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Faculty of Graduate Studies

Extraction, Characterization and Application of Essential oil from Native Palestinian Plants

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Dedication

Special dedication and thanks to my wonderful husband " Bilal" who was there step by step with me.

.

…..To my charming sons " Nidal, Mohannad and Mahmoud" .

….. To my lovely mum and great family ….. my brothers and sisters

…... To my sister- in law " Nihal"

…… To my friends and colleagues who supported and encouraged me, particularly " Diana Abu AL-Rub " the secretary of Qabatia Basic Girls' School. In addition to my faithful friend " Huda Abu Zaid".

….. To them and of everyone who wishes me the success and progress.

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Also I would like to present my thanks to the Head of Water resources Department for his help" Dr. Marwan Haddad"

الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Extraction, Characterization and Application of Essential oil from Native Palestinian Plants

أقر بأن ما شملته هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date **التاريخ**

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Extraction, Characterization and Application of Essential oil from Native Palestinian Plants

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Abstract

In this work, the effect of the extracted oils from three different plants(*Oleander*, *Rutagraveolens*, and *Basil*) were studied against insects such as *Sitophilus granaria*, *Mosquito*, and *Varroa mites* as it has been noticed recently that there's an increase in the use of chemically manufactured pesticides, which contributed to the pollution of the environment and human health. It's important to say that these extracted oils have some insecticidal activities.

These plants (*Oleander*, *Rutagraveolens*, and *Basil*) were selected since there are some previous studies showing activity of these extracts against some diseases, and showing antimicrobial and fungicidal activities. Therefore, in this study, the extracted oils have been tested as insecticides and insect repellent. They have been applied to *Sitophilus granaria*, *Mosquito*, and *Varroa mites* as these insects have harmful effects on the health and economy of human being.

The plants were collected from the NARC Center where they were collected through the flowering process and then taken to the laboratory to extract the oils. 800 g of each plant was taken and cut into small pieces , soaked with ethanol for 48 hours and then the solvent was filtered and

evaporated to get the extracted oil. Both TLC and GC-MS analysis were performed in order to identify the organic compounds.

In addition, The essential oil from these plants were tested against the above insects, obtained from the Faculty of Agriculture at An-Najah University. The extracted oils were tested as repellents for the *Sitophilus granaria* by placing them on filter-filled paper and leaving the paper slightly in the air to evaporate the solvent and then put the insects on it. These oils were then tested if they have some biocidal activity by the vaporization process.

The GC-MS analysis showed that the oleander and basil had a common compound number (VI)* which had insecticidal activity. Furthermore, the Ruta and Oleander had a common compound called (I)*. From the test conducted on the application of extracts, it was observed that oleander had 100% repellant activity against *Sitophilus granaria* followed by Ruta (98%), and Basil (95%) respectively. On the other hand, it was observed that of *Oleander* and *Ruta* essential oils showed the highest percent mortality against mosquito with nearly 100% mortality rate. However, the Basil had the highest mortality rate (80%) against mosquito. Other insects had lower mortality rates compared to the previous results. Further studies should be conducted in order to isolate and characterize the efficacy, the toxicity and the efficiency of the active ingredients as natural sources of insecticides.

* Table(3.1)and(3.2)

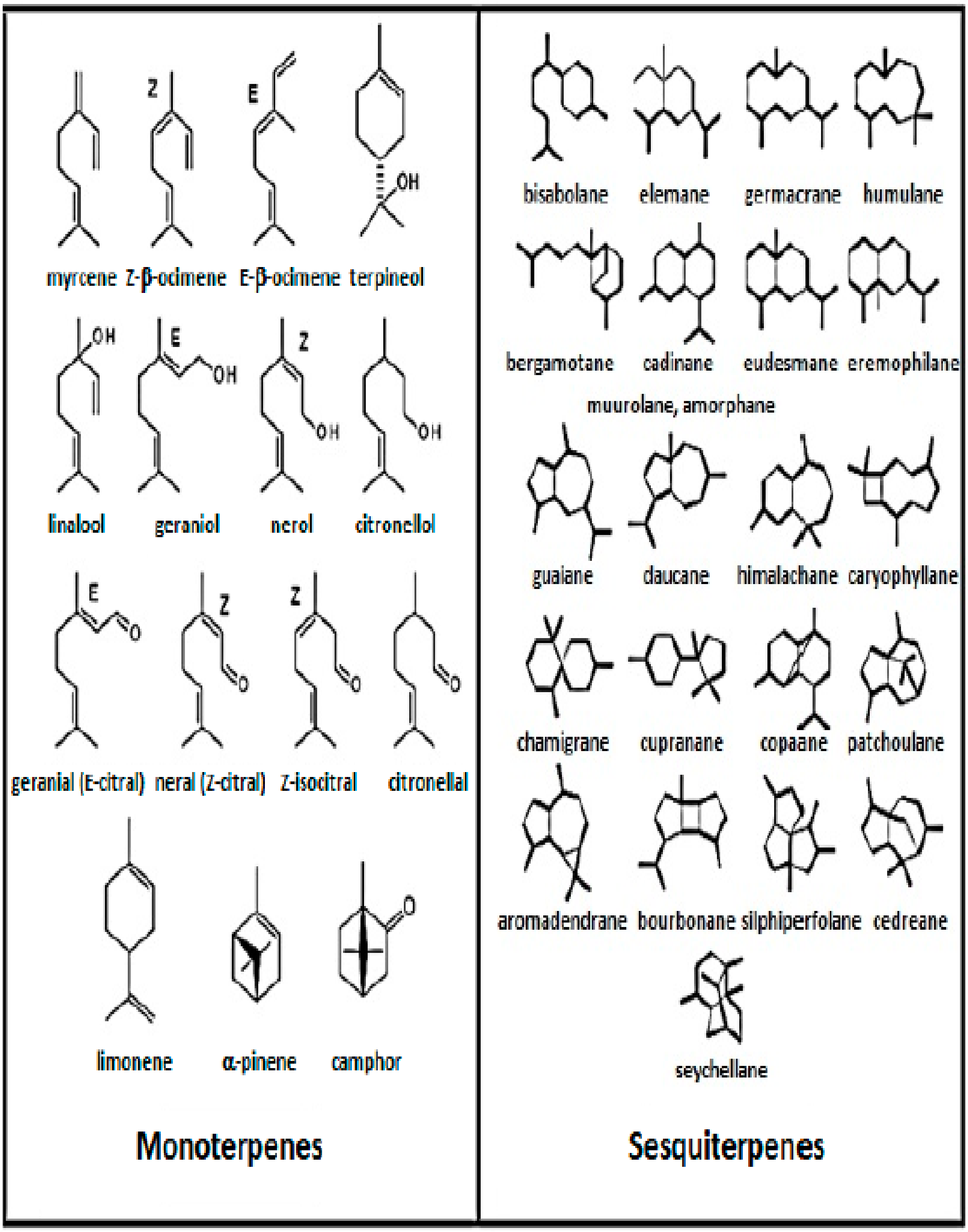
Chapter One

Introduction

There is a considerable interest in the use of natural products instead of synthetic insecticides. There is a great awareness of the need of new safe insecticides with a new mode of action. **[1]** Effectiveness and safeness over those chemical insecticides currently in use are to be improved. These chemicals are associated with dangers like environmental pollution, human toxicity, development of insects resistance and adverse effect on non-target organisms **[2]**

The term essential oil dates back to the sixteenth century and was derived from the drug Quinta essential, named by Paracelsus von Hohenheim of Switzerland [3]. Essential oils or “essences” owe their name to their flammability. Numerous authors have attempted to provide a definition of essential oils. The French Agency for normalizations: Agency Francoise de Normalization (AFNOR) gives the following definition (NF T 75-006): “The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicure of Citrus, or “dry”” distillation. The essential oil is then separated from the aqueous phase by physical means" [4]. This definition encompasses products obtained always from vegetable raw material, but using other extraction methods, such as using non-aqueous solvents or cold absorption. Thus, we can define two types of products hydrocarbons and oxygenated, These are further subdivided into sub-groups that are (Monoterpenes, Sesquiterpenes, Phenolics, Alcohols, Esters, Aldehydes, Ketones and Oxides) (table 1.1) [4].

Table (1. 1): 1. Structures of some terpenes



Common chemicals found in aromatic plant extracts.[5-8]

Essential oils are soluble in alcohol, ether, and fixed oils, but they are soluble partially in water. These volatile oils are generally liquid and colorless at room temperature. They have a characteristic odor, are usually liquid at room temperature and have a density less than unity, with the exception of a few cases (cinnamon(القرفة), sassafras(الغار), and vetiver (انجيل الهند)). They have a refractive index and a very high optical activity. These volatile oils contained in herbs are responsible for different scents that plants emit. They are widely used in the cosmetics industry, perfumery, and also aromatherapy [9]. The latter is intended as a therapeutic technique including massage, inhalations, or baths using these volatile oils. The last key will serve as chemical signals allowing the plant to control or regulate its environment (ecological role): attraction of pollinating insects, repellent to predators, inhibition of seed germination, or communication between plants (emission signals chemically signaling the presence of herbivores, for example). Moreover, essential oils extracts also possesses antifungal or insecticidal activity and deterrent activities. [3] All parts of aromatic plants may contain essential oils as follows:

- Flowers, including: orange, pink, lavender, and the (clove) flower bud or (ylang-ylang) bracts,
- Leaves, most often, including: eucalyptus, mint, thyme, bay leaf, savory, sage, pine needles, and tree underground organs, e.g., roots (vetiver),
- Rhizomes (ginger, sweet flag),
- Seeds (carve, coriander),

- Fruits, including: fennel, anise, Citrus epicures,
- Wood and bark, including: cinnamon, sandalwood, rosewood.

