Sudan University of Science and Technology
College of Graduate Study

Investigation of the Antimicrobial Activity and Chemical Composition of some Constituents of Garlic Extract

التحقق من النشاط المضاد للميكروبات والتركيب الكيميائي لبعض مكونات مستخلص الثوم

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

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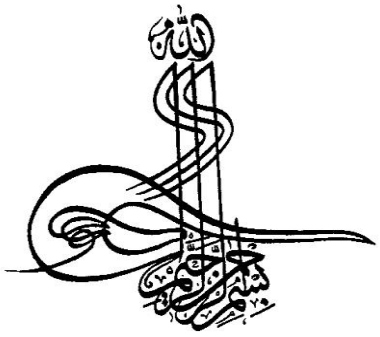
إستهلال



قال تعالى :

أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَسَلَكَهُ يَنَابِيعَ فِي الْأَرْضِ ثُمَّ يُخْرِجُ بِهِ زَرْعًا مُخْتَلِفًا أَلْوَانُهُ ثُمَّ يَهِيجُ فَتَرَاهُ مُصْفَرًّا ثُمَّ يَجْعَلُهُ حُطَامًا ۚ إِنَّ فِي ذَٰلِكَ لَذِكْرَىٰ لِأُولِي الْأَلْبَابِ﴿٢١﴾

سورة الزمر (21)

Dedication

I dedicate this work to:

My Family,

Friends,

All whom I love,

And To
THE NEW SUDAN

With my deepest love and

Respect.

Acknowledgements

First thanks go to Allah for giving me strength to complete this research.

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ABSTRACT

The Aims of this study were to evaluate the antimicrobial activity of different extract (Maceration and Soxhlet) of bulbs of *Allium satvium.L*, and to identify their chemical composition using GC/MS and ICPOES methods.

The calculated extraction percentages (%E) of ethanolic solvent revealed that soxhlet extract is the best having %E of (60.05%), followed by Maceration extract of fresh garlic (32.79%), and Maceration extract of dry garlic (9.5%).

The results of GC/MS analysis of garlic extract showed that it contains fifteen compounds and the target compounds is diallyl trisulfide, sucrose and acitc acid.

The ICPE Analysis of garlic extract showed that it contains sulfur (680ppm), phosphorus (2.1ppm), iodine (1.7ppm), beside other minor minerals.

The antimicrobial activity study showed that ethanol Maceration extract exhibited moderate anti-fungial activity (inhibition zone: 12mm, at 100% concentration) against Candida albicans fungus. On other hand the ethanol Soxhlet extract exhibited moderate antibacterial activity against *Escherichia coli* (inhibiton zone:10mm, at 100% concentration), *Pseudomonas aeruginosa* (inhibition zone: 9mm, at 100% concentration) , *Bacillus subtilis* (inhibiton zone:10mm, at 100% concentration) and *Staphylococcus aureus* (inhibiton zone: 9mm, at 100% concentration).

المستخلص

هدفت هذه الدراسة إلى تقييم النشاط المضاد للميكروبات لمستخلصات (النقع والإستخلاص المستمر) من بصيلات نبات الثوم، وأيضا شملت دراسة كيميائية لتحديد التركيب الكيميائي لمستخلص النقع الكحولي بإستخدام كروماتوغرافيا الغاز وجهاز حث البلازما.

كشفت نسبة الإستخلاص بمذيب الإيثانول أن مستخلص الإستخلاص المستمر أعطى أعلى نسبة مئوية وزنية (60.05%)، يليه مستخلص النقع للثوم الطازج (32.79%)، يليه مستخلص النقع للثوم المجفف (9.5%).

وضحت نتائج التحليل بإستخدام كروماتوغرافيا الغاز وجود خمسة عشرمركب وأهما مركب ثلاثي الكبريت ، السكروز وحمض الخل.

بين تحليل حث البلازما لمستخلص الثوم أنه يحتوي الكبريت (680 جزء من المليون) ، الفسفور(2.1 جزء من المليون) ، اليود(1.7 جزء من المليون) ، بجانب بعض المعادن الثانوية.

وضحت دراسة النشاط المضاد للميكروبات أن لمستخلص النقع الكحولي فعالية متوسطة ضد فطريات كانديدا (منطقة التثبيط: 12مم، بتركيز100%). ومن ناحية أخرى أظهر مستخلص الإستخلاص المستمر الكحولي فعالية متوسطة ضد بكتيريا العصيات القولونية (منطقة التثبيط: 10مم، بتركيز 100%) ، بكتيريا الزائفة الزنجارية (منطقة التثبيط: 9مم، بتركيز 100%)، المكورة العنقودية الذهبية (منطقة التثبيط: 9مم، بتركيز 100%) وبكتيريا العصوية الرقيقة (منطقة التثبيط: 10مم، بتركيز 100%)، بينما لم يظهر فعالية ضد فطر كانديدا (فطر المبيضة البيضاء).

List of Abbreviations

Ec	Escherichia coli
Pa	Pseudomonas aeruginosa
Sa	Staphylococcus aureus
Bs	Bacillus subtilis
Ca	Candida albicans
ATCC	American Type Culture Collection
NCTC	National Collection of Type Culture
GC\MS	Gas Chromatography Mass Spectrometer
ICPE	Inductively Coupled Plasma Emission spectrometer
DATS	Diallyl trisulfide
DADS	Diallyl disulfide
DAS	Diallyl sulfide

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Chapter one
Introduction and literature review

1. Introduction and Literature review

1.1 General introduction

Antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes in the past 60 years. The resistance to antibiotics is increasing at a faster pace than it can be controlled (Renuka et al,2004).

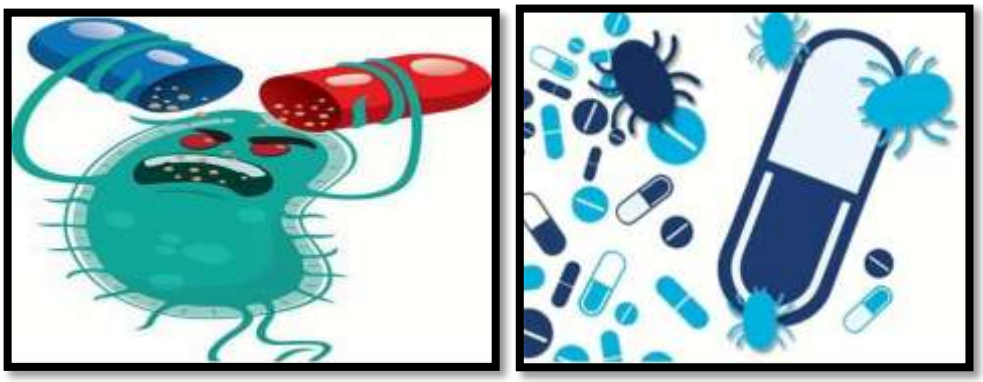


Figure (1.1): The resistance of bacteria to antibiotics

Natural medicines, such as traditional Chinese medicine (TCM) and Ayurveda, were formed and developed in the daily life of ancient people and in the process of their fight against diseases over thousands of years.They have produced a positive impact on the progress of human civilization. Today, natural medicines not only provide the primary health-care needs for the majority of the population in developing countries, but have attracted more and more attention in developed countries due tosoaring health-care costs and universal financial austerity. In the USA, approximately 49% of the population has tried natural medicines for the prevention and treatment of diseases (WHO,2017).

