plant is multiplied by vegetative reproduction rather than by sexual reproduction (seed). Individual garlic cloves are planted and they each produce a bulb in which the cloves all have the same genetic makeup as the original clove. Garlic has been used traditionally for ages to treat a wide array of diseases, namely respiratory infections, ulcers, diarrhea, and skin infections, to mention just a few reported garlic as plant with antibiotic, anticancer, antioxidant and immunomodulatory. hypoglycemic and cardiovascular protected effects.

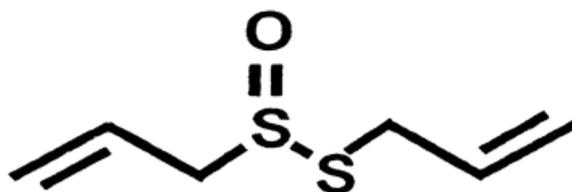
2.2.5 Traditional uses of Garlic:

2.2.6 Phytoconstituents and their pharmacological significance

Numerous phytochemical have been identified in garlic, including flavonoids, terpenoids, alkaloids, coumarin, saponins, and tannins. In addition, the major chemical constituents of garlic are allicin, diallylsulphide, diallyldisulphide, diallyltrisulfide, s-allylcysteine and allylisothiocyanate.[11]also bioactive compounds as α -linolenic and lauric acid reported by Sado-Kamdem to have antibacterial and antifungal activities. Martín-Lagos, Serrano & Lopez (1995) observed 2-vinyl-4(H)-1,3dithin to be the major compound present in garlic oil, dimethyl trisulfide being the least abundant. In addition, diallyldisulphide, 3-vinyl-4(H)-1,2-dithiin and allyl methyl disulphide were encountered .

2.2.6.1 .Allicin formation:

Garlic is therapeutically effective because of its oil and water soluble organosulfur and phenolic compounds, thiosulfates are mainly responsible for its antibiotic activity so if the extract is free from thiosulfates the antibacterial effect will be lost. Stoll and Sebeck showed that Allicin comes from a precursor alliin, an amino acid present in garlic intact cells formed by attaching an allyl group and an oxygen atom to the sulphur atom in the amino acid cysteine, within the cell there are vacuoles that contain an enzyme known as alliinase when the cells are sliced the enzyme comes in contact with alliin and causes it to break down into sulphur-containing compound allicin



Allicin

2.2.6.2 .Organosulfur compounds;

[12]Modern scientific study has discovered that large diversity of medicinal and dietary functions of garlic can be ascribed to organosulfur compounds [OSC]found in or generated from garlic . More than twenty kinds of sulfide compounds from a few sulfur-containing amino acid are produced by garlic with diverse functions such as allicin ,diallyltrisulfideand methyl allyltrisulfide ,which have shown antithrombotic ,anticancer ,and antibacterial activities

GBM:One of most common brain malignancies has an extremely poor prognosis current indication proposed treatment failure and adverse prognosis of GMB which may be result of presence of cancer stem cell

[CSC] in GBM and the uncommon subpopulation ,of CSC which is resistance to chemotherapy.

Z-ajone: that has revealed average of biological activates such as anti proliferative effects on various cancer.in articles researchers showed that z-ajone particularly hinder the growth of population of GBM.CSC.

DAS: One of the major active constituents of garlic is growth reversion of in vitro cancer cell and advanced in vivo immune response in trial setting .The study involved investigation of the anticancer effects of DAS in helahuman cervical cancer and to examine the fundamental mechanism invitro.

DADS: Have protecting impacts on colon carcinogenesis in rodent models, on other hand molecular mechanism of action is still not clear.

2.2.6.3. Type of garlic :

2.2.6.3.1 .Hard neck:

Preduceaflowerstalle ,are often termed as bolting or topsetting varieties .They are small aerial cloves which are genetically the same as the parent plant .hard neck produced 4-12 clove per plant and more productive as soft neck varieties.

2.2.6.3.2. Soft neck:

Do not preduceaflower steam normally and most common varieties used for commercial cultivations and have longer shelf life than hard neck store for 6-8month contain 10-40 cloves per plant and have anti chock and silver skin.

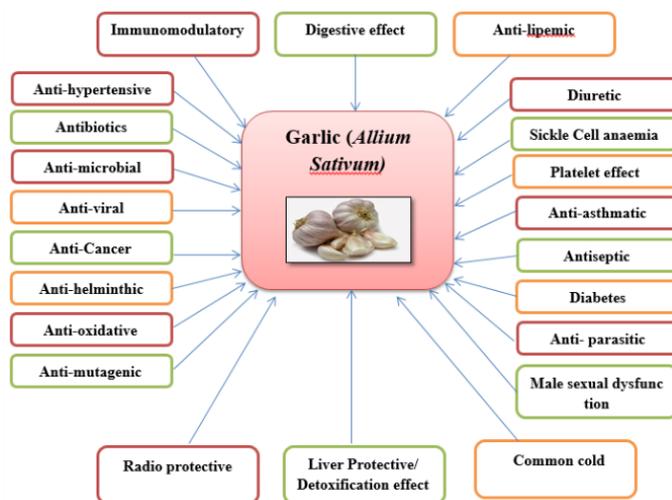
2.2.6.3.3.Black garlic :

Is garlic that have been fermented once the garlic has turned black the flavor is entirely different .

2.2.6.3.4. *White garlic:*

Garlic cultivated forms of *Allium sativum* with white bulbs .*Allium neapolitanum* ,a wild plant native to the Mediterranean and middle east.

2.2.6.3.5.SOME PRORTIES OF GARLIC



2.2.6.3 .5.1. *Anti-Cholesterolemic:*

[13]Organo-sulphur compounds are the main active substances responsible for the hypolipidemic and hypocholesterolemic effects of garlic, as much in humans as in experimentation animals . Several decades ago, reported the multiple inhibitory effects of garlic extracts in several different steps in cholesterol biosynthesis pathway in human hepatic cell. According to him defined compounds[allicin] present in water soluble extracts of garlic inhibit the biosynthesis of cholesterol in hepatocytes.

2.2.6.3.5.2 . *Anti-Hypertensive:*

[14]Several investigations have allowed the determination of the mechanism by which garlic exerts its anti-hypertensive action. Some studies of garlic effect on muscular contraction in vitro have concluded that its hypotensive action may be ,at least partly ,due to a direct relaxant effects on smooth muscles .

2.2.6.3. 5.3.Anti-Hyperglycemic or Anti-Diabetic Potential

[15]The garlic effectiveness as hypoglycemic agents has been scarcely investigated and the existing data are controversial, having not founds evidence of its effectiveness in all cases. The hypoglycemic effects of garlic and its individual components have been demonstrated in animal models whereas other researchers found no significant alteration of hyperglycemia in animals . Recently, it has been reported that long-term absorption of natural flavonoids as quercetin could be useful to prevent advanced glycation of collagens.

2.2.6.3.5.4 Anti- Platelet Pathway by Garlic Derivatives

[16]Antiplatelet activity is substantially affected by genotype, environment and storage duration of vegetable. It has been reported by several epidemiologic studies that, in garlic, the antiplatelet activity is determined, in part ,by the native concentration of organosulphur compound and genotypically determined sulphur content of the bulb . These compounds have structural similarity to ajoene, considered the major antiplatelet. compound in garlic extracts. In addition, other no sulphur compounds, such as β -chlorogeninandquercetin, have been also shown to inhibit platelet aggregation . The mechanism of platelet aggregation inhibition is associated at least with reduction of thromboxane formation from exogenous arachidonate and perturbation of the physicochemical properties of platetlet plasma membrane. The different results obtained are probably due to the use of different garlic

preparations and variable amounts of the active constituents in garlic in these studies .