Essential oils are produced by various differentiated structures, especially the number and characteristics of which are highly variable. Essential oils are localized in the cytoplasm of certain plant cell secretions, which lies in one or more organs of the plant; namely, the secretory hairs or trachoma's, epidermal cells, internal secretory cells, and the secretory pockets. These oils are complex mixtures that may contain over 300 different compounds [10]. They consist of organic volatile compounds, generally of low molecular weight below 300. Their vapor pressure at atmospheric pressure and at room temperature is sufficiently high and so they are found partly in the vapor state [11, 12]. These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohols, aldehydes, and ketones offer a wide variety of aromatic notes, such as fruity ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal (salience), etc. Furthermore, essential oil components belong mainly to the vast majority of the terpene family. Many thousands of compounds belonging to the family of terpenes have so far been identified in essential oils [12], such as functionalized derivatives of alcohols (geraniol, -bisabolol), ketones (menthone, p-vetivone) of aldehydes (citronellal, sinensal), esters (-tepinyl acetate, cedryl acetate), and phenols (thymol). Essential oils also contain non-terpenic compounds bio generated by the phenylpropanoids Pathway, such as eugenol, cinnamaldehyde, and safrole

1.2 Repellent and Insecticidal Activity

Chemical control is an effective strategy used extensively in daily life. However, the widespread use of synthetic insecticides has led to many negative consequences, resulting in increasing attention to natural products. Among bio pesticides, botanical ones are experiencing a revival due to their eco-toxicological properties. Plants play pivotal roles in ecological systems. They may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Essential oils are among the best-known substances tested against insects. These compounds act as fumigants, contact insecticides, repellents and anti-feedants and may affect some biological parameters such as growth rate, life span and reproduction [13]. Risks and problems associated with the use of chemicals lead to increasingly stringent environmental regulation of pesticides. There is therefore an urgent need to develop safer, more environmental friendly and efficient alternatives that have the potential to replace synthetic pesticides and are convenient to use. In this context, screening of natural products has received the attention of researchers around the world. Many secondary plant metabolites are known for their insecticidal properties, and in many cases, plants have a history of use as home remedies to kill or repel insects. In recent decades, research on the interactions between plants and insects has revealed the potential use of plant metabolites for this purpose [13]. It is known that some chemical constituents of essential oils have insecticidal properties. In some studies, essential oils obtained from commercial sources were used. Specific compounds isolated from plant extracts or essential oils were tested for fumigation purposes (Fumigation is a method of pest control that uses a toxic gas to exterminate pests in an enclosed space[3]. The space is sealed to prevent the gas escaping to areas that are not being treated, for environmental and public safety, and to keep the gas at the required concentration for the appropriate time to be effective). In the search for alternatives to conventional pesticides, essential oils extracted from aromatic plants will be investigated. Their toxicities on pests will be tested. [3]. A screening of plants extracts from many species selected for insecticidal activity could lead to the discovery of new agents for pest control

Essential oils are fragrant essences of plants, usually volatile oils obtained from an odoriferous, single species of plant. Most essential oils are primarily composed of terpenes and their oxygenated derivatives and are obtained by steam distillation or solvent extraction of different parts of the aromatic plants including the buds, flowers, leaves, seeds, roots, stems, bark, wood, and rhizomes etc. Chemical constituents of volatile oils can be divided into two broad classes:

1.) Terpene derivatives formed via acetate mevalonic acid pathway and
2.) Aromatic compounds formed via shikimic acid phenyl propanoid route. [10]

Essential oils are used in perfumery, aromatherapy, cosmetics, medicine, incense, household cleaning products, and for flavoring food and drinks etc. Variations in climatic conditions, type of soil in which the plant was grown etc will produce natural variations in the relative distribution of components in essential oils. For example, the same oil extracted from plants grown at different locations can manifest quantitative change in the oil composition. [14,15] Essential oils are very complex; hundreds of components can be present, and most of the components which confer aroma or flavor may be present only at ppm levels.

In the search for alternatives to conventional insecticides, essential oils extracted from aromatic plants have been widely investigated. Their toxicities toward insects were of special interest since they are derived from natural product.

1.3 Plants :

1.3.1 ***Oleander Nerium oleander L*** **(الدفلة).** is an evergreen shrub reaching up to four meters in height (Fig1. 1) . It belongs to the family – Apocynaceae, is a shrub or occasionally tree distributed in tropical Asia Nerium oleander L. is cultivated worldwide as an ornamental plant. It is native to the Mediterranean region [10, 11] and found in Southern Europe and Southwest Asia. The plant is naturalizing very easily and in many areas the plant is sub-spontaneous.

Fig 1.1 *Nerium oleander L*

Oleander is one of the most poisonous plants in the world and contains numerous toxic compounds, many of which can be deadly to people, especially young children. The toxicity of Oleander is considered extremely high and it has been reported that in some cases only a small amount had lethal or near lethal effects. The most significant of these toxins are oleandrin and nerine, which are cardiac glycosides [15] .They are present in all parts of the plant, but are most concentrated in the sap, which can block out receptors in the skin causing numbness. It is thought that Oleander may contain many other unknown compounds that may have dangerous effects.

Oleander bark contains rosagenin, [fig 1.2] which is known for its strychnine-like effects. The Oleander is also known to hold its toxicity even dry. At the same time, it is believed that a 10-20 leaves consumed by an adult can cause an adverse reaction, and a single leaf could be lethal to an infant or a child.

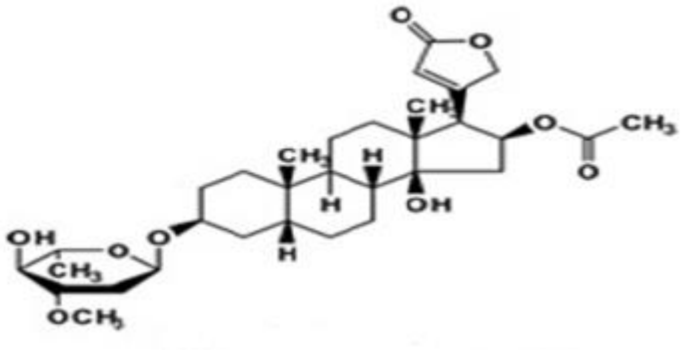


Figure (1. 2): Structure of rosagenin

Oleandrin is a toxic cardiac glycoside. Ingestion of Oleander can cause both gastrointestinal and cardiac effects. The gastrointestinal effects can consist of nausea and vomiting, excess salivation, abdominal pain, diarrhea that may or may not contain blood.

1.3.2 *Ruta graveolens* (الفيجن):

It is known as Rue or *R. graveolens* (figure 1.3), its extracts and essential oil are important areas in drug development with numerous pharmacological activities in many countries. For a long time *R. graveolens* has been used in traditional medicine for the relief of pain, eye problems, rheumatism and dermatitis. *R. graveolens* has recently been shown to have antibacterial, analgesic, anti-inflammatory, antidiabetic and insecticidal activities[2]. Rutin, quercetin, psoralen, methoxypsoralen, rutacridone epoxide and gravacridondiol are phytochemical compounds which are reported from this plant[12]. α-Pinene, limonene and 1,8-cineole were identified as the main monoterpene constituents for the essential oil of *R. graveolens* . Due to the easy collection of the plant and being widespread and also remarkable biological activities, this plant has become a medicine good in many countries especially in Mediterranean region. [16]

Figure (1. 3): Ruta graveolens (Rue).

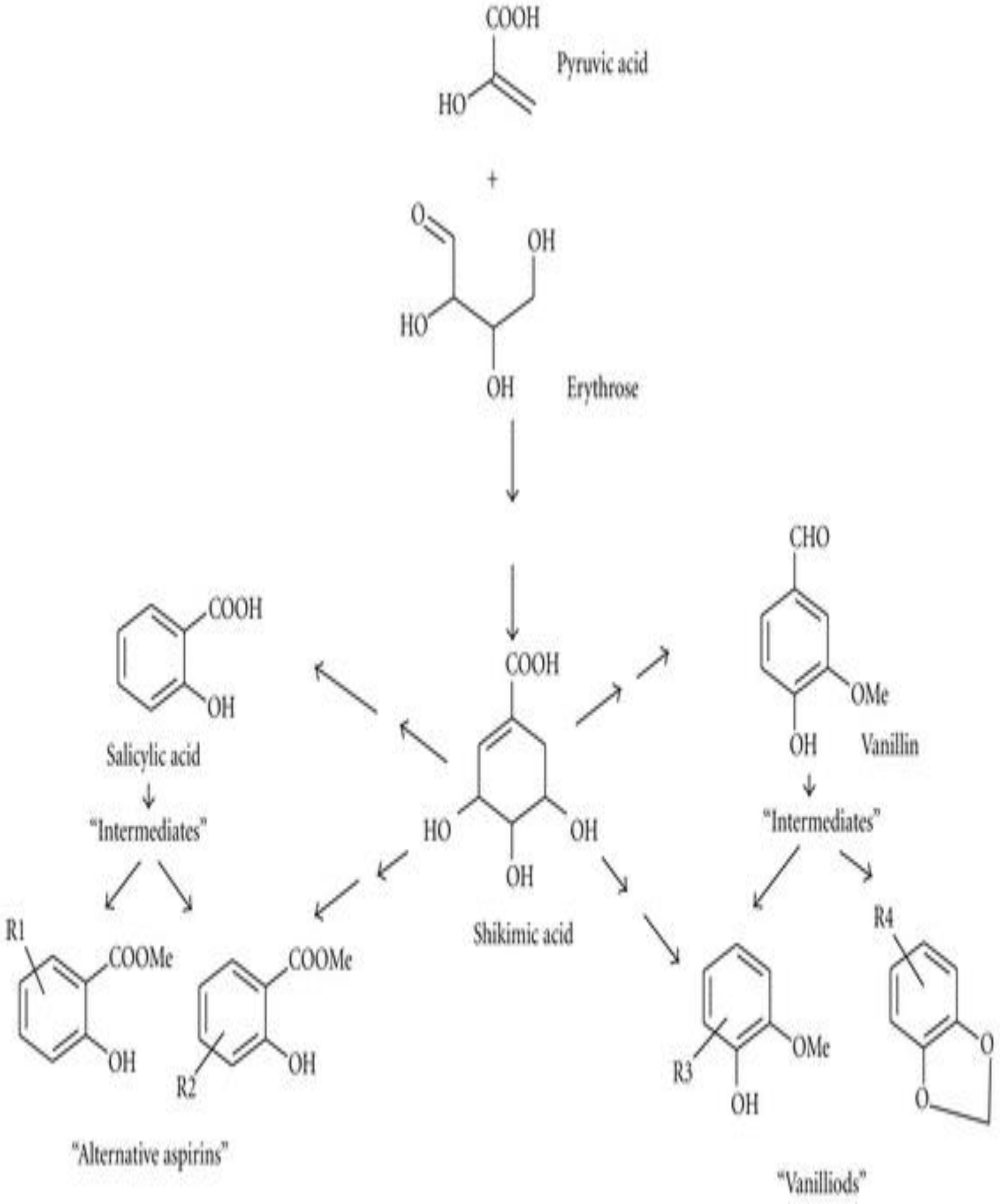
1.3.3 *Basil (Ocimum spp.)* (الريحان):