Chemicals known to have medicinal benefits are considered to be “active ingredients” or “active principles” of natural medicines. Natural products have provided the primary sources for new drug development (Newman and Cragg,2016)

1.2 Literature review

1.2.1Garlic plant

Garlic *(Allium sativum.L*), like other plants, has an exquisite defense system, composed of as many different components as the human immune system. In order to protect itself from insects and fungi, garlic produces allicin by enzymatic reaction when it is injured. Thus, allicinis mother-nature's insecticide. Since ancient times, garlic has been used worldwide, not only as a food, but also as a medicine (Rahman, 2007).

1.2.2 Taxonomy and local Names

Table (1:1) Classification of *Allium sativum.L*

Domain	**Eukaryota**
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Equisetopsida
Subclass	Magnoliidae
Superorder	Lilianae
Order	Asparagales
Family	Amaryllidaceae
Genus	Allium

- **Local Common Names:**

Taum, Thoum (Alam et al,2016).

1.2.3 Botanical description

Other members of the onion genus, Allium, include A. cepa (onion), A. schoenoprasum (chives), A. ascalonicum (shallots), and A. porrum (leeks). Allium sativum is further divided into two subspecies, A. sativum var. sativum, also known as softneck garlic, and *Allium sativum* var. ophioscorodon, also known as hardneck garlic. Both varieties are composed of an underground bulb made up of cloves, which are prophylls enclosed by dry membranous skins and held together by a basal plate. The variations differ in that hard neck garlic's bulb is composed of six to eleven cloves, circled around a centralized woody stalk. This variety of garlic has a scape that curls at the top, but it is generally removed after it curls one to three times. This is because if it continues to grow, less energy can be utilized towards the bulb. Eventually, the scape would give rise to bulbils, containing miniature cloves. The bulbils are occasionally accompanied by white or light purple flowers, although these are sterile. Softneck garlic does not have a flowering top and contains up to twenty-four cloves per bulb. The stem is central and soft, hence the name, and the cloves are layered with larger ones on the outside. *Allium sativum* is the more common variation, many studies involving garlic do not specify which subspecies is used, but chemical and biological action are assumed to be similar. *Allium sativum* is sterile and hence is grown asexually from cloves, not requiring a pollinator. It grows best in mild climates, through hardneck varieties are better adapted to colder environments. *Allium sativum* is a perennial species, as are most members of the genus. Garlic is composed of very strong organosulfur compounds that serve as secondary metabolites as described in the section entitled chemistry and pharmacology. These compounds are responsible for the very pungent smell and taste of garlic and act as defenses against predators (Block 2010).

Bulb Rounded, composed of up to about 15 smaller bulblets known as cloves. Cloves and bulbs are covered by a whitish or pinkish tunic (papery coat).

Leaves Four to twelve long, sword-shaped leaves attached to an underground stem.

Flowers Borne in a dense, spherical cluster on a spike (flower stalk) up to 25 cm long. The young flower head is enclosed in a long-beaked pair of enclosing bracts, which become papery and split to reveal the flowers. Individual flower stalks arise from a common point. Flowers are greenish-white or pinkish with six perianth segments (sepals and petals) about 3 mm long. Bulbils (asexual propagules), which resemble tiny cloves, are often interspersed among the flowers.

Fruit Flowers usually abort before developing to a stage at which fertilisation could take place.

Seeds Not usually produced in the wild but have been produced under laboratory conditions. With a black coat, similar to onion seeds, but approximately half the size (Block,2010).

Figure (1.2): Species of Garlic (bulbs)

1.2.4 Geographical distribution

Allium sativum.L is supposed to originate from Central Asia, from where its cultivation has spread to Southwest Asia and the Mediterranean region. Today garlic is cultivated in regions with moderate or subtropical climate all over the world (Koch *et al*,1938).

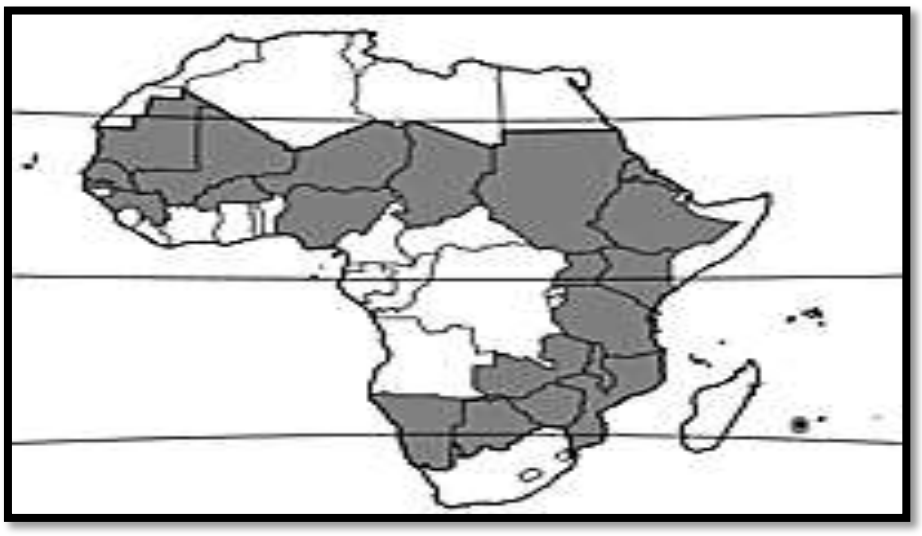


Figure (1.3): Distribution map of garlic

1.2.5 The Chemictry of Garlic

Smashing garlic grain releases some enzymes called alliinase that converts sulfoxide materials due to sulfonic acid. Then, these acids are converted to aromatic compounds such as allicin and dimmer by losing water.

1.2.5.1Components of garlic

- Amino acids: Glutamic acid, arginine, aspartic acid, leucine, lysine, valine..etc
- Minerals: Mainly: manganese, potassium, calcium, phosphorus and in minor

quantities: magnesium, selenium, sodium, iron, zinc and copper.

- Vitamins: Mainly: vitamin B6, also Vitamin C and in minor quantities, folic acid, panthotenic acid and niacin.

- Essential oil with many sulphur containg components: allyl disulfide , allyl trisulfide, etc
- Alliin, which, by means of enzyme alliinase, is converted into allicin.
- Ajoene, produced by allicin condensation.
- Quercetin.
- Sugars: fructose and glucose. (Mardomi,2017).