2.2.6.3.5.5. Antiviral Activity

[17]Garlic has long been stated to possess antiviral properties; however, hardly any work has been done to investigate these properties reported in vivo antiviral effect of garlic in mice against intranasally-inoculated influenza virus . Garlic extract also enhanced the production of neutralizing antibodies when it was inoculated with the influenza vaccine. Other study reported the effectiveness in vitro of allicin and its various transformation products against Herpes Simplex Virus 1 and 2, Vesicular Stomatitis Virus, Vaccinia Virus and Parainfluenza Virus type 3. Garlic extract was effective against each virus tested the highest concentration tested (1 g/mL), the infectivity of each virus was substantially reduced .

2.2.6.3.5.6. Antiparasitic Activity

[18]Literature on the antiparasitic capacity of garlic focuses mainly on protozoan parasites. African trypanosomiasis, amoebiasis and giardiasis are all serious threats to humans and livestock in vast regions of Africa , South America and Asia. Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals recommended for the treatment of these diseases , garlic has been investigated as a potential alternative. Results of a clinical study carried out on patients with trypanosomiasis, amoebiasis, and giardiasis demonstrated that DATS, an allicin breakdown product, is effective against *Trypanosoma brucei* (ssp. *brucei*, ssp. *rhodesiense*, ssp. *gambiense*, ssp. *evansi*, ssp. *congolense* and ssp. *equiperdum*), *Entamoeba histolytica*, *Giardia lamblia* and *Giardia intestinalis*. Moreover, several studies have demonstrated that garlic extracts are also effective against *Opalina ranarum*, *O. dimidiata*, *Balantidium* entozoon,

Leishmania, Leptomonas and, is commercially available as a preparation, called Dasuansu, prescribed for the treatment of giardiasis and infection by Entamoebahistolyticagiardiasis and infections by and Trichomonas vaginalis. In addition, ajoene and other organo-sulphur compounds from garlic are also effective antiprotozoals

2.2.6.3.5. 7. Antimicrobial activity of Garlic

[19]Allicin exhibits anti-parasitic activity against major human intestinal parasites such as Entamoebahistolytica, Ascarislumbricoides and Giardia lamblia. Among the viruses which are sensitive to garlic extracts are the human cytomegalovirus, influenza B, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2.

2.2.6.3.5.8. Antifungal Activity:

- [20]Several *in vitro* and *in vivo* studies have shown the great effectiveness of garlic against a broad spectrum of yeasts and fungi, including *Epidermophyton* and *Trichophyton*, two of the three filamentous fungal genera classified as dermatophytes, *Candida*, *Torulopsis*, *Cryptococcus*, *Rhodotorula* and *Trichosporon*. Likewise, Adetumbi and Lau reported that an aqueous extract of dehydrated garlic preparation inhibits the growth of the dimorphic fungus *Coccidioides immitis* and *in vitro* fungal spore germination. Aqueous extract of garlic has been successfully used in treating cryptococcal meningitis, which is caused by the fungus *Cryptococcus neoformans*.

2.2.6.3.5.9. Effect on Hyperhomocysteinemia

[21]Homocysteine (Hcy) is a sulphur-containing amino acid formed during metabolism of methionine, an essential amino acid derived from the diet. The determination of total plasma Hcy has become a very useful tool because moderately elevated values of circulating Hcy constitute an important risk factor for the development and progress of occlusive

vascular affections . In addition, hyperhomocysteine is a risk factor for ischaemic heart disease (IHD) in diabetic patients .

2.2.6.3.5.10. Immuno modulatory Activity

[22]Garlic has been suggested as a promising candidate for maintaining the homeostasis of immunomodulatory activity .Since the immune dysfunction plays an important role in the development and progress of several diseases, modification of immune functions by garlic can contribute to their treatment and prevention.

2.2 6 .3.5.11 .The Garlic Side Effects :

- **When taken by mouth:** is likely safe for most people. Garlic has been used safely for up to 7 year . It can cause side effects such as bad breath , heartburn ,gas, and diarrhea . These side effects are often worse with raw garlic. Garlic might also increasing the risk of bleeding and cause allergic reactions in some people .
- **When applied to the skin:** Garlic products are possibly safe ,pastes, and mouthwashes containing garlic have been used up to 3 month . But garlic might cause skin damage that is similar to burn .
- RAW garlic is possibly unsafe when applied to skin . It might cause severe skin irritation .

2.2.6.3.5.12.People are contraindication to garlic :

1- Breastfeeding .

2- Pregnant, or may became pregnant while on this supplement.

3- Hemophilia or for two weeks before surgery, due to increasing danger from bleeding .

4- Stomach ulcer .

5- HIV , AIDS treatment .

2.2.6.3.5.13. The Type Of Bacteria

Gram Negative Bacteria					
Name	Morphology	O ₂ Requirements	Commensal	Reservoirs / Sites of colonization, Transmission	Types of Infections
Enterobacteriaceae (<i>E. coli</i> , klebsiella, salmonella, shigella)	Rods	facultative anaerobe	Some species	GI tract, animals / Endogenous, fecal-oral	Diarrhea, urinary tract, food poisoning, sepsis
Bacteroides	Rods	anaerobic	Yes	GI tract / Endogenous	Abscesses, intraabdominal infections
Pseudomonas	Rods	aerobic	No	Water, soil / Endogenous, breach of skin barrier	Infections in immunocompromised hosts, Cystic Fibrosis
Vibrio (cholera)	Rods, curved shape	microaerophilic	No	Water / Contaminated food, water	Diarrhea
Campylobacter	Rods, curved shape	microaerophilic	No	Food / Ingestion of contaminated food	Diarrhea, Bacteremia
Legionella	Rods, poorly stained	microaerophilic	No	Water / Inhalation of aerosol	Pneumonia, febrile illness
Neisseria	Cocci, kidney-bean shaped	Microaerophilic	No (<i>N. meningitidis</i> sometimes)	Humans / Sexual, aerosol	Meningitis, pelvic inflammatory disease
Hemophilus	Coccobacillary - pleomorphic	facultative anaerobe	Some species	Respiratory tract / Endogenous, aerosol	Respiratory, sinusitis, otitis meningitis
Bartonella	Small, pleomorphic rods	aerobic / microaerophilic	No	Cats, fleas, lice / cat bites, lice or fleas?	Cat scratch disease, endocarditis, bacillary angiomatosis

Miscellaneous Bacteria					
Name	Morphology	O₂ Requirements	Commensal	Reservoirs / Sites of Colonization, Transmission	Types of Infections
Helicobacter	GN, but not visible on Gram stain - helical (corkscrew) shaped	microaerophilic	Yes	Stomach / Endogenous, Fecal-oral	peptic ulcer disease, gastric ulcer
Mycobacteria	Rods, Weakly Gram positive, Acid fast stain positive	aerobic	No	Lungs / Fomites	Tuberculosis
Treponemes	Not visible on Gram stain, spiral shaped on dark field exam	nonculturable on routine media	No	Humans / Sexual transmission	Syphilis
Borrelia	Not visible on Gram stain, spiral shaped on dark field exam	nonculturable on routine media	No	Rodents, Ticks / Tick bites	Lyme, Relapsing fever
Mycoplasma	Not visible on Gram stain, no cell wall, pleomorphic	Non-culturable on routine media	Some species	Humans / aerosol	Respiratory tract infections
Rickettsia/ Ehrlichia	Obligate intracellular (Gram negative but not visible on Gram Stain)	Non-culturable on routine media	No	Ticks, Mites/ transmitted from the feces of infected lice, fleas, ticks	Cause a variety of illnesses including systemic vasculitis (e.g. Rocky Mountain Spotted Fever), rash, pneumonia