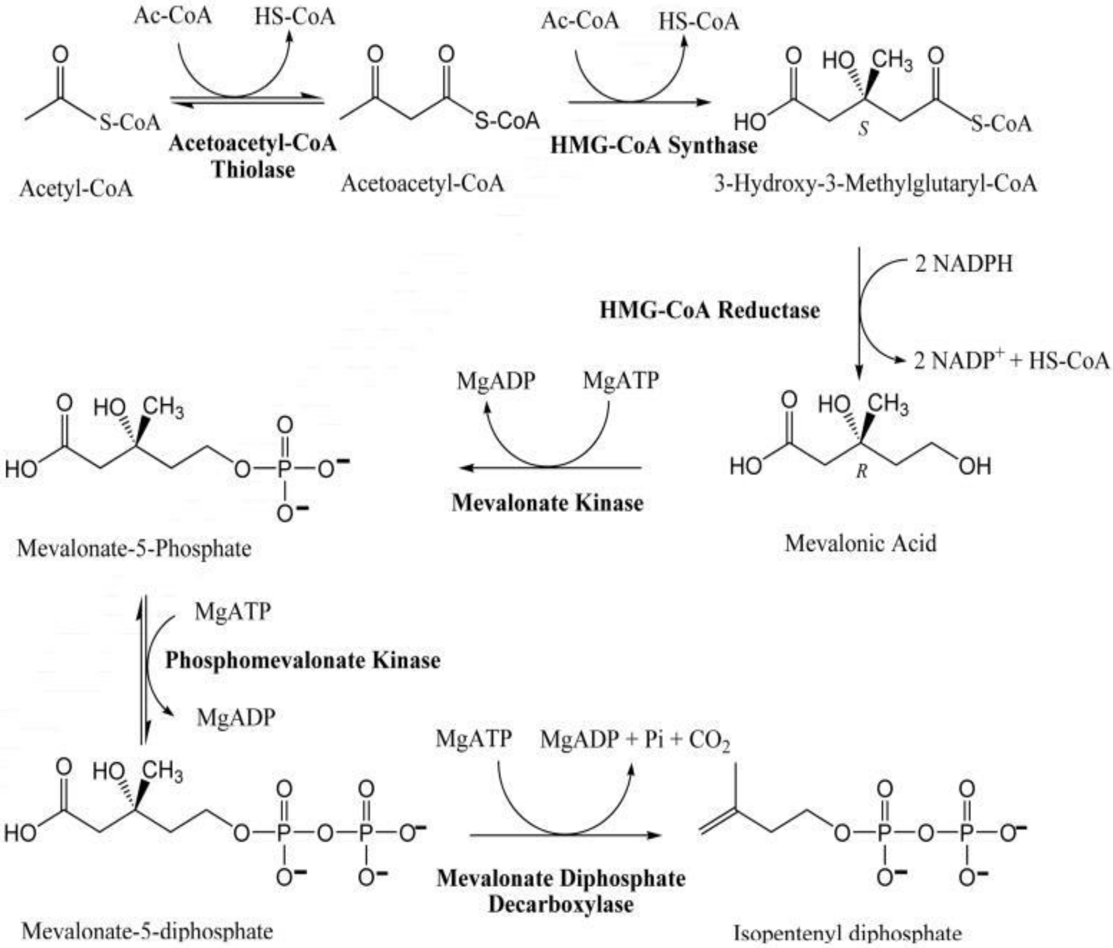
Belonging to the Lamiaceae family (fig1.4), it has a pleasant by smelling perennial shrub which grows in several regions all over the world. Basil is one of the species used for the commercial seasoning. It is commonly known that the presence of essential oils and their composition determine the specific aroma of plant and the flavour of the condiments. Many species of aromatic plants belonging to the Lamiaceae family grow wild in the Mediterranean basin. There are usually considerable variations in the contents of the major components within this species.

Figure (1. 4): Basil (Ocimum spp.)

In a study of essential oils of different geographical origins, researchers found that the main constituents of the essential oil of basil are produced by two different biochemical pathways, the phenylpropanoids (methyl chavicol, eugenol, methyleugenol and methyl cinnamate) by the shikimic acid (trihydroxycylcohexene caroxylic acid) pathway(Scheme 1), and the terpenes (linalool and geraniol) by the mevalonic acid pathway (Scheme2).[9]

Scheme 1: The shikimic acid pathway is a key biosynthetic pathway for several phytochemicals known for their medicinal attributes





Scheme 2 : Mevalonate Pathway for Isopentenyl Diphosphate Biosynthesis.

1.4 Insects :

Most insects cause damage and destruction to humans, directly or indirectly. As these insects caused the disease or caused human material losses or damage to agricultural crops and others.

Stored product insect, Mosquito and Varroa mites are examples of these insects that had a clear impact on human's life.

1.4.1Stored product insect (سوسة القمح)

Sitophilus granaries is types of insects that cause considerable and economic losses of stored wheat grain fig (1.5). Heavy infestations of these insects may cause weight losses of as much as 30–40% in hot and humid areas. insect’s actions are able to cause up to 90% loss of cereal stocks after 5 months of storage. Control on such insects depends largely on pesticides, artificial and vaporization materials, which have caused many problems. Therefore, there was an urgent need to develop safe, convenient, environmental and low-cost alternatives. Considerable efforts have been focused on plant derived materials to make useful products as bio insecticides. The protection of integrated stored – products and phytochemicals may be used for different porpuse such as:

(1) pest control and pest repelling away from goods.

(2) Early detection of pests, attracting pests to lures

(3) pest control by using toxic compounds.

It is possible that agricultural extracts and essential oils can be used to protect crops, since these may contain compounds that have toxic effects on a wide range of insects. [3]

Figure (1. 5): Sitophilus granaries

1.4.2Mosquito (البعوض)

Mosquitoes are nuisance pests and a major vector for the transmission of several life threatening diseases. Aedes aegypti is known to carry dengue(حمى الضنك) and yellow fever; malaria is carried by Anopheles stephensi; and filarial disease by Culex. The present proliferation of these diseases is due basically to increasing resistance of mosquitoes to current insecticides. In many parts of the world, plant-derived products have been used to repel or kill mosquitoes and other domestic insect pests. Solvent extracts and essential oils of many plants show varying levels of insect-repellent properties [17]. Most mosquito control programs target the larval stage at their breeding sites with larvicides since adulticides may reduce the adult population only temporarily. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae. The search for new strategies or natural products to control destructive insects and vectors of diseases is desirable. This is due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic non-biodegradable residues contaminating the environment and undesirable effects on non-target organisms. An alternative to conventional chemical control is the utilization of natural products from plants and essential oils as an alternative source for larvuicidal agents. [3]

In this work, the active ingredient responsible for the repellent of the insect or bugs will be extracted and evaluated. Selected local plants known “traditionally” for its biocidal potency will be studied.

1.4.3 Varroa mites (الفاروا)

Varroa mites are a ubiquitous parasite of honey bee colonies. They are common nearly everywhere honey bees are found, and every beekeeper should assume they have a Varroa infestation.

Varroa mites are tiny red-brown external parasites of honey bees. Although Varroa mites can feed and live on adult honey bees, they mainly feed and reproduce on larvae and pupae in the developing brood, causing malformation and weakening of honey bees as well as transmitting numerous viruses[18]

Figure (1.6a) Varroa destructor on Bees

Figure (1. 6b): Varroa destructor

Colonies with low infestation generally show very few symptoms, however as the mite population increases symptoms become more apparent. Heavy Varroa mite infestations can build up in 3–4 years and cause scattered brood, crippled and crawling honey bees, impaired flight performance, a lower rate of return to the colony after foraging, a reduced lifespan and a significantly reduced weight of worker bees. Colony symptoms, commonly called parasitic mite syndrome, include an abnormal brood pattern, sunken and chewed capping and larvae slumped in the bottom or side of the cell. This ultimately causes a reduction in the honey bee population, supersedure of queen bees and eventual colony breakdown and death

Varroa mites (Varroa destructor) are now the most serious pest of honey bee colonies and one of the primary causes of honey bee decline. A honey bee colony with Varroa, that is not treated to kill the pest, will likely die within one to three years.[18]

Varroa mites affect beekeepers economy. It is thus necessary to control the growth of the mite. may need a chemical application (chemical pesticides), but it will negatively affect the bees because the chemical pesticide will kill the bees as kills Varroa mites we should thus find a replacement for chemical pesticides to eradicate this insect; such as oils extracted from plant. .[17]

CHAPTER TWO

METHODOLOGY

2.1 Chemicals:

Polar and non-polar solvents such as, ethanol, diethyl ether, hexane cyclohexane, ethyl acetate, and other chemicals were of analytical grade and used for the extraction of essential oils. Thin Layer Chromatography -TLC- sheets were (obtained from chem, samual or sun pharm drug store).

The spectra of the extracts were obtained using a *Thermo finnigan* GC-MS Instrument(2002) fig (2.1), serial No(20022740), equipped with Quadrupole detector capable of analyzing masses up 600 Da.

Figure(2.1) Thermo finnigan GC-MS Instrument(2002)

2.2 Plants:

The used plants and their . scientific names, along with their importance are tabulated in (table 2.1).

Table (2. 1): Scientific name and uses of the three plants

No	Plant	scientific name	*Importance*
1	Oleander	*Nerium Oleander* L	*Nerium oleander*(Apocynaceae family) is very important to say that *N.oleander* has various features and advantages. One of these features is their insecticidal properties. In addition to that, it have the antibacterial properties which *N.oleander* leaves possess. Also, we have its medical effects which researches have reported that this plant can be used to treat some diseases like cancer.[19]
2	Rue	*Ruta graveolens*	*Ruta graveolens* (Rutaceae family)*R. graveolens* extracts and essential oil are important areas in drug development with numerous pharmacological activities in many countries. For a long time *R. graveolens* has been used in traditional medicines for the relief of pain, eye problems, rheumatism and dermatitis. *R. graveolens* has recently been shown to have antibacterial, analgesic, anti-inflammatory, antidiabetic and insecticidal activities.The essential oil also has a depressing effect on the central nervous system. At high doses, it works as a narcotic poison[20]
3	Basil	Basil (Ocimum spp.)	Basil is a famous plant in folk medicine due to its high medical value. In folk medicine, the flowers and the oil are used for different purposes and needs. In addition. It has antifungal, physicochemical and insect-repelling activity. It is also regarded as highly antiseptic .[21]

2.2.1 Plants sample Collection:

The plants were collected in June from Qabatia region, and identified by Dr. Banan Al Sheikh, National Agricultural Research Center (NARC) , Jenin-Palestine. Voucher of plants were preserved there.

2.3 Insects:

Insects were delivered from the Agriculture collage of An-Najah National University

Table (2. 2): Scientific names for the three insects

No	Insect name	Scientific name
1	Varroa mites	*Varroa destructor*
2	Mosquitoe	*Culex pipie's Mosquitoes*
3	Stored product insect	*Sitophilus granaria*

2.4 Experimental

Essential oils from plants

Various plants have compounds within them that yield familiar smells or aromas. These fragrances are the result of unstable essential oils. These essential oils are often isolated from the particular plant to produce concentrated normal extracts. These essential oils are a blend of organic complexes. The oil can be removed by extraction with alcohol. This process, known as decoctions, [22] was formerly used extensively in the production of perfumes and pomades.

2.5 Methodology:

Essential oils were extracted and tested by TLC to show many components each isolated and analyzed by GC-MS.