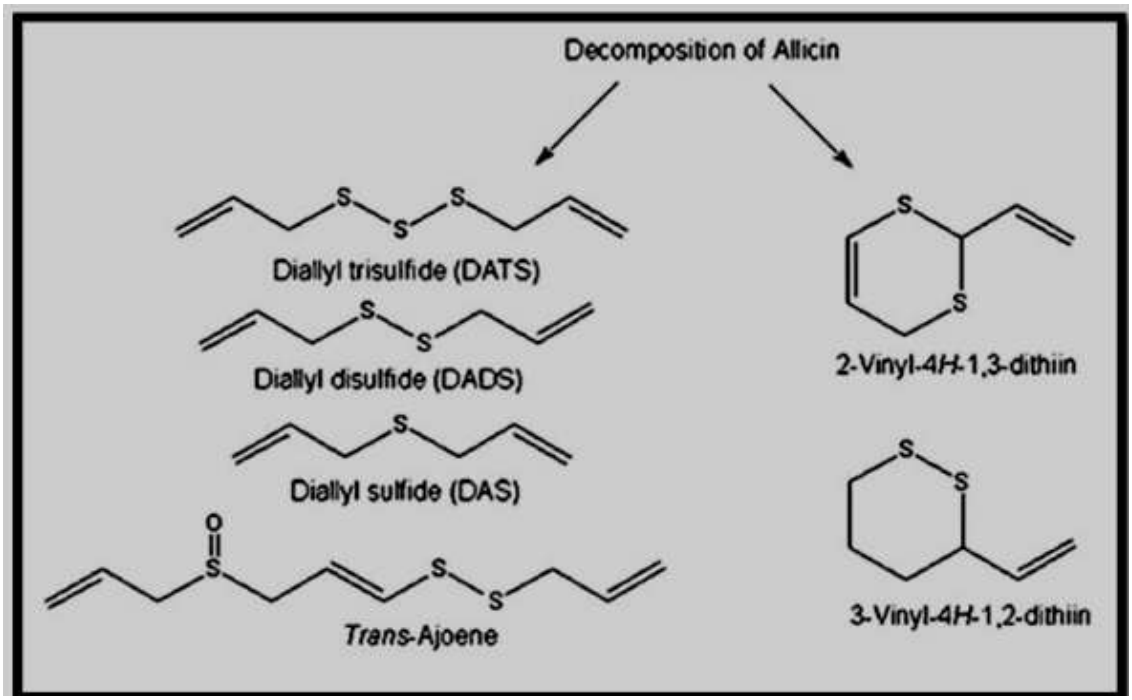


Figure (1.4): Organsulfur constituents in garlic

1.2.6 Traditional uses

Garlic used traditionally as expectorant, for the treatment of asthma, antipyretic, sedative ,aphrodisiac,diuretic, emmenagogue, carminative ,to promote hair growth, for the treatment of dyspepsia , urinary and respiratory tract infections and cardiac complains (Al-Snafi,2013).

1.2.7 Pharmacological aspects

Antimicrobial actions:

Principals from garlic have been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. They also modulate the cardiovascular and immune system as well as having antioxidant and anticancer properties (Harris *et al*,2001).

Some *Allium sativum* pharmacological uses are detailed as follows:

1.2.7.1 Antibacterial properties

Garlic has been used for centuries in various societies to combat infectious disease. In India, garlic has been used to prevent wound infection and food spoilage (Arora and Kaur,1999). In Ireland, at the turn of the twentieth century garlic was used to combat pulmonary infection (Delaha and Garagusi,1985). During World War I garlic was used to alleviate intestinal infections in soldiers stationed in the Balkans. Historically it is believed that Louis Pasteur first described the antibacterial effect of garlic 'juices' in 1858, although no reference is available. More recently garlic has been proven to be effective against a plethora of gram-positive, gram-negative and acid-fast bacteria. These include *Pseudomonas ,Proteus ,Staphylococcus Aureus* (Cavallito and Bailey, 1944), *Escherichia coli*, *Salmonella* (Johnson and Vaughn,1969), Klebsiella (Jezowa *et al*,1966), *Micrococcus , Bacillus subtulis* (Sharma *et al*,1977), Clostridium (De Witt *et al*,1979) Mycobacterium (Delaha and Garagusi,1985) and Helicobacter (O'Gara *et al*,2000). It has been documented that garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful entero bacteria (Rees *et al*,1993). Inhibition observed in *E. coli* was more than 10 times greater than that seen in *Lacto bacillus* casei for the same garlic dose (Skyrme,1997).

Exactly why this differential inhibition should occur is not clear, but may be due to differing compositions of bacterial membranes and their permeability to allicin (Miron *et al*,2000). The antibacterial activity of garlic is widely attributed to allicin. This is supported by the observation that if stored at room temperature the antibacterial effectiveness of garlic extract is greatly reduced.

This reduction occurs to a much lesser extent if the extract is stored at 0–4°C, suggesting thermal instability of the active components. The intracellular effects of allicin are not well understood. It is known that allicin has sulfydryl modifying activity (Wills,1956), and as such is capable of inhibiting sulfydryl enzymes. Cysteine and glutathione counteract the thiolation activity of allicin, and on addition to the reaction mixture the antibacterial activity is reduced. Garlic extract and allicin have been shown to exert bacteriostatic effects on some vancomycin resistant enterococci. An inhibitory synergism was observed when used in combination with vancomycin (Jonkers *et al*,1999). It is thought that allicin modifies the sulfydryl groups on the enzymes of the TN 1546 transposon, which encodes vancomycin resistance, enhancing susceptibility to vancomycin.

1.2.7.2 Antifungal properties

Antifungal activity was first established in 1936 by Schmidt and Marquardt whilst working with epidermophyte cultures. Many fungi have proven susceptible, including *Candida, Torulopsis, Trichophyton Cryptococcus* (Fromtling and Bulmer,1978), *Aspergillus* (Hitokoto *et al*,1980), *Trichosporon* and *Rhodotorula* (Tansey and Appleton,1975). Garlic extracts were shown to decrease the oxygen uptake (Szymona,1952), reduce the growth of the organism, inhibit the synthesis of lipids, proteins and nucleic acids (Adetumbi *et al*,1986) and damage membranes (Ghannoum,1988). A sample of pure allicin was shown to be antifungal. Removal of the allicin from the reaction by solvent

extraction decreasesd the antifungal activity (Hughes and Lawson,1991). Activity has also been observed with the garlic constituents, diallyl trisulfide, against cryptococcal meningitis (Cai,1991), and ajoene, against Aspergillus (Yoshida *et al,* 1987). Again thiol addition to the test reduced the activity, suggesting the blocking of thiol oxidation by allicin.

Inhibition of respiratory activity is thought to be due to inhibition of succinate dehydrogenase. The adhesion of Candida is also greatly reduced in the presence of garlic extract (Ghannoum,1990). Again this effect is diminished by the addition of thiol compounds. The addition of Ajoene to some fungal growth mixtures, including *Aspergillus Niger*, *C. albicans and Paracoccidiodes* (Reimers *et al*,1993), has resulted in inhibition at concentrations lower than that experienced with allicin.