General Phenotypic Classification of Bacteria

Gram Positive Bacteria					
Name	Morphology	O₂ Requirements	Commensal	Reservoirs / Sites of colonization, Transmission	Types of Infections
Staphylococci	Cocci in grape-like clusters	facultative anaerobe	Yes	Skin, nares / endogenous, direct contact, aerosol	Soft tissue, bone, joint, endocarditis, food poisoning
Streptococci	Cocci in pairs, chains	facultative anaerobe	Some species	Oropharynx, skin / endogenous, direct contact, aerosol	Skin, pharyngitis, endocarditis, toxic shock
Pneumococci	Diplococci, lancet shaped	facultative anaerobe	±	Oropharynx, sinus / aerosol	Pneumonia, otitis, sinusitis, meningitis
Enterococci	Cocci in pairs, chains	facultative anaerobe	Yes	GI tract / endogenous, direct contact	UTI, GI, catheter-related infections
Bacilli	Rods, spore-forming	aerobic	±	Soil, air, water, animals / aerosol, contact	Anthrax, food poisoning, catheter-related infections
Clostridia	Rods, spore formers	anaerobic	Some species	GI tract, soil / Breach of skin, endogenous, ingestion	Tetanus, diarrhea, gas gangrene, botulism
Corynebacterium	Rods, nonspore forming	facultative anaerobe	Some species	Skin	Catheter-related infections, diphtheria
Listeria	Rods, nonspore formers	facultative anaerobe	No	Animals, food products / Ingestion	Meningitis
Actinomyces	Irregular, filamentous, form sulfur granules	anaerobic	Yes	GI tract / endogenous	Skin, soft tissue

2.2.6.3.5.14. The previous study;

14.1. Anti microbial

This study examined the antimicrobial activity of both fresh, and heat treated *Allium sativum* as well as the garlic of domestic origin (kayani) and the imported (china) garlic . The study used three Gram –positive bacteria : *staphylococcus aureus* , methicillin- resistant *staphylococcus*

Staphylococcus aureus (MRSA), and Bacillus subtilis, two Gram –negative bacteria : Escherichia coli , salmonella enteritidis and fungus candida albicans. The results in this research based on the measured zones of inhibition , it determined that the fresh homemade garlic manifests the best antimicrobial effects . It had the highest level of inhibition on the fungus candida albicans (62mm), while the lowest level of antimicrobial recorded in bacillus subtilis (13mm) . Heat-treated domestic garlic demonstrated the weakest inhibitory effect in MRSA and the fungus candida albicans , wherein the zone of inhibition was 0 mm , while the other type of bacteria , both Gram –positive and negative show slightly better antimicrobial activity . The highest inhibitory effects recorded for salmonella (11mm).

Chapter Three

Methodology

3. Material and Method:

3.1 Study design:

Experimental laboratory based study

3.2 Study area:

This study was done in National research center in Khartoum. Mammon Hamida university Napata Collage

3.3 Study population and sample size:

The study utilized the garlic clove to explained the antimicrobial activity

3.4. Study duration:

This study was done between October 2021 to Janauary 2022.

3.5 Data collection:

3.5.1 Primary data collection:

The primary data were collected from the laboratory of National research center and Napata collage. Mammon Hemida university .

3.5.2. Secondary data collection:

The secondary data were collected from references (books, journals, published articles and websites .)

3.6 Materials:

3.6.1. Plant material: Garlic has been used as spice and food ingredient in cooking all over the world because of it combines well with an enormous range of foods, adding its own aroma and flavor as well as enhancing the flavors of the foods with which it is mixed (Woodward, 1996). Besides to be used like food, garlic has long been used in folk medicine with protective and curative purposes.

3.6.2. Garlic Extraction:

3.6.2.1. Maceration Method;

Extraction was carried out according to the method described by Sukhdevet .al. [2008].

The plant sample was coarsely powdered using mortar and pestle . Coarsely sample was soaking with ethanol Extraction carried out for three days with daily filtration and evaporation the solvent under reduced pressure using rotary evaporator apparatus . Sample extract was allowed to air in evaporating dish ,till complete dryness and the yield percentages were calculated as follows;

Weight of extract obtained /Weight of plant sample *100

3.6.2..2.2.Method of analysis:

GC/MS Conditions

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 3 minutes hold time , the injection port temperature was 300c, the ion source temperature was 200c and the interface temperature was 250c.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes .Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology(NIST), then result was recorded and major organo-sulfur compounds were determined.

3.6.2.2. Anti microbial activity assay;

3.6.2.2.1. Test microorganisms;

Bacterial microorganisms:

Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve Bacteria)
Escherichia coli	ATCC 25922(Gram -ve bacteria)
Pseudomonas aeruginosa	ATCC 27853 (Gram -vbacteria)

- National Collection of Type Culture (NCTC), Colindale, England.
- American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Candida albicans	ATCC7596
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Fungal microorganisms:

3.6.2.2.2 *Methods:*

Preparation of the test organisms:

Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10⁸- 10⁹ C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor

to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Test microorganisms:

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

Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used .**Testing of antibacterial susceptibility**

3.6.2.2.3.Disc diffusion method

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

Chapter Four

Results

4.4.1 /Results and Discussion

4.1. Yield percentage of the extract and physical properties:

The weight of the plant material before drying = 283,5 g

The weight of the plant material after drying = 350,9 g

The yield % = $283,5/350,9 \times 100\% = 80,7\%$. **Table 1: Yield and Physical Characteristic of methanolic Extracts of A.:-**

Types of Extract	Yield %	Physical State	Color	Texture
Ethanollic extract	80,7%	Liquid	Pale yellow	Pungent

4.4.2. Gas Chromatography-Mass Spectrometry (GC-MS):-

In the present study, GC-MS procedure was applied for the identification of chemical profile of the methanolic extracts. The relative retention times (Rt) and mass spectra of the extract's components were compared with the standard mass spectra in the library and with data from the literature. Thirty-two compounds belonging to different classes of secondary metabolites, were identified from the methanolic extract .

- **Figure 1: GC-MS Chromatogram of A.sativaMethanolic Extract :**
- **Table 2: Chemical Composition of A.sativaethanollic leaves Extract:**
- **Figure 2: GC-MS Chromatograms of the Major Identified Components of A.sativa ethanollic Extract:-**

Figure 1: GC-MS Chromatogram of *A.sativum*Ethanollic Clove Extract

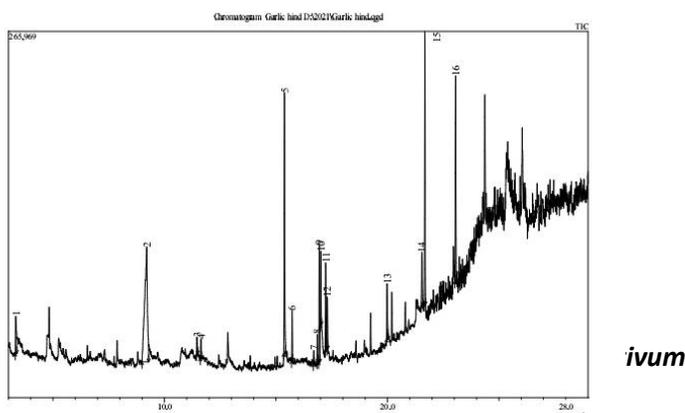


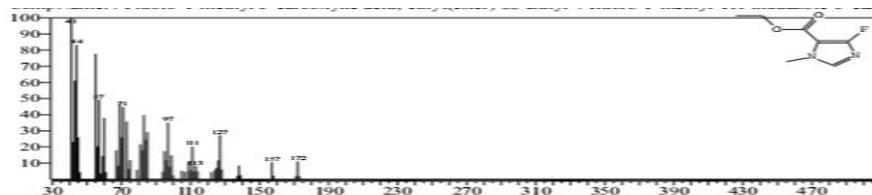
Figure 2: GC-MS Chroma Clove Ethanollic Extract:-