2.5.1 Extraction of essential oils :

The plants were collected and dried in the shadow for 48h. The dry plant 250g was grinded and soaked in 800ml ethanol for 48 hours then filtered. The filtrate was evaporated under reduced pressure (rotary evaporator) to leave the essential oil. [22]

2.5.2 Thin Layer Chromatography (TLC) :

The essential oil was separated on TLC sheet (5 × 20) and the best eluent (1: 2) Ethyl acetate and Hexane (fig 2.2). [23]

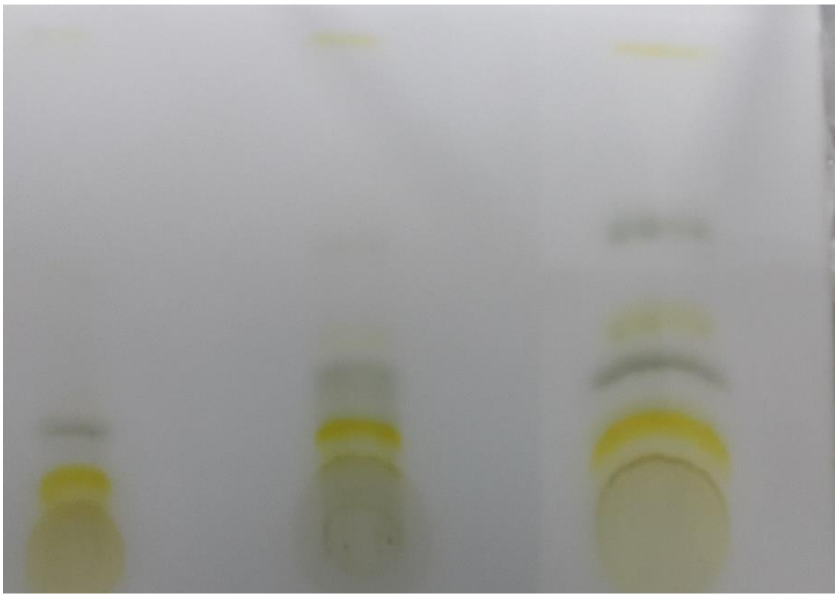


Figure (2.2) TLC of essential oil extracts from Nerium oleander L, Ruta graveolens& Basil (Ocimum spp.)

Then the different bands were scraped (fig 2.3) and the compound was extracted by ethanol and analyzed by GC-MS.

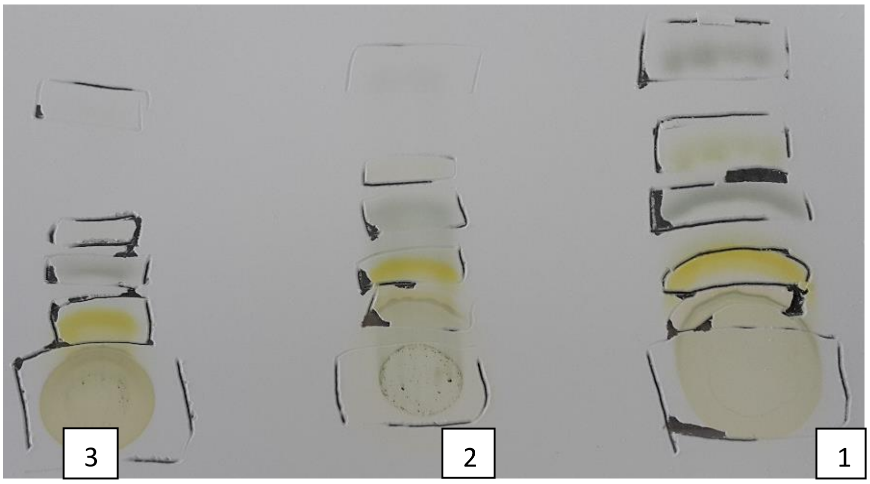


Figure (2.3):TLC of essential oil extracts from 1- *Nerium oleander L*, 2- *Ruta graveolens*& 3- *Basil (Ocimum spp.)* scraped

2.5.3 GS-MS Analysis :

Each sample analyzed by GC-MS device was equipped by a MS-Library for most of organic compounds.

The major compounds of the essential oils were characterized (Table 3.1)

2.6 Application on insects

Insecticidal activity and repellent effect of essential oils was obtained from three Palestinian plants against harmful insects .

2.6.1 Repellent effect :

Repulsive effect of the oil on the *Sitophilus granaria* was carried out using the area preference method. The test area consisted of (125mm) filter paper (Filtermann®), which was cut into 2 equal parts.

One half of filter paper was treated with 1ml of each prepared extract of essential oil from plant as uniformly as possible while the other half (control) was only treated with 1ml of alcohol.

These two halves were then air dried to evaporate the solvent completely.

A full disc was carefully remade by attaching the treated half to the control half with the help of an adhesive tape. Each remade filter paper was placed in a petridish and 10g of wheat grains were uniformly distributed over it with the purpose of providing the natural condition for the *Sitophilus granaria.*

Then 10 adult *Sitophilus granaria* were released on to the center of each filter paper disc, and the cover was placed over the petridish and kept in the dark. Each extract of essential oil solution and the control were replicated 5 times. The number of insects present on each strip was recorded after 30minutes [20]

2.6.2 Fumigant test :

The bioassay of fumigant test was determined by using a modified closed container method, [1] which consists of isolated groups of ten insects(*Culex pipie's Mosquitoes* , *Sitophilus granaria* and *varroa*) and then, fixing paper discs, treated by essential oil extract (5ml oil /50ml air) on the top of the beakers. Three replicates were done to test extracts and control. The mortality of insects was observed and determined after 5,10, 24 h of exposure

Chapter Three

Results And Discussion

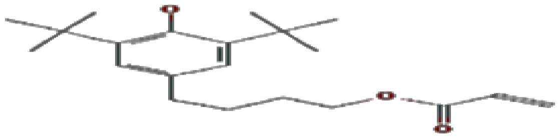
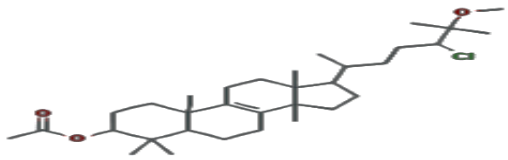
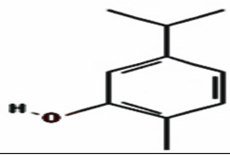
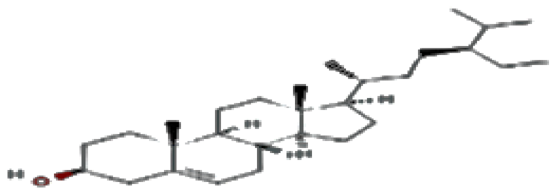
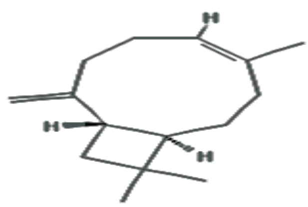
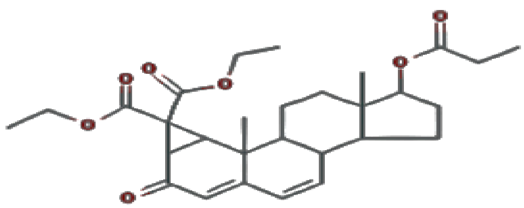
In search for another way to control insects to the current applications of artificial insecticides, extracts from medicinal plants are being obtained. These extracts can be developed commercially as active personal protection measures against harmful insects and thus to control ailments caused by mosquito-borne pathogens. Essential or unstable oils of plants have been variously described to have many therapeutic claims. Methanol, acetone and petroleum ether extracts of selected plants were screened for their action against mosquito, vaorra, and Sitophilus.

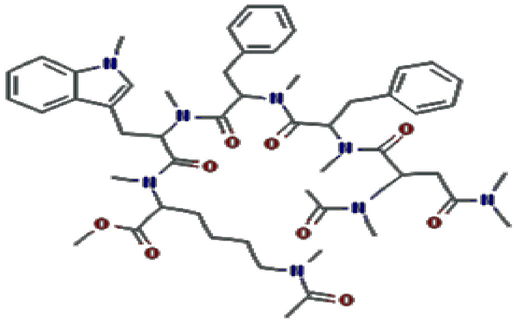
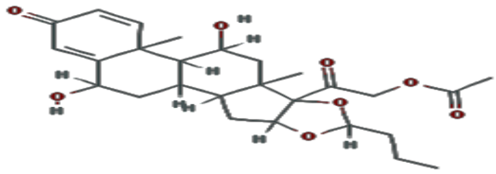
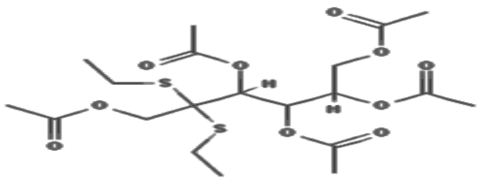
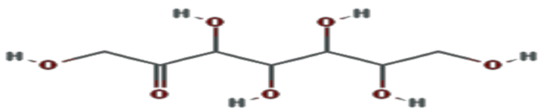
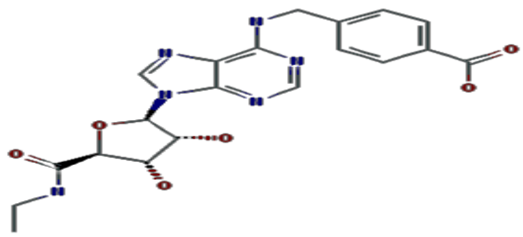
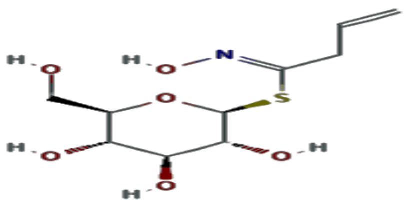
Of all mosquito-borne virus-related diseases, dengue is spreading most rapidly worldwide. [24] Conventional chemical insecticides (e.g., organophosphates and carbamates) effectively destroy mosquitoes at their larval stage, but are toxic to humans.. Natural product-based insecticides may be highly specific.

3.1 Chemical Results :

GC-MS spectra showed that plants *Nerium oleander L, Ruta graveolens and Basil (Ocimum spp.)* contain a mixture of chemical compound (Table 3.1 and Table 3.2)

Table(3.1): chemical compounds found in Nerium oleander L, Ruta graveolens and Basil (Ocimum spp.)