Studies with aged garlic extract (with no allicin or allicin-derived constituents) showed no in vitro activity. However, when given to infected mice the number of organisms that were seen was reduced by up to 80% (Tadi *et al*,1990).

1.2.7.3 Antiviral properties

In comparison with the antibacterial action of garlic very little work has been done to investigate antiviral properties. The few studies have reported that garlic extract shows in vitro activity against influenza A and B (Fenwick and Hanley, 1985), cytomegalovirus (Meng *et al*,1993; Nai-lan *et al*,1993), rhinovirus, HIV, herpes. simplex virus (Tsai *et al*,1985) and (Weber *et al*,1992) , viral pneumonia and rotavirus. Allicin, diallyl trisulfide and ajoene have all been shown to be active(Hughes *et al*,1989; Weber *et al*,1992). In the case of HIV, it is thought that ajoene acts by inhibiting the integrin-dependent processes (Tatarintsev *et al,* 1992). Allyl alcohol and diallyl disulfide have also proven effective against HIV-infected cells (Shoji et al,1993). No activity has been observed with alliin

or S-allyl cysteine; it appears that only allicin and allicin-derived substances are active.

1.2.7.4 Anti-cancer Activity

In looking at multiple studies, (Powolny and Singh,2008). conclude that organosulfur compounds such as DAS, DADS, and DATS act by arresting the cell cycle of cancerous cells. Data from (Miroddi *et al*,2011) and (Omar and Al-Wabel,2010) support this, showing that these allyl derivatives act as antioxidants and arrest the cell cycle. DATS was found to have the most significant role by (Powolny and Singh,2008), and is even a potential skin cancer fighting compound (Wang *et al*, 2010). More specifically, garlic acts as anti-inflammatory agent by altering cytokines and inhibiting NF-kB activity in surrounding tissues (Keiss *et al*,2003). In a study using human promyeloleukemic cells, (Dirsch *et al,1998)* found that ajoene prompted apoptosis in cancerous cells but not healthy ones; this might be due to peroxide production. In an in-vitro study involving rats, (Jastrzebsk *et al*,2007) found that raw garlic had the strongest antioxidant activity. (Tsai *et al*,2012) reviewed animal and cell studies and found an inverse correlation between consumption of garlic and presence of cancerous cells, suggesting it has anticancer effects.

1.2.7.5 Anti-tumor Effects

Garlic extracts used as inhibition of cancer development in the presence of known tumor promoters and Sulphurous components present in garlic are believed to be liable to evade the developing of cancerous cells in stomach, liver, and other organs of human as described by(Pendbhaje *et al*.2011).

1.2.7.6 Anti- Diabetic activity

A number of animal studies support the effectiveness of garlic in reducing blood glucose in streptozotocin-induced as well as alloxan -induced diabetes mellitus in rats and mice. Most of the studies showed that garlic can reduce blood glucose level in diabetic mice rats and rabbits. One Iranian study evaluated oral administration of garlic extract for 14 days on the level of serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, in normal and streptozotocin-induced diabetic rats. Administrations of the garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase and alanine amino transferase levels, while increased serum insulin in diabetic rats but not in normal rats ($p<0.05$). Interestingly, a comparison was made between the action of garlic extract and glibenclamide, a well -known antidiabetic drug. The antidiabetic effect of the garlic was more effective than that observed with glibenclamide (Eidi *et al*,2006). Unfortunately, the effect of garlic on humans with diabetes is not well studied as is fraught with conflicting results (Zhang *et al*,2001).

1.2.7.7 Alzheimer' Disease Protective activity

Known for its neuro protective abilities in vitro (Peng *et al*,2002), aged garlic has been looked to for multiple benefits that some researchers believe may address a number of underlying mechanisms which contribute to the classic Alzheimer beta-amyloid plaque. According to one author, garlic: "is expected to produce cumulative benefits and exhibit enhanced neuro protection by virtue of being "natural statin", "natural NSAID", "natural anti-oxidant", "natural anti-apoptotic agent" and "memory enhancer", a combination of many single-ingredient synthetic pharmaceutical drugs currently used for Alzheimer's therapy, only with least adverse effects (Chauhan,2006)." Unfortunately, there is a dearth of clinical studies showing of aged garlic extract in relation to

Alzheimer's pathology, except for reports showing improved behavior in senescence accelerated mice after garlic treatment (Nishiyama *et al*,2001). Given the multiple-mechanistic possibilities and minimal risk associated with its use, garlic seems a prudent recommendation for prevention and treatment. Since aged garlic is best studied in relation to Alzheimer's it may be the best form to employ.

1.2.7.8 Liver Protective/Detoxification Effects

It has been reported that aged Garlic Extract have liver protective effects. It has demonstrated in vivo from the liver toxins: carbon tetrachloride, paracetamol (acetaminophen) and bromobenzene (Amagase,2006). It has been shown to inhibit both the formation and bioactivation of liver carcinogenic nitrosamines and has prevented the mutagenic effects of aflatoxin B1as pronounced (Borek,1998).

1.2.7.9 Antiprotozoal properties

Very little work has been done to establish the mechanisms of effects of garlic on protozoa. Several studies have shown the extract to be effective against a host of protozoa including Opalina ranarum, O. dimidicita, Balantidium entozoon, Entamoeba histolytica, Trypanosomes, Leishmania, Leptomonas and Crithidia (Reuter *et al*,1996).

Early work establishing garlic as a possible treatment for giardiasis was carried out in the former Soviet Union (Kramarenko,1951). (Bolton *et al*,1982) noted that garlic was often used for gastrointestinal complaints whilst investigating the historical use of garlic. By this time it had already been established that it had antibacterial, antifungal and antiviral properties. This prompted an investigation into its possible use as an antiprotozoal against Entamoeba histolytica (Mirelman *et al*,1987).

Inhibitory activity was noted with crude extract at 25 μg ml^{-1} and the lethal dosage was established as approximately 50 μg ml^{-1}.Encouraged by these results, a clinical trial was carried out on patients that had giardiasis (Soffar and Mokhtar,1991). Garlic was established as an antigiardial, removing the symptoms from all patients within 24 h and completely removing any indication of giardiasis from the stool within 72 h at a dosage of 1 mg ml^{-1} twice daily aqueous extract or 0.6 mg ml^{-1} commercially prepared garlic capsules. No in vitro calculations were possible, as the workers could not culture the protozoa in vitro.

Under certain conditions allicin, a major component of garlic shown to be antibacterial, degrades to diallyl trisulfide. This chemical is more stable than the extremely volatile allicin and is easily synthesised. In China it is commercially available as a preparation called Dasuansu and as been prescribed for *Entamoeba histolytica* and *Trichomonas vaginalis* infections (Lang and Zhang,1981). The antigiardial activity of this garlic component was assessed (Lun *et al*,1994). It gave an IC50 of 14 μg ml^{-1} and was shown to affect cell morphology.