Peak2	# Name	R.Time	Area	Area%
-------	--------	--------	------	-------

1	Erythritol	3.326	76074	3.17
2	1,2-Cyclopentanediol, 3-methyl-	9.199	702139	29.29
3	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta	11.456	25328	1.06
4	3-Trifluoroacetoxypentadecane	11.639	19506	0.81
5	n-Hexadecanoic acid	15.382	336210	14.02
6	Hexadecanoic acid, ethyl ester	15.723	55245	2.30
7	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)	16.694	15614	0.65
8	Decane, 2,3,5,8-tetramethyl	16.854	27501	1.15
9	9,12-Octadecadienoic acid (Z,Z)-	16.942	170836	7.13
10	Cyclopropaneacetic acid, 2-hexyl-	17.011	74150	3.09
11	Cyclopropanoic acid, 2-[[2-[(2- ethylcyclopro	17.229	102368	4,27
12	E-11-Hexadecenoic acid, ethyl ester	17.301	56487	2.36
13	Pentadecanoic acid, 2-hydroxy-1- (hydroxymethyl	19.983	79672	3.32
14	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester	21.541	76702	3.20
15	2-methylhexacosane	21.676	320251	13.36
16	2-methyltetracosane	23.059	259244,2397327	10.81,100.00

Figure 2: GC-MS Chromatograms of the Major Identified Components of *A.sativum* Clove Ethanolic Extract:-

Chromatogram of 1,2-Cyclopentanediol, 3-methyl.



1. Chromatogram of Diallyldisulphide.

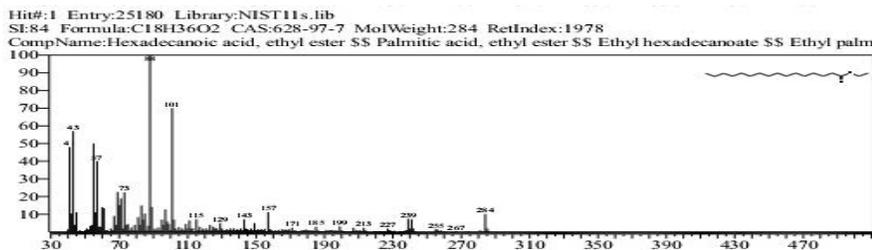
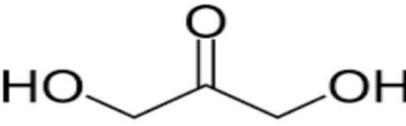
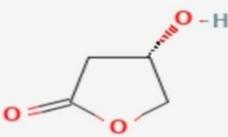


Table 3: Activity of Bioactive Compounds Present in Ethanolic Clove Extract of *A. sativum*:-

Name of Compound	Biological activity	Chemical Structue
------------------	---------------------	-------------------

1/Allyltrisulfide	Anti-cancer effects, platelet aggregation, blood pressure reduction, antioxidant,	
2/1,3-Dihydroxyacetone dimer	cholesterol lowering, papain inactivation, detoxification, glutathione transferase activity	
2/Hydroxy-gammabutyrolactone	Antioxidant, analgesic, anti-diabetic, antibacterial, and antifungal activity	

4.4.3 Antimicrobial activity

Disc diffusion method was employed to explore the antimicrobial activity of ethanolic extract of *A. sativum* clove against some pathogenic bacterial strains ;Gram positive bacterial strains(*S. aureus* and *B. subtilis*), Gram negative (*E. coli*, and *P. aeruginosa*).Results exhibited significant antimicrobial activity; against all the tested pathogenic microorganisms; with the zone of inhibition ranged between 10.5-13.5 mm. Additionally ,antimicrobial activity of the ethanolic extract increased when higher concentrations were used(Table3) . Strong inhibitory activity could be observed against *B. subtilis* (13.5 mm) whereas the less effective was against *P. aeruginosa* ,with the Standard deviation 7.07

Table (3) :Antimicrobial activity of ethanolic extract of *A. sativum* clove

Zone of inhibition ZD(mm)			Zone of inhibition ZD(mm)		
	S. aureus	B. subtilis	P. aeruginosa	E.coli	C. albicana
10% Garlic	12	13.5	10.5	11	12.5

Interpretation of results: ZD (mm):>18mm → sensitive; 14-18 → intermediate; <14mm → resistant; (0) → no inhibition zone.

	ZI-Sa	ZI-BS	ZI-Pa	ZI-Ec	ZI-Ca
N valid	2	2	2	2	2
Missing	0	0	0	0	0
Mean	11.50	13.50	10.50	11.00	12.00
Std.Deviation	2;121	707	707	000	1.414

4.4.4. Discussion:

Garlic (Allium sativum), is broadly used around the world for its numerous culinary and medicinal uses, it has been used in food as well as in herbal medicine. The present study investigated the chemical compositions of *A. sativum* clove ethanolic extract and its antibacterial activities.

Through analysis of the yields and GC-MS, the ethanol concentration range with the optimum extraction efficiency 80.9% yield of the extract (Table 1), this ratio of the amount actually produced when a target substance is obtained from a raw material through a certain chemical process and is generally expressed as a percentage.

GC-MS results revealed the presence of Sixteen identified compounds ranged between fatty acids and hydroalkane the major were: 1,2-Cyclopentanediol, 3-methyl- (29.29%), Diallyldisulphide (14.26%), 1,2,3- thiadiazole, 5-methyl- (4.24%, 5.91%) and Trisulfide, di-2-propenyl (19.84%, 14.36%). In contrast Na *et.al.*, 2017 examined the GC-MS of the *A. sativum* pulp, results indicated the presence of, allyltrisulfide, 2-hydroxy- γ -butyrolactone, 1, 3-dihydroxyacetone dimer, propanoic acid, 2-propone, these compounds were confirmed as predominant components of garlic extract.

GC/MS analysis, antimicrobial and Effect of Ethanol Garlic Extract

July 2017

International Journal of Phytomedicine

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Antimicrobial activity also was examined for the ethanolic extract, results indicated that the Strong inhibitory activity could be observed against *B. subtilis* (13.5 mm) whereas the less effective was against *P.*

aeruginosa .These findings of antimicrobial activity warrant future studies determining/identifying the individual bioactive compounds that are responsible for the observed antimicrobial activity.

The inhibitory effect is also found to be dependent on the type of solvent used for the extraction, these facts were not surprising, as methanol has been reported to be more efficient in the extraction of bioactive compounds in a garlic matrix .

Thus ,Garlic has been proved to be effective against a wide range of microorganisms .

Chapter five

Conclusion and Recommendations

5.5.1 CONCLUSION:

The results indicate that this garlic clove should be studied more extensively to explore its potential in the treatment of infectious diseases. Recently, there has been enormous interest in the medicinal plants for their antimicrobial activities in controlling pathogens.

In that way, active molecule present in the *A. sativum* having both antimicrobial activity and it could be a potential source of novel therapeutic drugs.

5.5.2 Recommendations

Other phytochemical screening are recommended to isolate and characterize specific compounds that responsible for the biological properties.

Modern techniques e.g. HPLC or LC-MS is recommended to identify the chemical composition of *A.sativum* clove and other plant parts.

Other organic solvents are recommended to cover more types of microorganism.

More biological activities are required to define the other pharmacological activities of *A.sativum* clove.

Further researches to formulate the different extracts of the of *A.sativum* clove into suitable dosage form to treat different disease.

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The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.