S. No.	Phytochemical compound	Chemical structure
I	4-(3,5-ditert-butyl-4-hydroxyphenyl)butyl prop-2-enoate	
II	Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl	
III	2-Methyl-5-(1-methylethyl)phenol(carvacrol)	
IV	Sitosterol	
V	(E)-beta-caryophyllene	
VI	1',1'-Dicarboethoxy-1.beta.,2.beta.-dihydro-17.beta.-propionoxy(3'H)cyhclop rop[1,2]androsta-1,4,6-trien-3-one	

VII	L-Lysine, N6-acetyl-N2-[N-[N-[N-(N2-acetyl-N,N,N2-trimethyl-L-asparaginyl)-N-methyl-L-phenylalanyl]-N-methyl-L-phenylalanyl]-N,1-dimethyl-L-tryptophyl]-N2,N6-dimethyl-, methyl ester	
VIII	(22S)-21-Acetoxy-6.alpha.,11.beta.-dihydroxy-16.alpha.,17.alpha.-propylmethylenedioxypregna-1,4-diene-3,20-dione	
IX	D-Fructose, diethyl mercaptal, pentaacetate	
X	D-Mannoheptulose	
XI	1. Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	
XII	Desulphosinigrin	

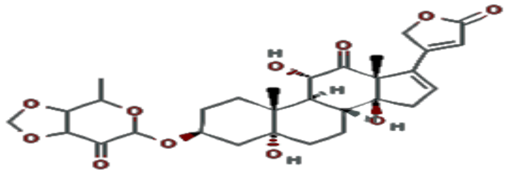
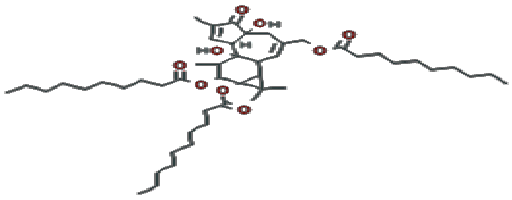
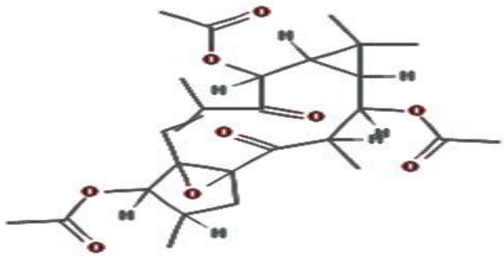
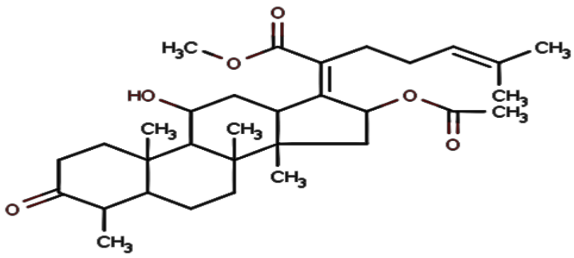
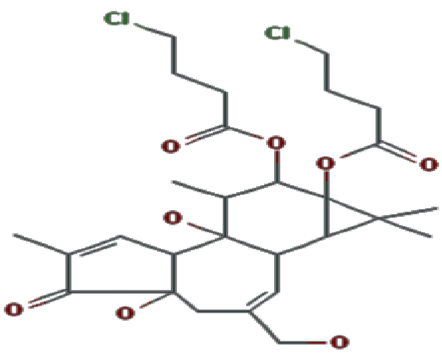
XIII	Anodendroside E 2	
XIV	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-5-oxo-3-[[(1-oxodecyl)oxy]methyl]-9aH-cyclopropa[3,4]benz[1,2-	
XV	1. 3,8,12-Tri-O-acetoxy-7-desoxyingol-7-one	
XVI	2-(16-Acetoxy-11-Hydroxy-4,8,10,14-Tetramethyl-3-Oxohexadecahydrocyclopenta[A]Phenanthren-17-Ylidene)-6-Methyl-Hept-5-Enoic Acid, Methyl Ester	
XVII	Rhodopin	
XVIII	Butanoic acid, 4-chloro-, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1ar-(1a.alpha.,1b.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	

Table 3.2 : molar masses corresponding to structures found in the essential oil extracts

S. No.	Exact Mass g/mole	Plant
I	332.235	*Ruta graveolens and Oleander*
II	534.384	*Ruta graveolens*
III	150.104	*Ruta graveolens*
IV	414.386	*Ruta graveolens*
V	204.188	*Ruta graveolens*
VI	498.126	*Ruta graveolens and Basil*
VII	964.542	*Ruta graveolens*
VIII	488.241	*Basil (Ocimum spp.)*
IX	496.144	*Basil (Ocimum spp.)*
X	210.074	*Basil (Ocimum spp.)*
XI	442.160	*Basil (Ocimum spp.) and L Nerium oleander*
XII	279.078	*Basil (Ocimum spp.)*
XIII	574.241	*Basil (Ocimum spp.)*
XIV	826.596	*Basil (Ocimum spp.)*
XV	490.22	*Nerium oleander L*
XVI	528.345	*Nerium oleander L*
XVII	554.449	*Nerium oleander L*
XVIII	572.28	*Nerium oleander L*

4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)butyl acrylate

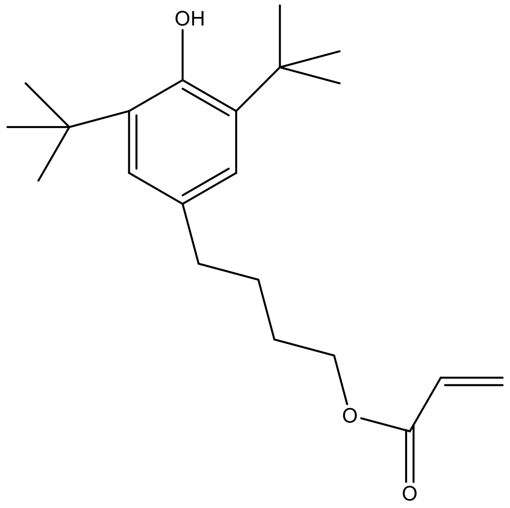


Figure (3.1.a) represents a mass spectrum of the components extracted from oleander

This components was identified as 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate. This compound has a calculated molar mass of 332.477 Da. The M+1 molar mass is shown in figure 3.5 with several fragments

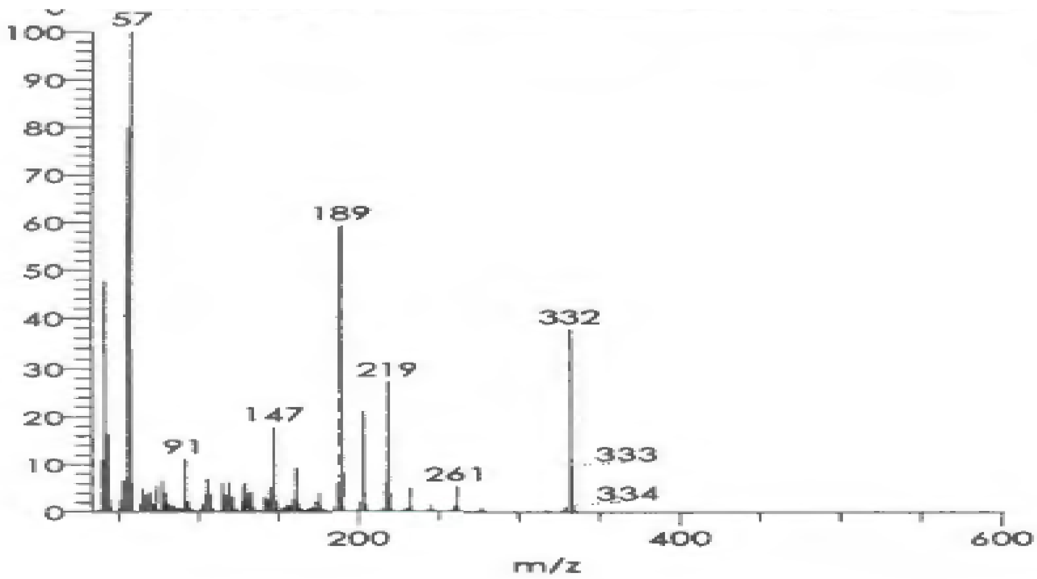


Figure (3. 1.b): Mass spectrum of **(I)**

The mass at 261 m/z represent the molecule after losing m/z of 72, which represents the loss of acrylate group. This same compound was also found in *Ruta graveolens*.

Compound number (VI)that is shown in Table 3.5 were also found in both *Ruta graveolens* and *Basil (Ocimum spp).*

Furthermore, compound numbr(XI) is als cmmon to both *Oleander* and *Basil (Ocimum spp)*.

In addition to that, the compounds of number(I) and (XVIII) are common in *Ruta* and *N-oleander*. Consequently, it was noticed that these common compounds may have poisonous effects (active ingredients) in the three plants (*Ruta* , *Basil* and *N-oleander*).

4-(3, 5-di-tert-butyl-4-hydroxyphenyl)butyl acrylate

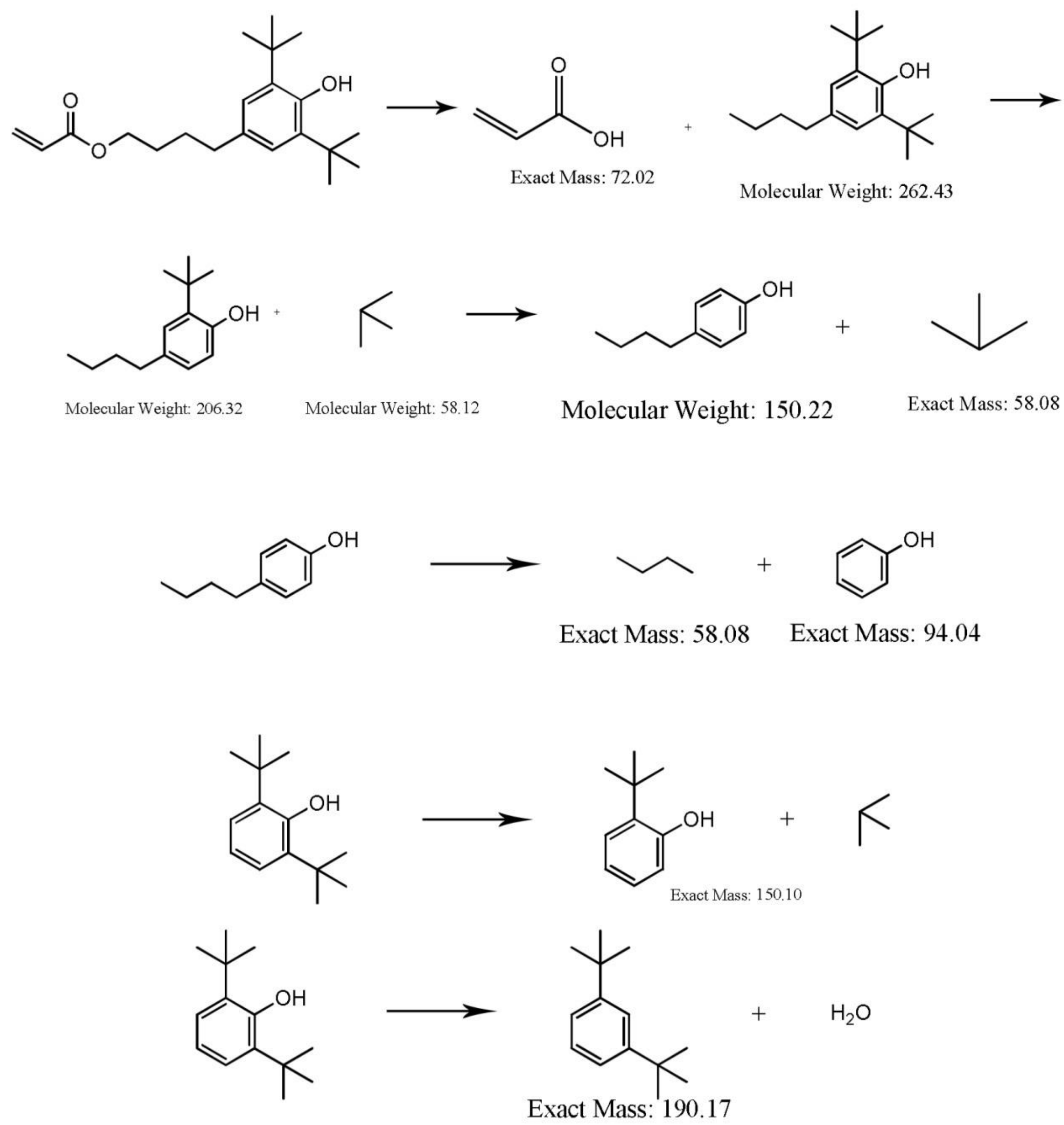
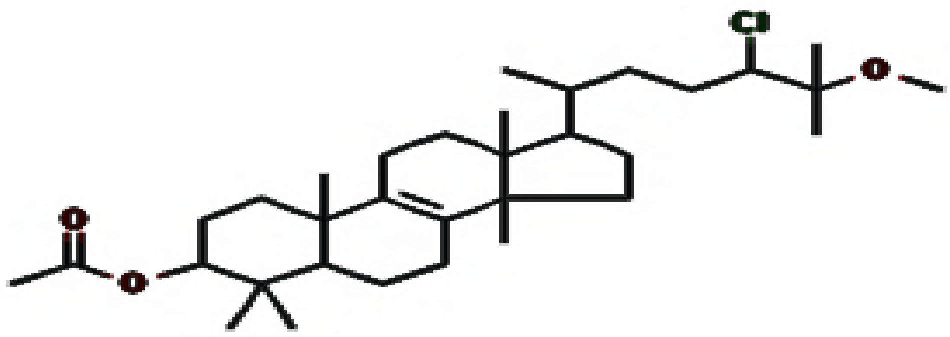