Allicin, ajoene and organosulfides from garlic are also effective antiprotozoals. It has been suggested that microbial cells are more affected than human cells because they do not have an intracellular thiol content adequate to counterbalance the thiol oxidation by allicin and allicin-derived products. Ajoene has been shown to inhibit phosphatidyl choline synthesis in trypanosomes (Urbina *et al*,1993).

1.3 Objectives of the study

The aims of this study were to assess antimicrobial properties of Garlic and to investigate the chemical composition of its extract using GC/MS and ICP-OES. In order to achieve Objectives, The following Specific objectives are address:

- To extract the garlic components with different extraction methods; soxhlet and maceration extraction.
- To analyze the garlic extract using GC-MS.
- To determination the elements in extract using ICP-OES.
- To investigate the effectiveness of garlic extracts as Antimicrobial agent.

Chapter two
Materials and method

2. Materials and Methods

2.1Materials

2.1.1Plant material

2.1.1.1 Dry garlic

Fresh garlic was obtained from local market. The outer skin of the garlic cloves was peeled off. The garlic cloves were cut into small cubes using a kitchen knife It was then shade dried at ambient temperature. The product was grinded in a grinder prior to extraction.

2.1.1.2 Fresh garlic

Fresh garlic was obtained at local market , The outer skin of the garlic cloves was peeled off. Then was grinded in a grinder prior to extraction.

2.1.2. Chemicals

Ethanol 95.5% , Nitric acid 70.5% , sulfuric acid 98% , deionizer water , distilled water.

2.1.3 Instrumentations and Equipments

Gas Chromatography Mass Spectrometer, GC-2010 Plus , Shimadzu, Japan.

Plasma Atomic Emission spectrometer , ICPE-9000 , Shimadzu, Japan.

Soxhlet extractor , Analytical balance, Buchner Funnel , Rotary evaporator

Conical flasks, volumetric flask, Shaker, Paraffin Foil.

2.2Methods

2.2.1 Extraction methods

2.2.1.1 Preparation of Ethanol Soxhlet extract

Garlic plant powder(90g) using Soxhlet extractor was successively extracted with ethanol. The solvent was carefully evaporated from extract and the extractability was determined.

2.2.1.2 Preparation of Ethanol Maceration extract (dry garlic)

100 g finely ground powder of garlic transferred into a conical flask. a solution of 500 ml (95.5%) ethanol was added. The content was placed in a shaker for 24 hours at room temperature . The extract was filtered through Wagner funnel . The clear extract was evaporated through the rotary vacuum , the weight was recorded and % yield was calculated**.**

2.2.1.3 Preparation of Ethanol Maceration extract (fresh garlic)

278.06g of fresh garlic was transferred into a conical flask A solution of 1000 ml (95.5%) ethanol was added. The content was placed in a shaker for 24 hours at room temperature . The extract was filtered through Wagner funnel. The clear extract was evaporated through the rotary vacuum, The weight was recorded and % yield was calculated**.**

2.2.2 Anti-microbial activity

2.2.2.1 Bacterial microorganism materials:

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 -ve bacteria)

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA

2.2.2.2 Fungal microorganisms materials:

Candida albicans	ATCC 7596

2.2.2.3 Preparation of the test organisms:

2.2.2.3.1 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 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 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 (Miles and Misra, 1938). 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.

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

2.2.2.3.2 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 100 ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.2.2.3.3 Testing of antibacterial susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS,1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts.The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.
The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active.

2.2.3 Method of ICPE Analysis

2.2.3.1 preparation of garlic Maceration extract

1ml of garlic Maceration extract transferred into test tube ,10 ml of nitric acid 70.5% and 10 ml of sulfuric acid 98% was added. Then The content was placed in a digestion for 24 hours, then transferred into a volumetric flask 25ml. The volume was completed with deionizer water.

2.2.3.2 preparation of Blank

10 ml of nitric acid 70.4% and 10 ml of sulfuric acid 98% placed into volumetric flask 25 ml. The volume was completed with deionizer water.

2.2.4 Method of GC/MS Analysis

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra,Shimadzu Company, japan).

The capillary column was (Rtx-5ms-30m×0.25 mm×0.25µm). 10µ/l of the sample was diluted by 1ml of ethyl alcohol, then 1µ/L of oil solution was injected by using split mode, instrument operating in EI mode at 70eV. Helium as the carrier gas passed with flow rate 1.80 ml/min, the temperature program was started from $80C^0$ with rate $7C^0$/min to $180C^0$ then the rate was changed to $10C^0$/ min until reaching $300C^0$ as final temperature degree. The injection port temperature was $250C^0$, .the ion source temperature was $200C^0$ and the interface temperature was $250C^0$.The sample was analyzed by using scan mode in the range of m/z 35-400 charges to ratio and the total run time was 22 minutes. Identification of components for the sample was achieved by comparing the retention index and mass fragmentation patterns with this available in the library of the National Institute of Standards and Technology (NIST). Then the results were recorded.

Chapter three
Results and discussions

Results and Discussion

3.1 Extraction

Tables (3.1) and (3.2) summarize the weights, percentage yeild and consistency of different methods (maceration and soxhlet) respectively.

3.1 Maceration extract

Table (3.1): Weight, % yield and properties of Maceration extract

Extract		Solvent	Weight (gm)	Yield(%)	Consistency
1	Dry Garlic	Ethanol	9.5	9.5	Brown-Crude oil
2	Fresh garlic	Ethanol	91.2	32.79	Yellow-oil

Figure (3.1): Maceration Extraction of fresh Garlic

Figure (3.2): Maceration Extraction of dry Garlic

Figure (3.3): Maceration Extract (fresh and dry) Garlic.

3.2 Soxhlet extract

Table (3.2): Weight, % yield and properties of soxhlet extract

NO	Extract	Weight (gm)	Yield(%)	Consistency
1	Ethanol	54.05	60.05	Darkly-Brown paste

Figure (3.4): Soxhlet Extraction

The results of extraction with ethanol using different methods in tables (3.1) and (3.2) show that soxhlet extract gave the highest value of total weight in percentage yield 60.05%, followed by Maceration extract of fresh garlic 32.79%, and finally Maceration extract of dry garlic 9.5% .

The difference in percentage and consistency of extracts clarify that choice of method to be used for the extraction of garlic components depends on what kind of extract we need which depends on extract uses. for example: aged garlic extract in relation to Alzheimer' Disease Protective activity. And the antibacterial activity attribute to allicin and allicin derivatives (DATS, DADS, DAS).

3.3 Evaluation of anti-microbial activity of Garlic extracts

The extracts of Garlic were tested for anti-microbial activity against *Bacillus subtilis* and *Staphylococcus aureus* as (Gram + ve bacteria). *Escherichia coli* and *Pseudomonas aeruginosa* as (Gram -ve bacteria**)** and anti-fungal against *Candida albicans*.