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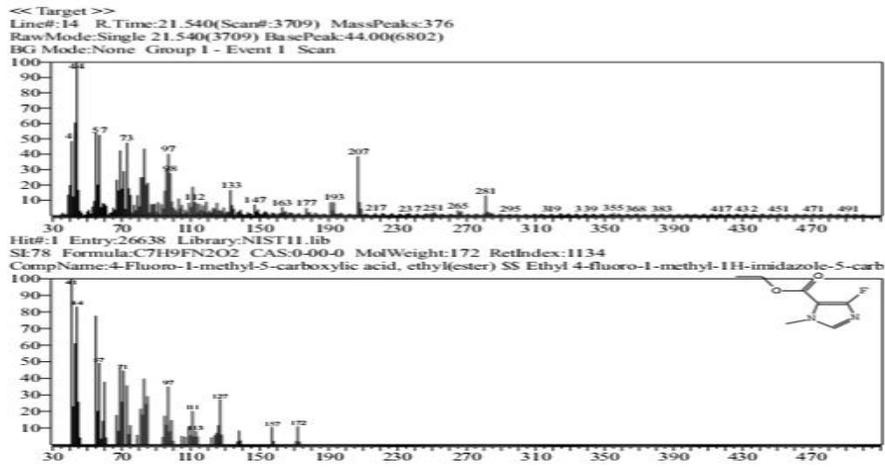
Appendix

Figure 2: GC-MS Chromatograms of the Major Identified Components of *A.sativum*

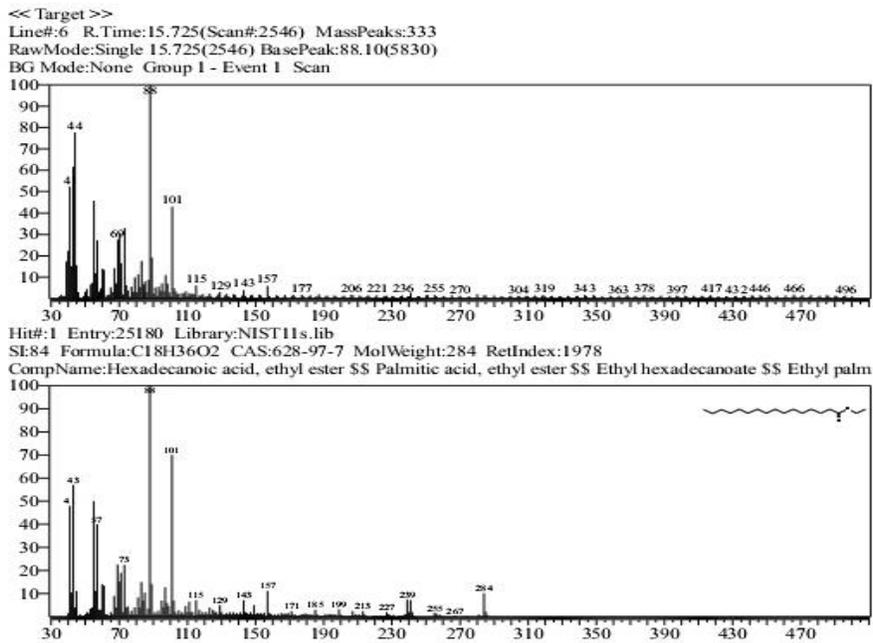
Clove Ethanolic Extract:-

Peak2	# Name	R.Time	Area	Area%
1	Erythritol	3.326	76074	3.17
2	1,2-Cyclopentanediol, 3-methyl-	9.199	702139	29.29
3	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta	11.456	25328	1.06
4	3-Trifluoroacetoxypentadecane	11.639	19506	0.81
5	n-Hexadecanoic acid	15.382	336210	14.02
6	Hexadecanoic acid, ethyl ester	15.723	55245	2.30
7	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)	16.694	15614	0.65
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9	9,12-Octadecadienoic acid (Z,Z)-	16.942	170836	7.13
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11	Cyclopropanoic acid, 2-[[2-[(2-ethylcyclopro	17.229	102368	4.27
12	E-11-Hexadecenoic acid, ethyl ester	17.301	56487	2.36
13	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl	19.983	79672	3.32
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15	2-methylhexacosane	21.676	320251	13.36
16	2-methyltetracosane	23.059	259244,2397327	10.81,100.00