Figure (3. 1.c): fragmentation of compound number (I)



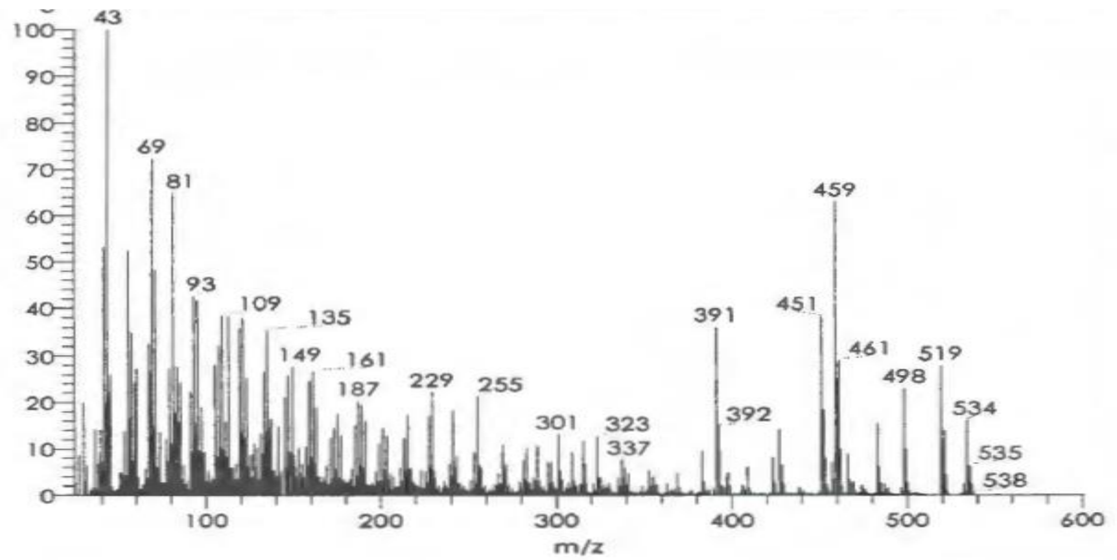
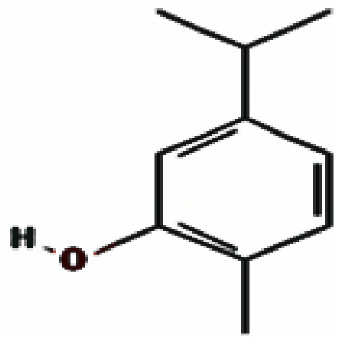


Figure (3. 2): Mass spectrum of compound number (II)



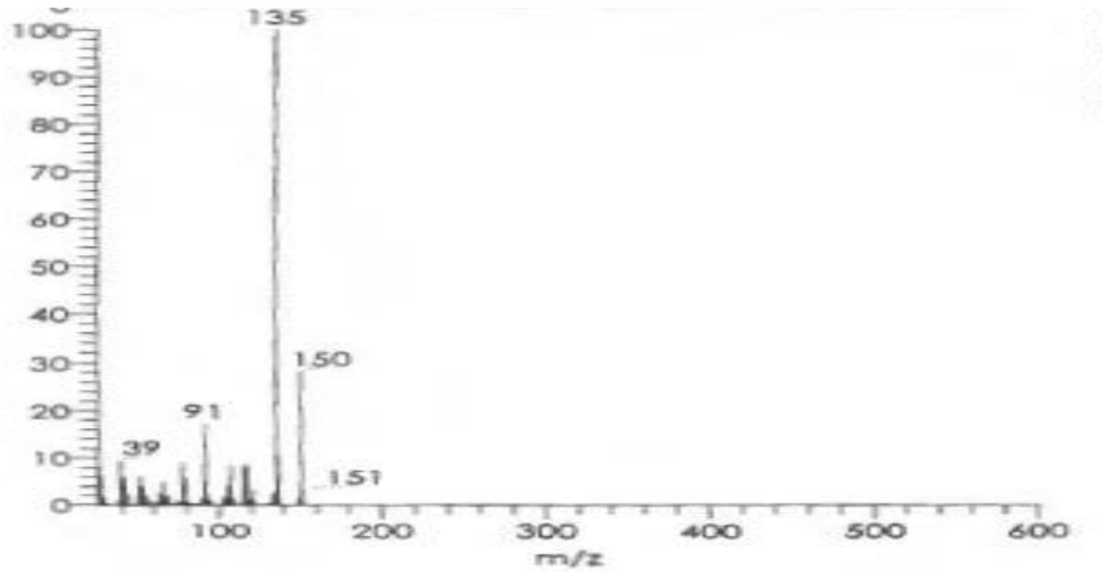


Figure (3. 3.a):mass spectrum of compound number (III) it is monoterpenes and related phenols with insecticidal activities.

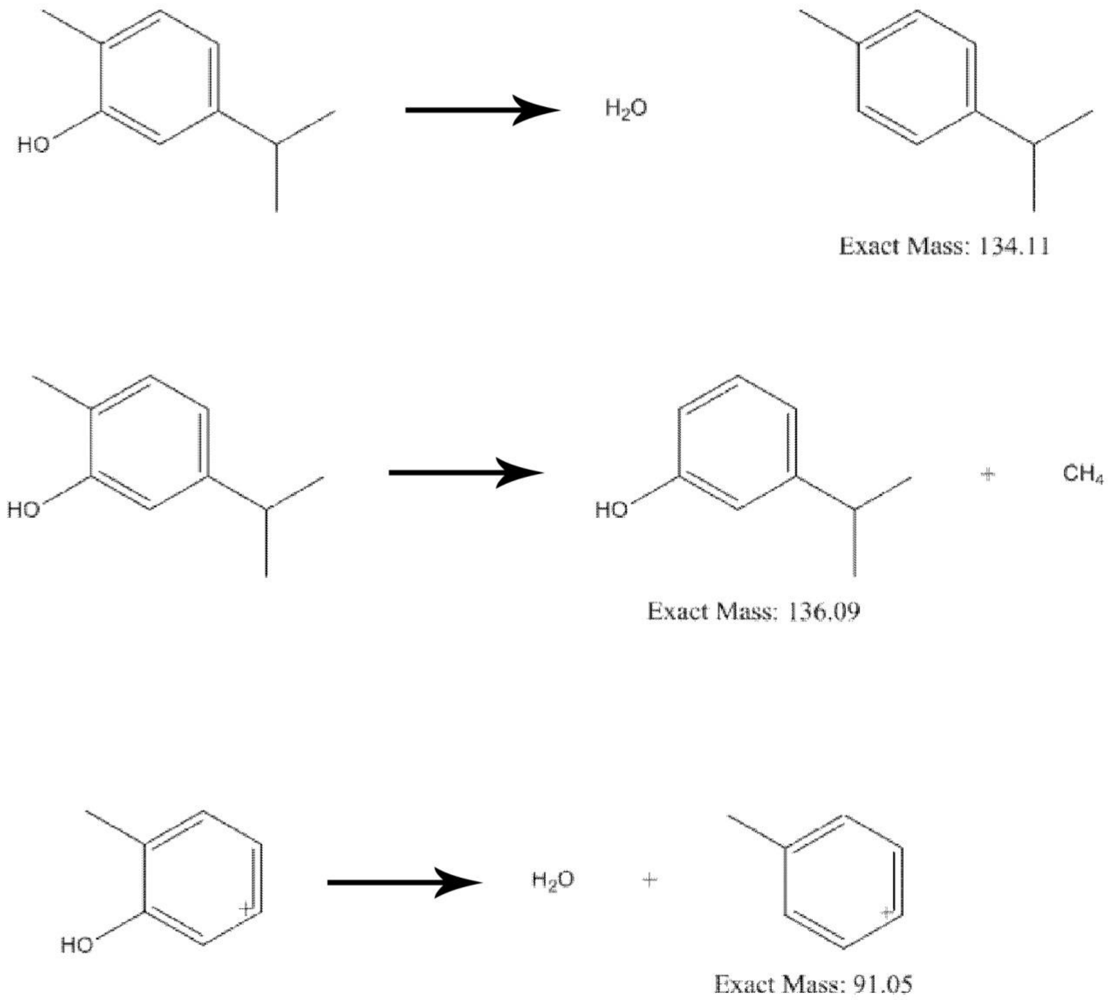


Figure (3. 3.b): fragmentation of compound number (III)