Table (3.3):Evaluation of antimicrobial properties of Garlic extracts

Extract	Conc.mg/L	Ec	Ps	Sa	Bs	Ca
Ethanol Soxhlet	100	10	9	9	10	-
Ethanol Maceration	100	-	12	-	-	12

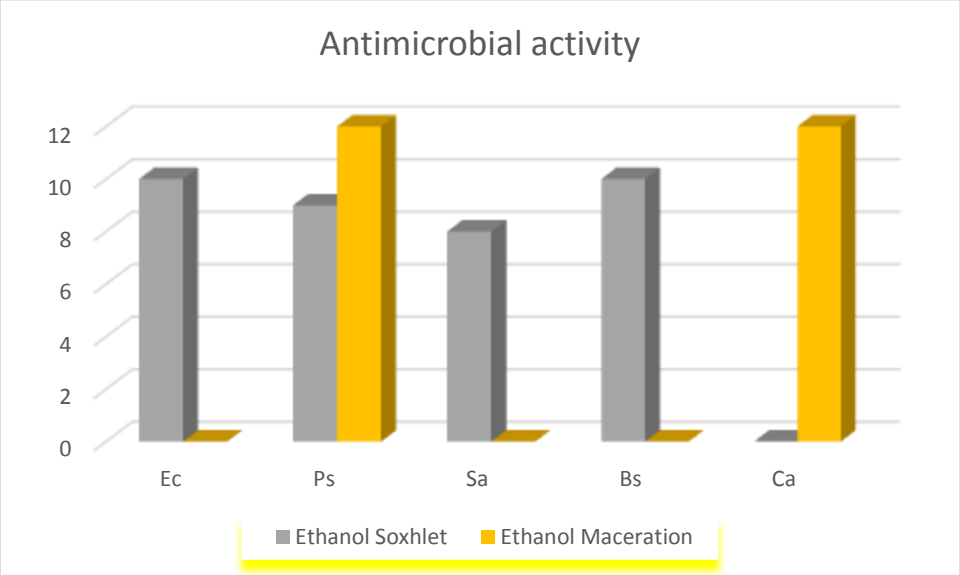


Figure (3.5): Antibacterial activity of garlic extracts

Key:

X axis: Ec = *Escherichia coli*. Ps = *Pseudomonas aeruginosa*, Bs = *Bacillus subtilis*, Sa = *Staphylococcus aureus*, Ca = *Candida albicans*.

Y axis: Inhibtion zone.

3.3.1 Inhibition zones of garlic soxhlet extract:

Plate (3.1): Inhibition zone of garlic soxhlet extract against Escherichia coli

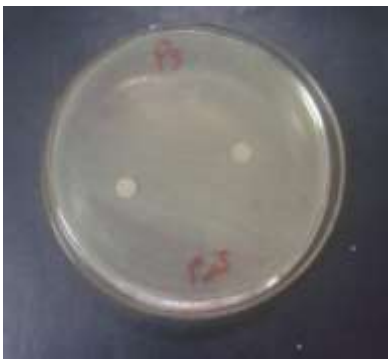


Plate (3.2): Inhibition zone of garlic soxhlet extract against Pseudomonas aeruginosa

Plate (3.3): Inhibition zone of garlic soxhlet extract against Staphylococcus aureus

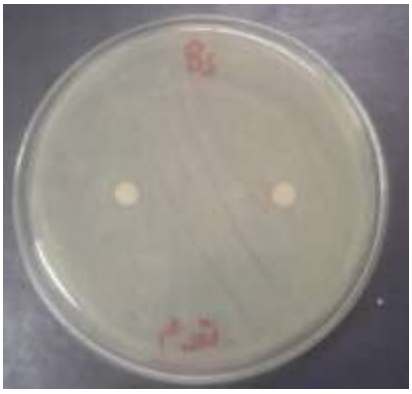


Plate (3.4): Inhibition zone of garlic soxhlet extract against Bacillus subtilis

Plate (3.5): Inhibition zone of garlic soxhlet extract against Candida albicans

3.3.2 Inhibition zone of garlic Maceration extract against:

Plate (3.6): Inhibition zone of garlic maceration extract against Escherichia coli

Plate (3.7): Inhibition zone of garlic maceration extract against Pseudomonas aeruginosa

Plate (3.8): Inhibition zone of garlic maceration extract against Staphylococcus aureus

Plate (3.9): Inhibition zone of garlic maceration extract against Bacillus subtilis

Plate (3.10): Inhibition zone of garlic maceration extract against Candida albicans

The antibacterial activity results were expressed in term of the diameter of zone of inhibition and < 9mm zone was considered as inactive; 9-12 mm as partially active; while13-18mm as active and >18mm as very active.

The results of antimicrobial activity show that *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus*, have no susceptibility to maceration extract and partially susceptibile to soxhlet extract. The fungus *Candida albicans* and bacteria *Pseudomonas aeruginosa* were moderately susceptible to ethanolic maceration extract with diameter of inhibition zone (12mm).

The results of antimicrobial activity show that garlic exerts has a differential inhibition for the same dose. why this differential inhibition should occur is not clear, but may be due to differing compositions of bacterial membranes and their permeability to sulfur compounds.

The antibacterial activity of garlic is widely attributed to allicin and allicin derivatives This is supported by the observation that if stored at room temperature the antibacterial effectiveness of garlic extract is greatly reduced because allicin (a major component of garlic shown to be antibacterial) is extremely volatile. Its degrades to diallyl trisulfide. This chemical is more stable than allicin.

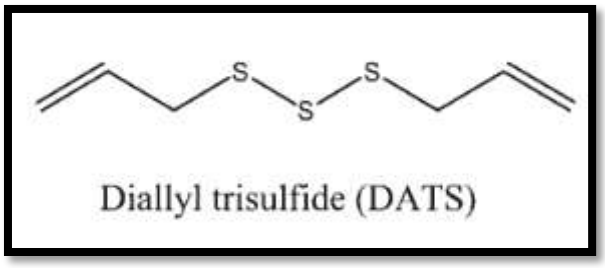
3.4 Analysis of maceration Extract by GC/MS

Table (3.4): Results of GC/MS

Peak Report TIC

Peak	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.109	4.067	4.158	4158575	7.50	2422450	14.74	1.72		Acetic acid, cyano-
2	4.190	4.158	4.225	1521676	2.75	925489	5.63	1.64	V	1-Tridecanamine
3	4.635	4.592	4.692	1078333	1.95	568592	3.46	1.90		$H_2N(CH_2)_7NH_2$
4	4.750	4.692	4.808	1394414	2.52	719206	4.38	1.94	V	1-Octanamine
5	6.378	6.342	6.483	10144096	18.30	4910626	29.88	2.07		Trisulfide,di-2-propenyl
6	6.630	6.483	6.675	1012228	1.83	212912	1.30	4.75	V	1-H-Azepin-1-amine,hexahydro
7	7.532	7.433	7.750	30980934	55.89	4670867	28.42	6.63		Sucrose
8	12.096	12.050	12.142	1199850	2.16	579433	3.53	2.07		Leucine
9	15.556	15.517	15.617	531424	0.96	212217	1.29	2.50		1-Octadecanamine,N-methyl
10	16.565	16.533	16.600	302151	0.55	164183	1.00	1.84		1-Octadecanamine,N-methyl
11	17.217	17.183	17.392	829106	1.50	208802	1.27	3.97		Piperazine, 2-methyl-
12	17.408	17.392	17.533	505991	0.91	134833	0.82	3.75	V	
13	17.558	17.533	17.608	601550	1.09	290710	1.77	2.07	V	Piperazine, 2-methyl-
14	18.187	18.150	18.233	487227	0.88	194213	1.18	2.51		D-Alamine
15	19.417	19.375	19.475	686205	1.24	219955	1.34	3.12		1-Octadecanamine,N-methyl
				55433760	100.00	16434488	100.00			

The results of GC/MS Analysis show that it contains Trisulfide,di-2-propenyl and sucrose as target content , acetic acid , amino acids.