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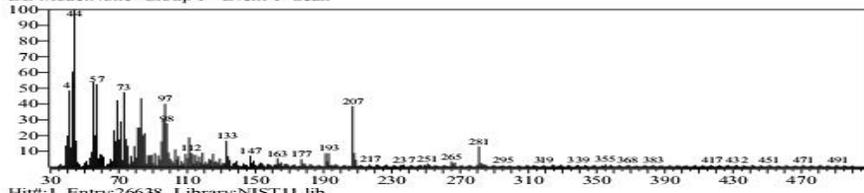
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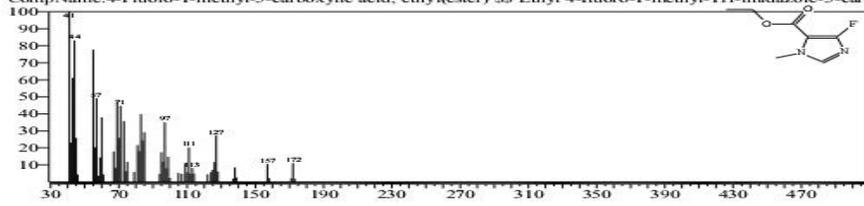
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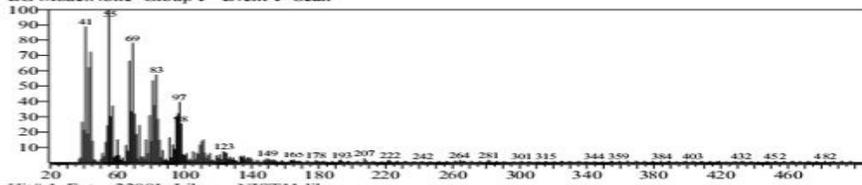
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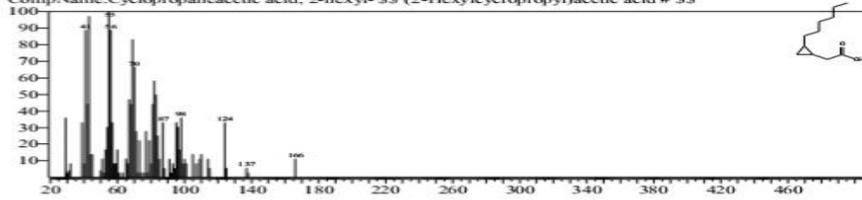
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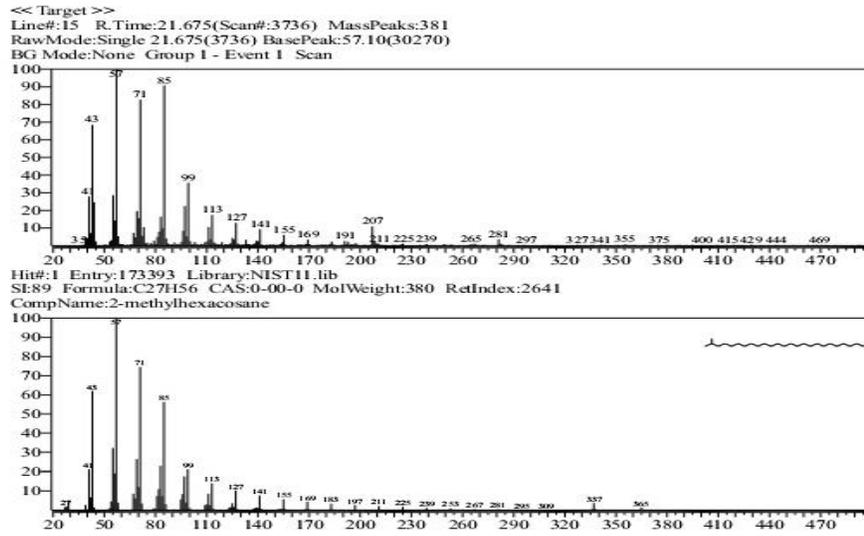
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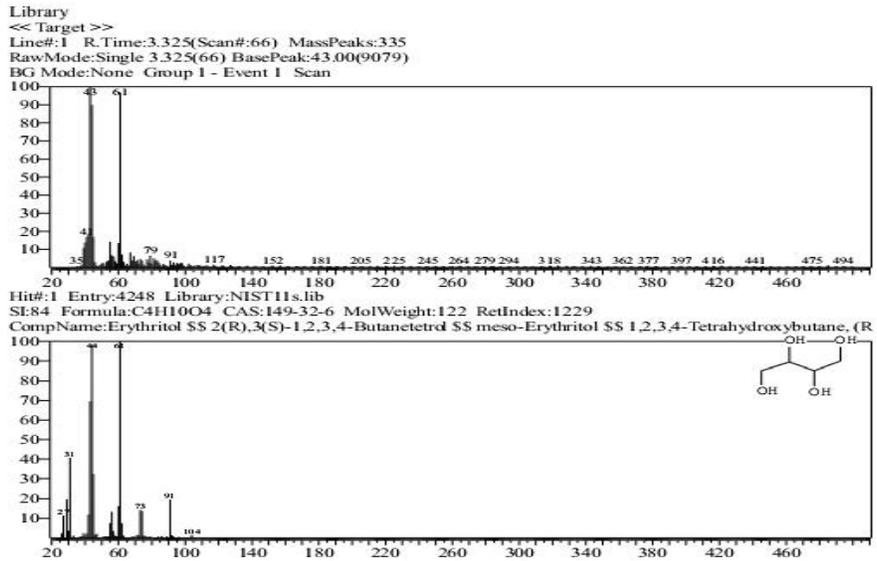
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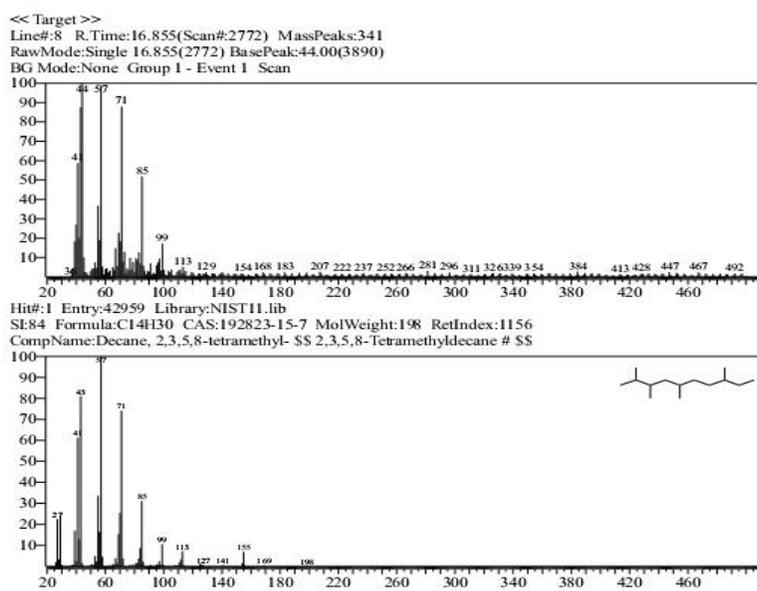
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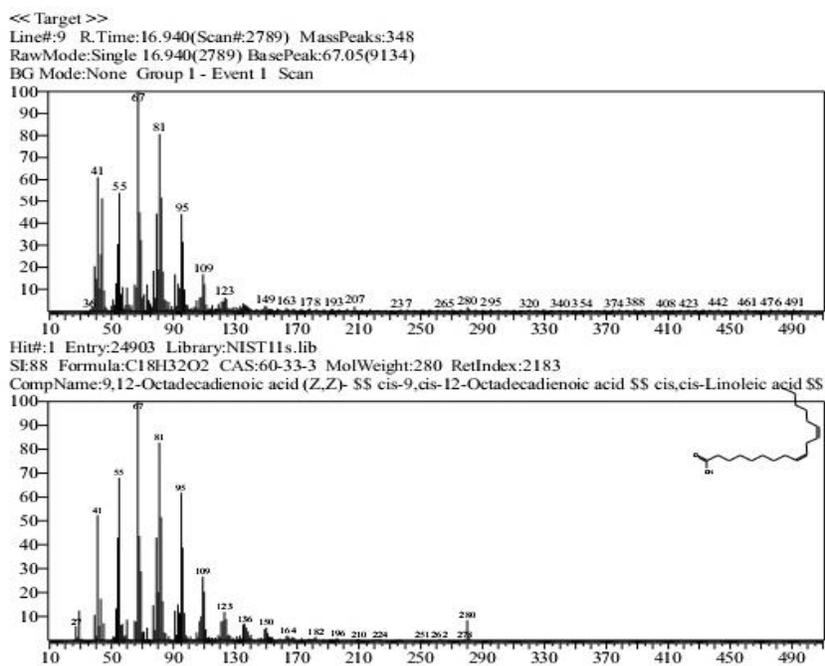