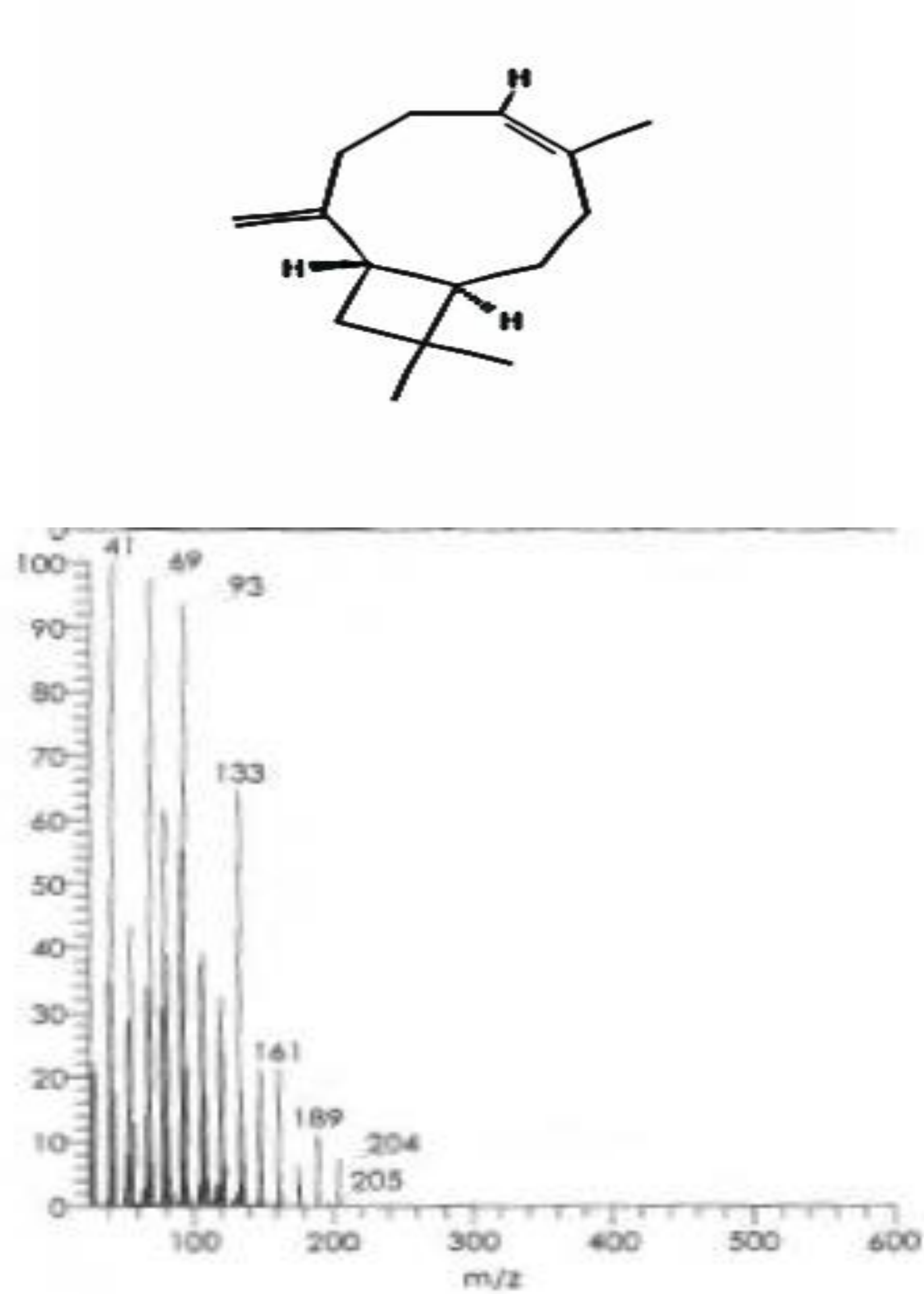


Figure (3. 4): mass spectrum of compound number (IV)

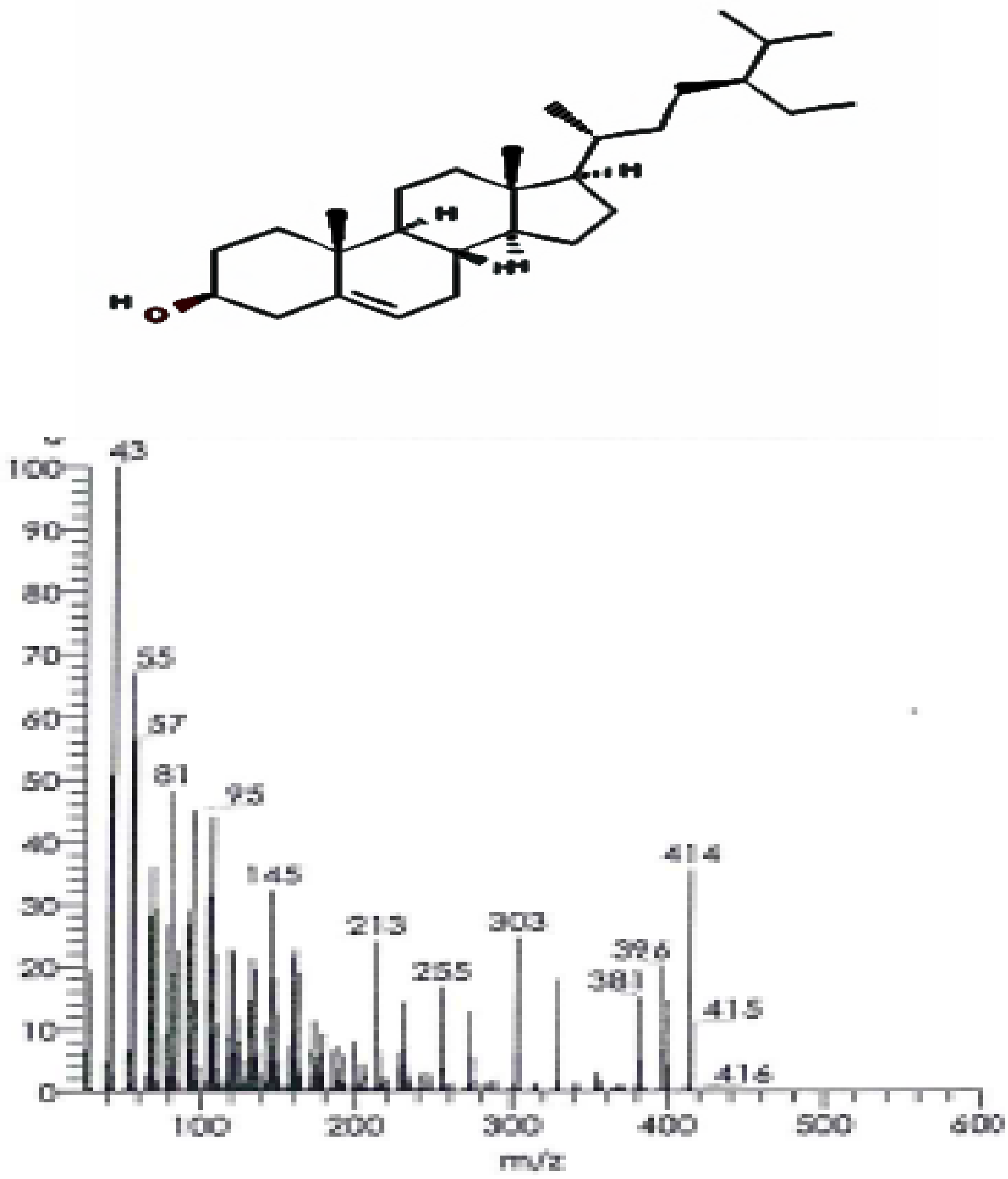


Figure (3. 5): mass spectrum of compound number (V)

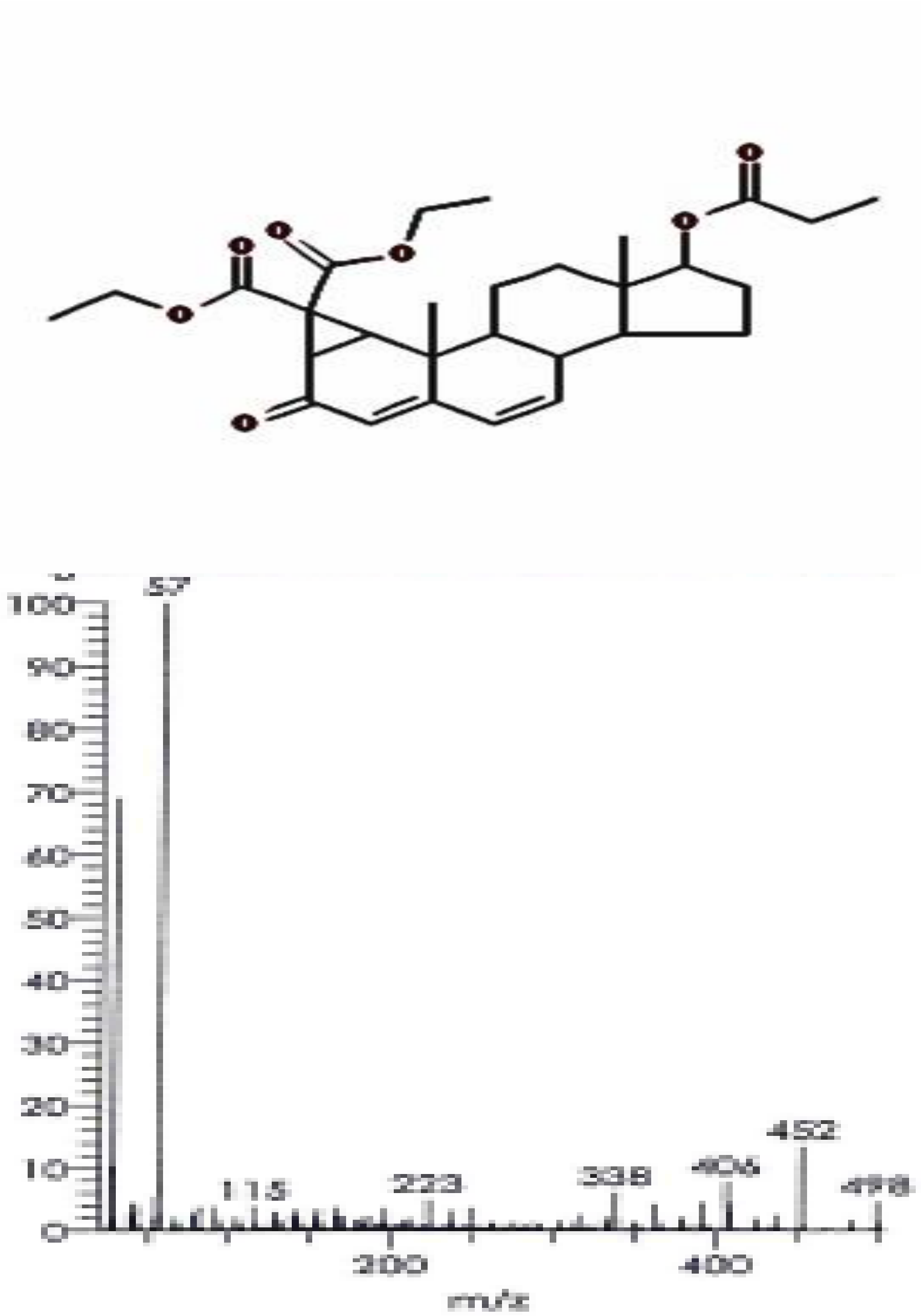


Figure (3. 6.a): mass spectrum of compound number (VI)