Diallyl trisulfide (DATS)

The Trisulfide,di-2-propenyl also known as allitridin , allyl trisulfide or DATS (diallyl trisulfide) is a major component of the essential oil of garlic belongs to the class of organic compounds known as organic trisulfide with the general formula RSSSR` ( R,R` = alkyl, aryl) , These sulfur compounds confer antimicrobial property.

3.5 Analysis of maceration Extract by ICP-OES

The results of ICP Analysis show that it contains:

Mainaly : Sulfur 680ppm , phosphorus 2.1ppm , iodine 1.7ppm. Beside other minor minerals.

3.6 Conclusion and Recommendations

3.6.1 Conclusion

The results from this study on *Allium satvium.L* have led to the following points:

- The results of GC/MS Analysis of garlic extract revealed that it contains Trisulfide and sucrose as target content , acetic acid , amino acids.
- The ICPE Analysis of garlic extract showed that it contains:
  Mainaly : sulfur , phosphorus ,iodine. Beside other minor minerals.
- The antimicrobial activity obtained in this study is indicated that ethanolic soxhlet extract of *Allium satvium.L* moderately effective as antibacterial and no effect on *Candida albicans* fungus.
- Most of tested bacterial strains were not susceptible to ethanolic maceration extract except *Pseudomonas aeruginosa*.
- The fungus *Candida albicans* was moderately susceptible to ethanolic maceration extract.
- The *Allium satvium.L* plant rich in sulfur element content which make the sulfur compounds that confer antibacterial property.

3.6.2 Recommendations

According to the results it recommended that:

- The results of this study are promising because ethanolic extracts of *Allium satvium.L* considerable a moderate antimicrobial activity.
- The study also supports the use of *Allium satvium.L* not only as folk medicine but also as agent to prevent or control some diseases.
- A *Allium satvium.L* extracts warranting more investigations, fractionations and purification for active antimicrobial compounds.
- It is need for modern equipment to extract, purify the plant constituent to enable researcher to accomplish their goal successfully and obtain reliable results.
- On going research on pharmacology is highly recommended to synthesize antimicrobial drugs for treatment the multiple infections. And to solve the problems of antibiotics side effects and the resistance to antibiotics.

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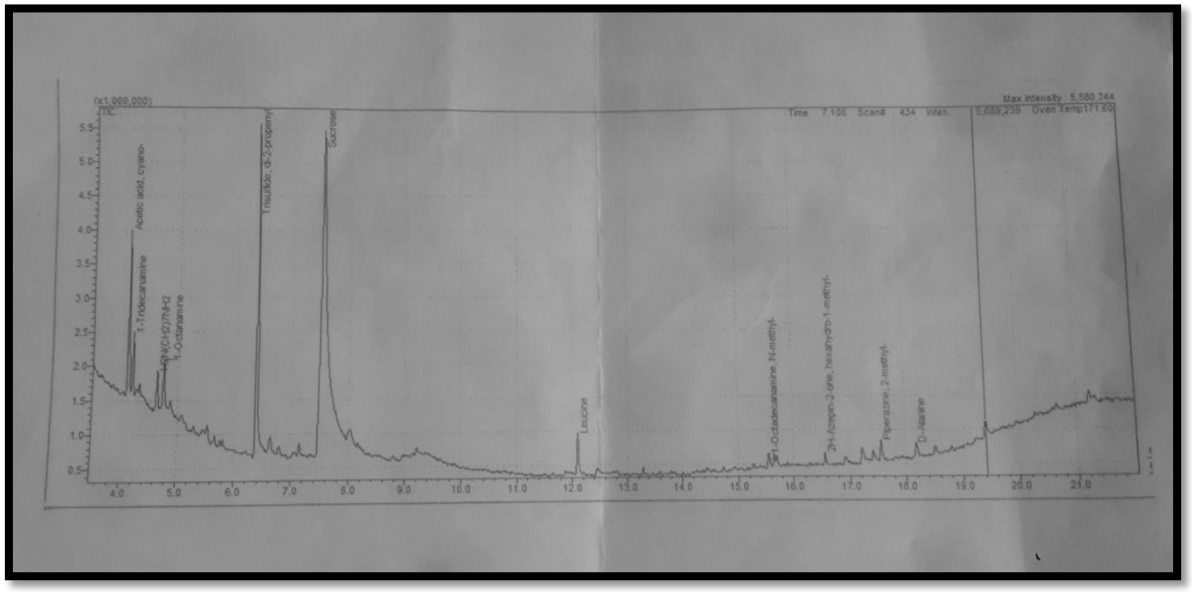
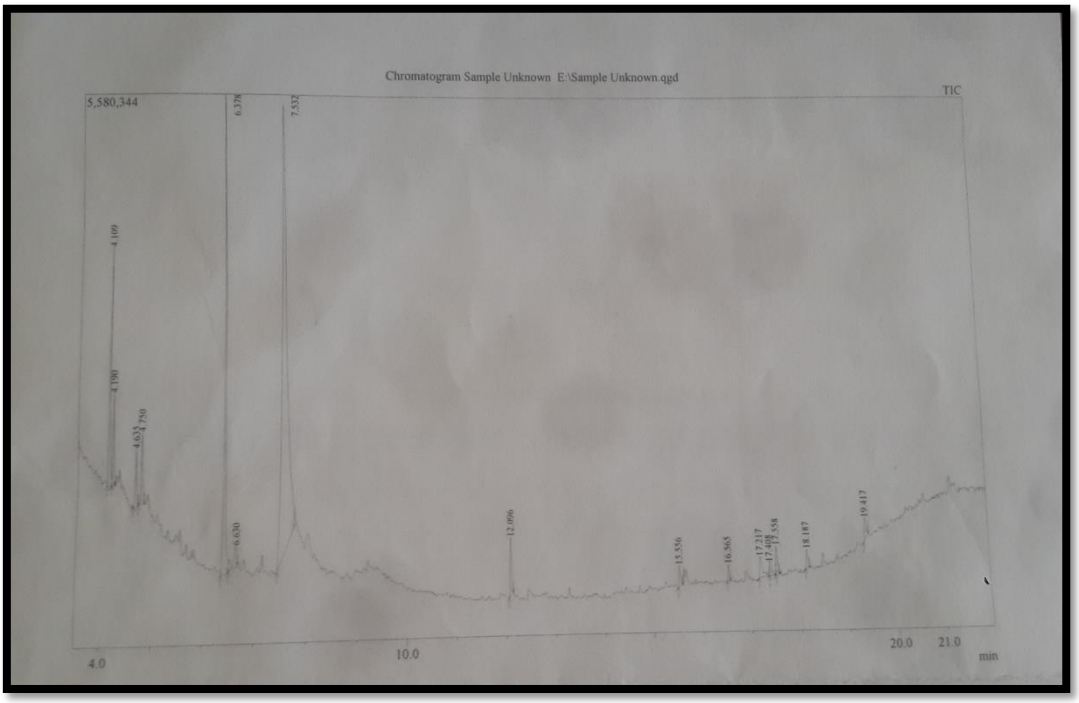
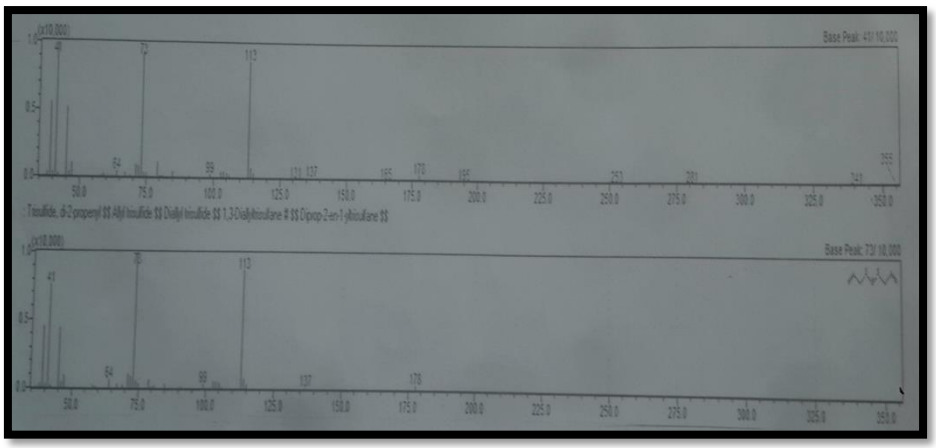
Appendixes