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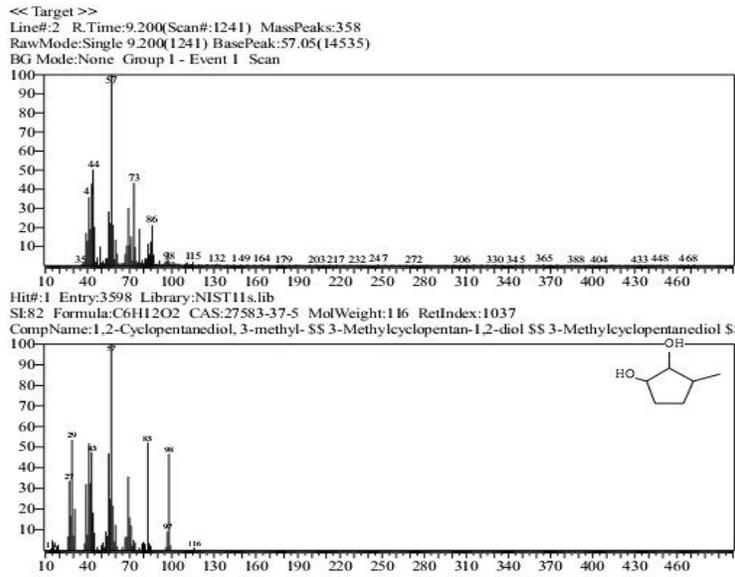
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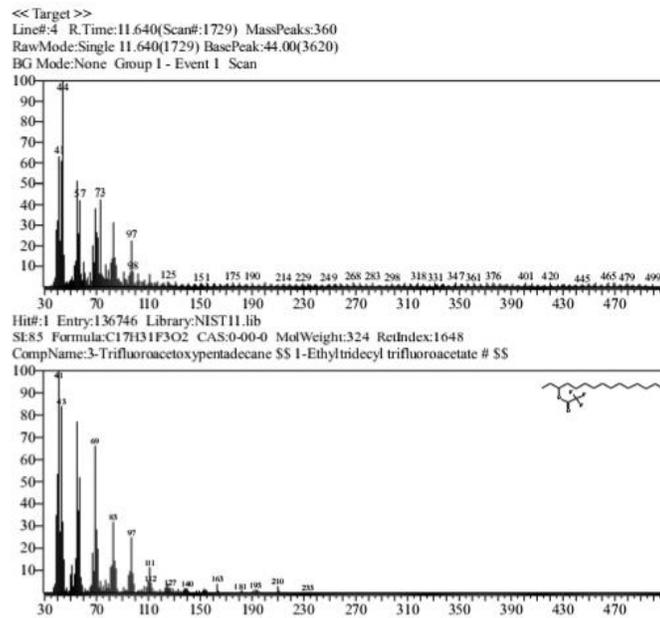
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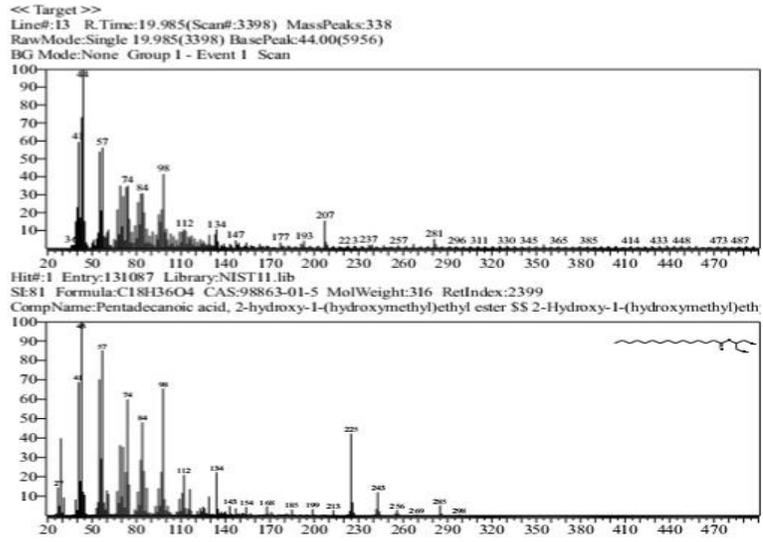
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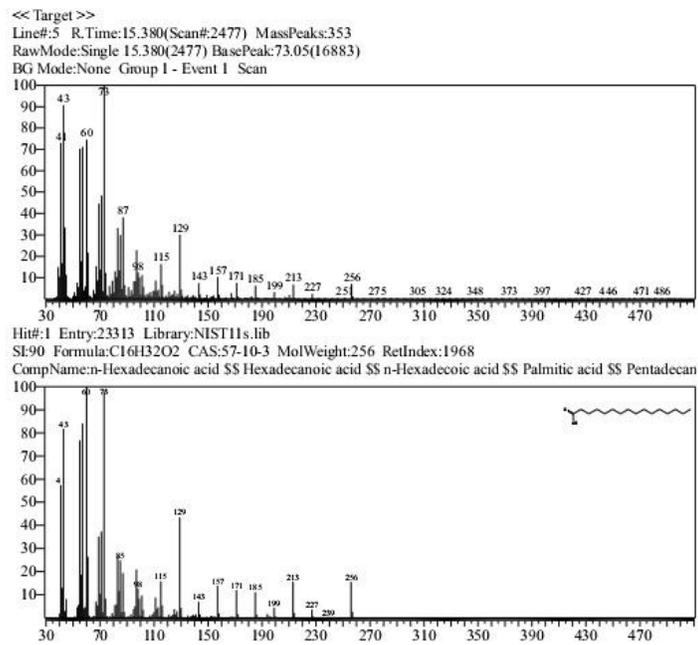
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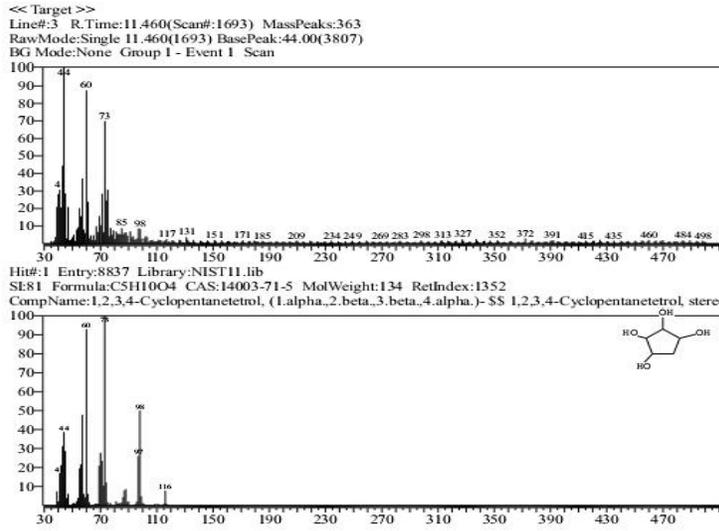
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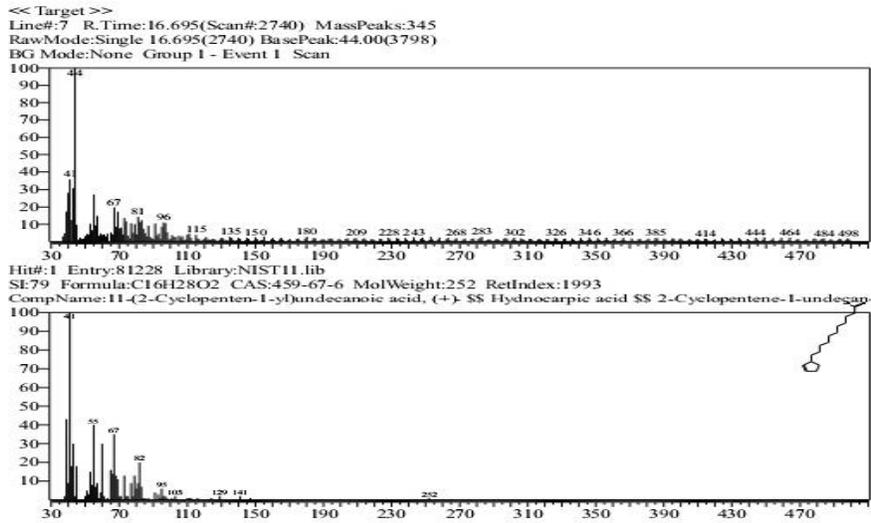
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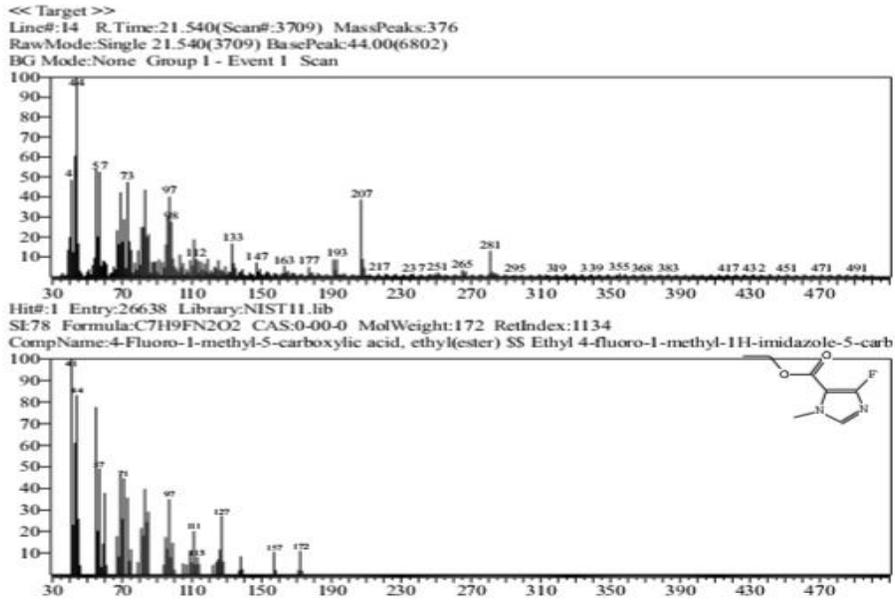
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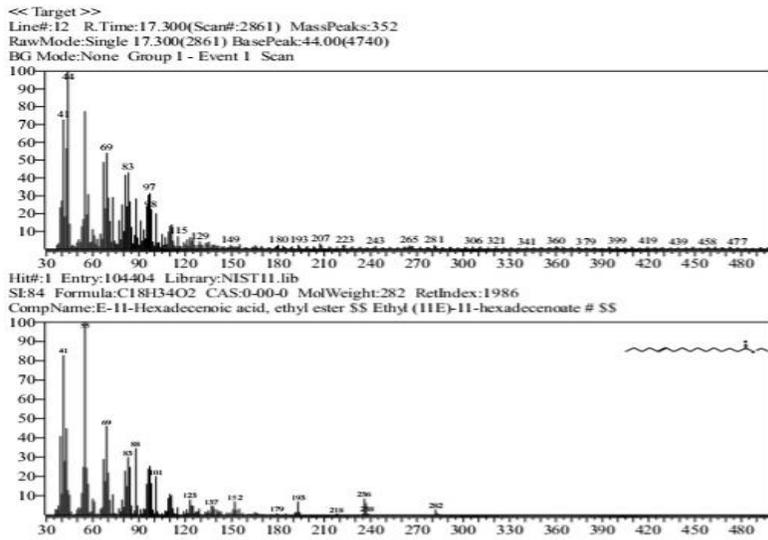
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4.3.16



4.3.17

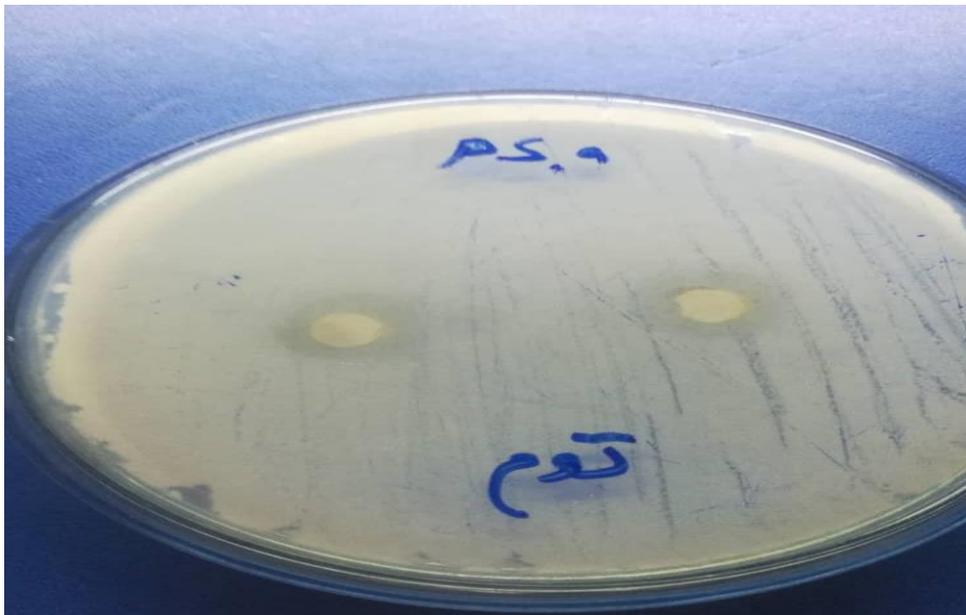


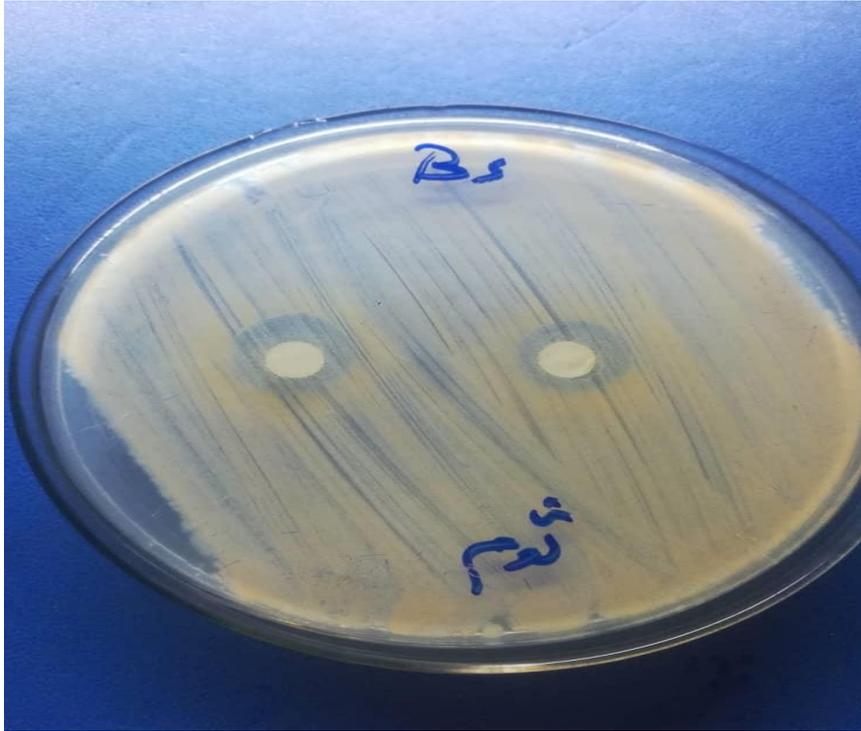












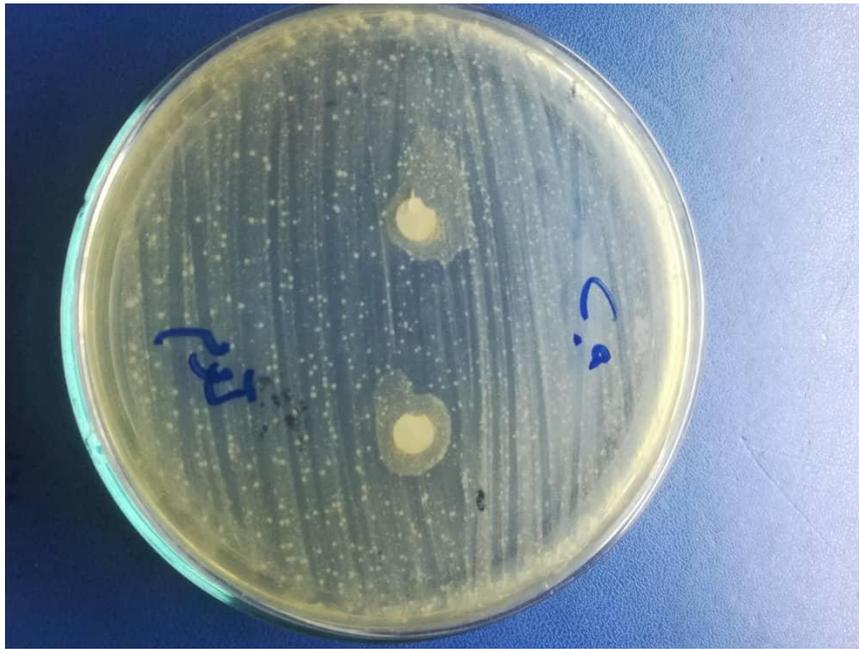


Table 1: Organo-sulphur compounds present in commercial garlic preparations.21

Sr no.	Organo-sulphur compounds	
1	Allicin (Diallylthiosulfinate)	
2	Allyl methane sulfinate	
3	Alliin (S-AllylL-cysteine sulfoxide)	
4	Diallyl disulfide (DADS)	
5	S-allyl cysteine	
6	Allyl methyl trisulfide	
7	Diallyltrisulfide (DATS)	
8	Allyl methyl trisulfide	
9	Allyl methyl disulfide	
10	Diallyltetrasulfide	
11	Allyl methyl tetrasulfide	
12	Dimethyl trisulfide	
13	Diallylsulphide	
14	2-vinyl-4-H1,3-dithiin	
15	3-vinyl-4.-H1,2-dithiin	
16	E-ajoene	
17	Z-ajoene	