Appendixes:

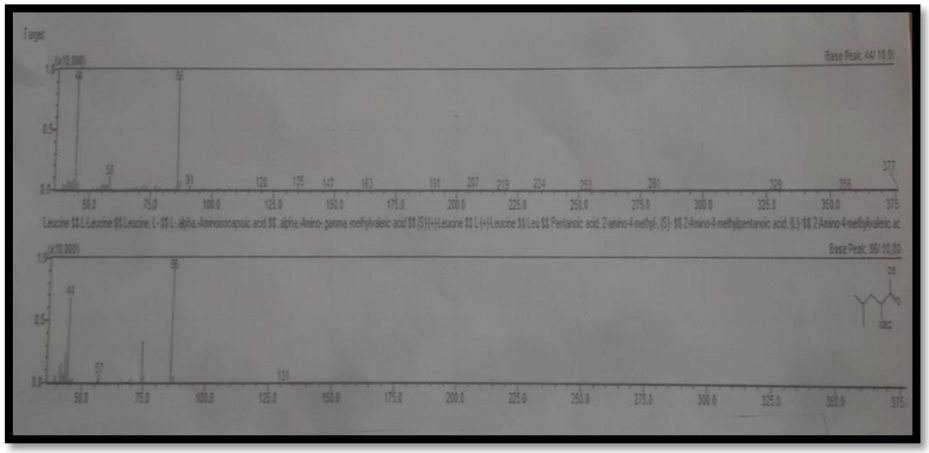




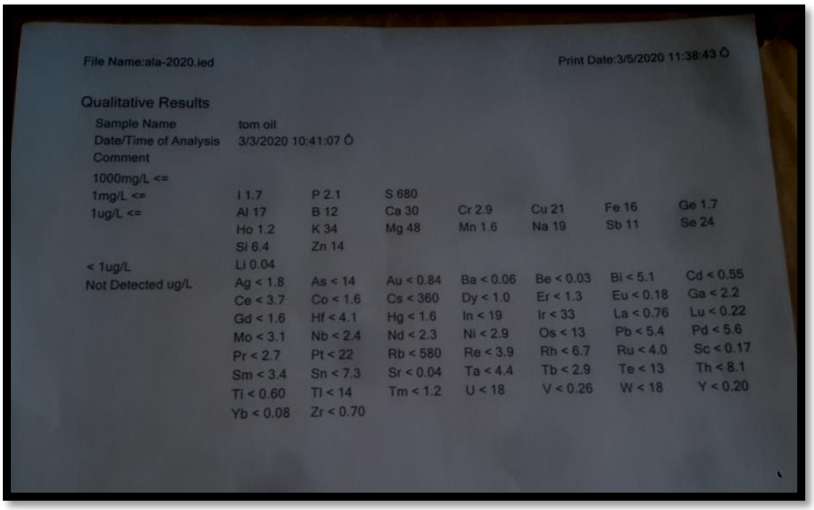
(1) Chromatogram Analysis of maceration Extract by GC/MS



(2) Diallyl trisulfide



(3) Leucine



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Print Date:3/5/2020 11:38:43 Ô

Qualitative Results

Sample Name	tom oil						
Date/Time of Analysis	3/3/2020 10:41:07 Ô						
Comment							
1000mg/L <=							
1mg/L <=	I 1.7	P 2.1	S 680				
1ug/L <=	Al 17	B 12	Ca 30	Cr 2.9	Cu 21	Fe 16	Ge 1.7
	Ho 1.2	K 34	Mg 48	Mn 1.6	Na 19	Sb 11	Se 24
	Si 6.4	Zn 14					
< 1ug/L	Li 0.04						
Not Detected ug/L	Ag < 1.8	As < 14	Au < 0.84	Ba < 0.06	Be < 0.03	Bi < 5.1	Cd < 0.55
	Ce < 3.7	Co < 1.6	Cs < 360	Dy < 1.0	Er < 1.3	Eu < 0.18	Ga < 2.2
	Gd < 1.6	Hf < 4.1	Hg < 1.6	In < 19	Ir < 33	La < 0.76	Lu < 0.22
	Mo < 3.1	Nb < 2.4	Nd < 2.3	Ni < 2.9	Os < 13	Pb < 5.4	Pd < 5.6
	Pr < 2.7	Pt < 22	Rb < 580	Re < 3.9	Rh < 6.7	Ru < 4.0	Sc < 0.17
	Sm < 3.4	Sn < 7.3	Sr < 0.04	Ta < 4.4	Tb < 2.9	Te < 13	Th < 8.1
	Ti < 0.60	Tl < 14	Tm < 1.2	U < 18	V < 0.26	W < 18	Y < 0.20
	Yb < 0.08	Zr < 0.70					

(4) ICP results