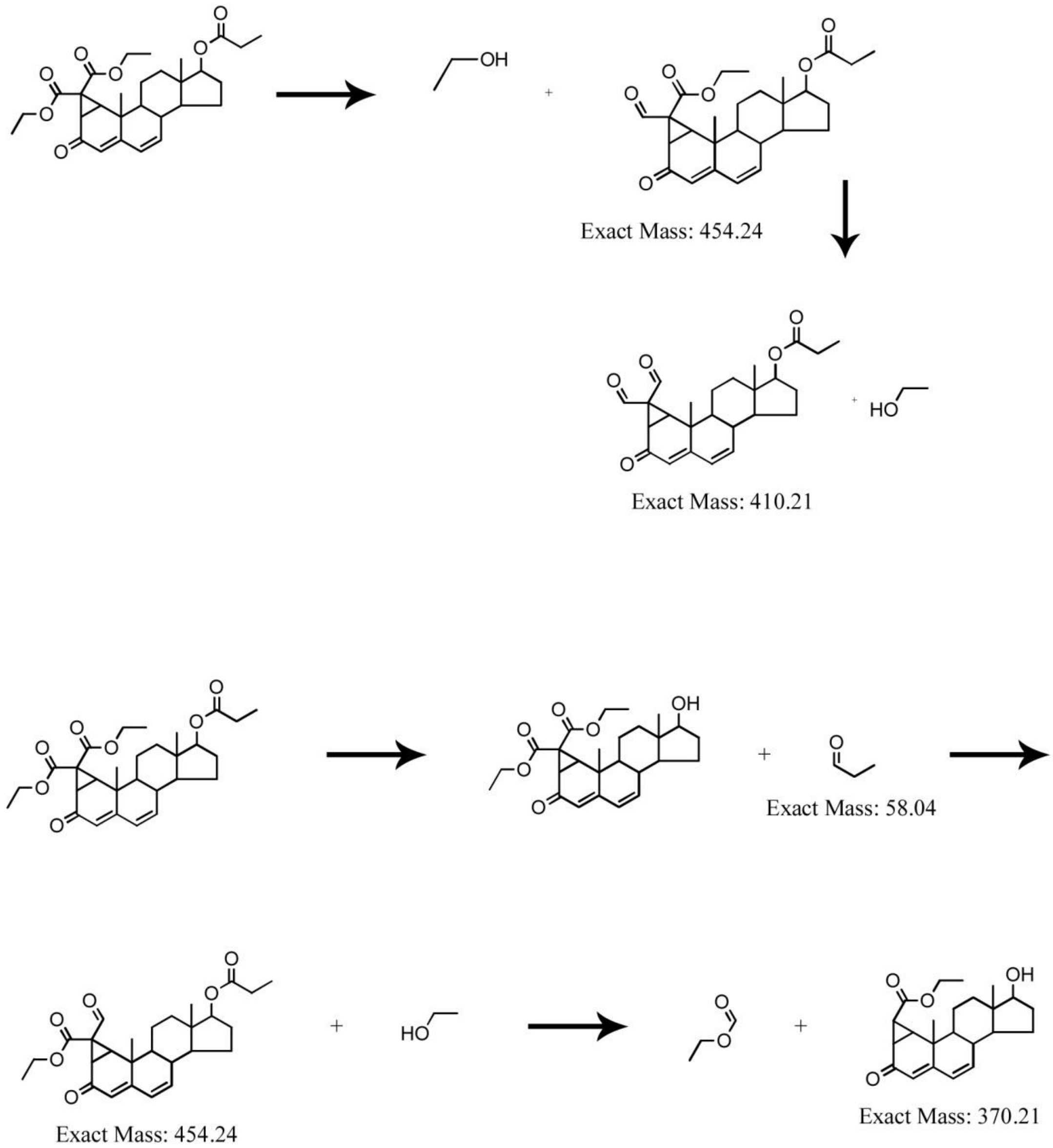
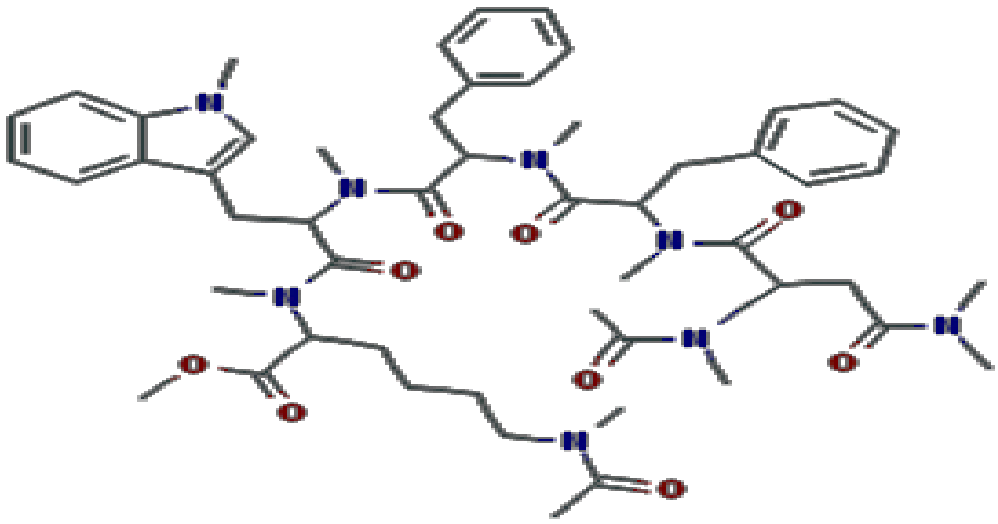


Figure (3. 6.b): fragmentation of compound number (VI)



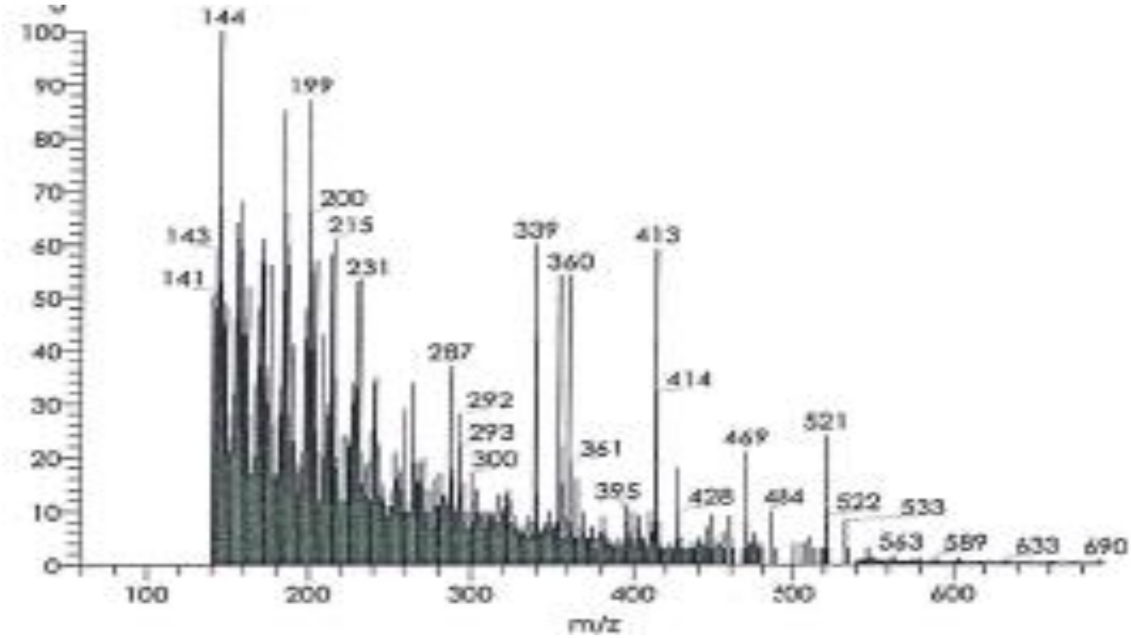
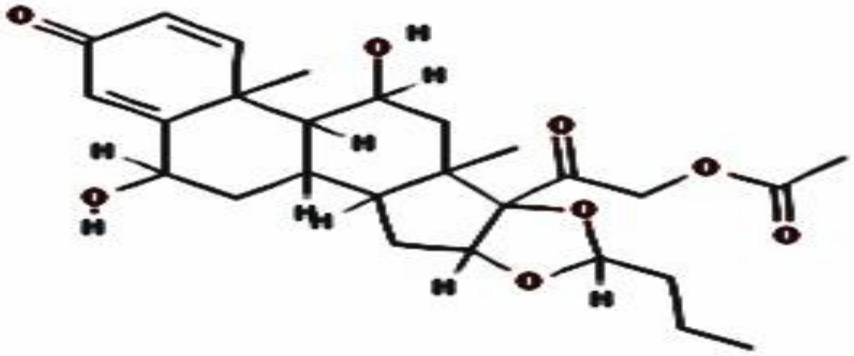


Figure (3. 7): mass spectrum of compound number (VII)



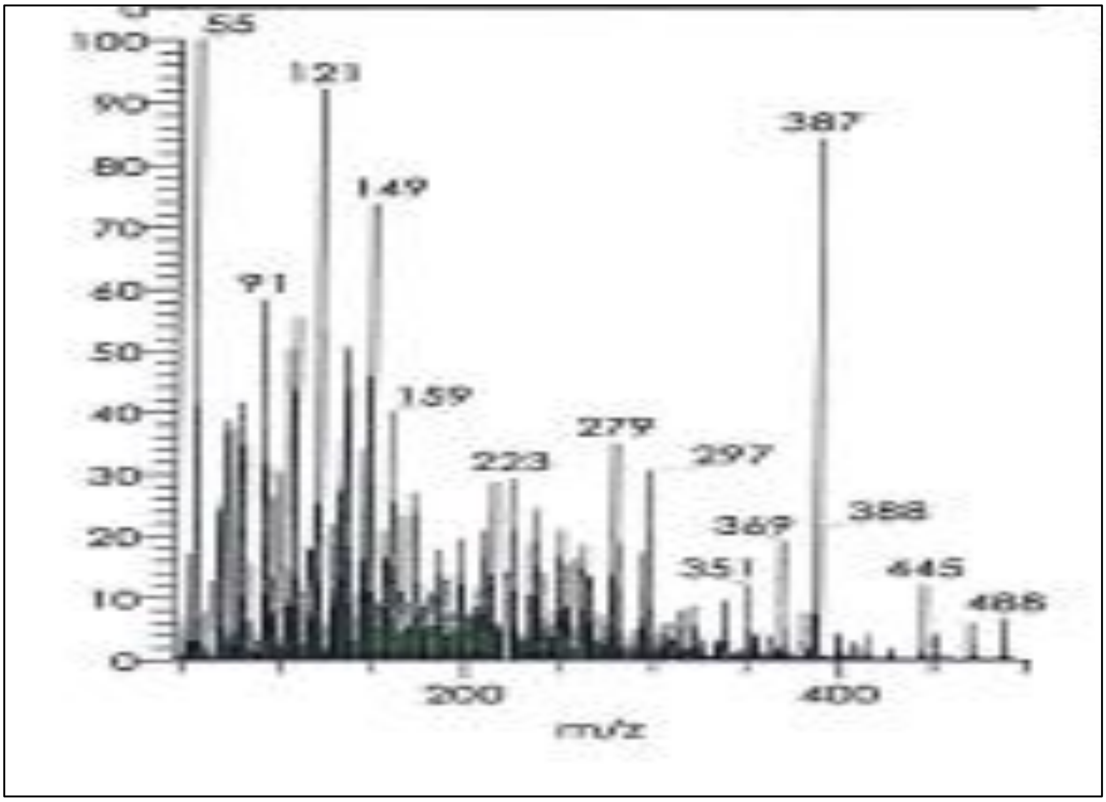


Figure (3.8): mass spectrum of compound number (VIII)

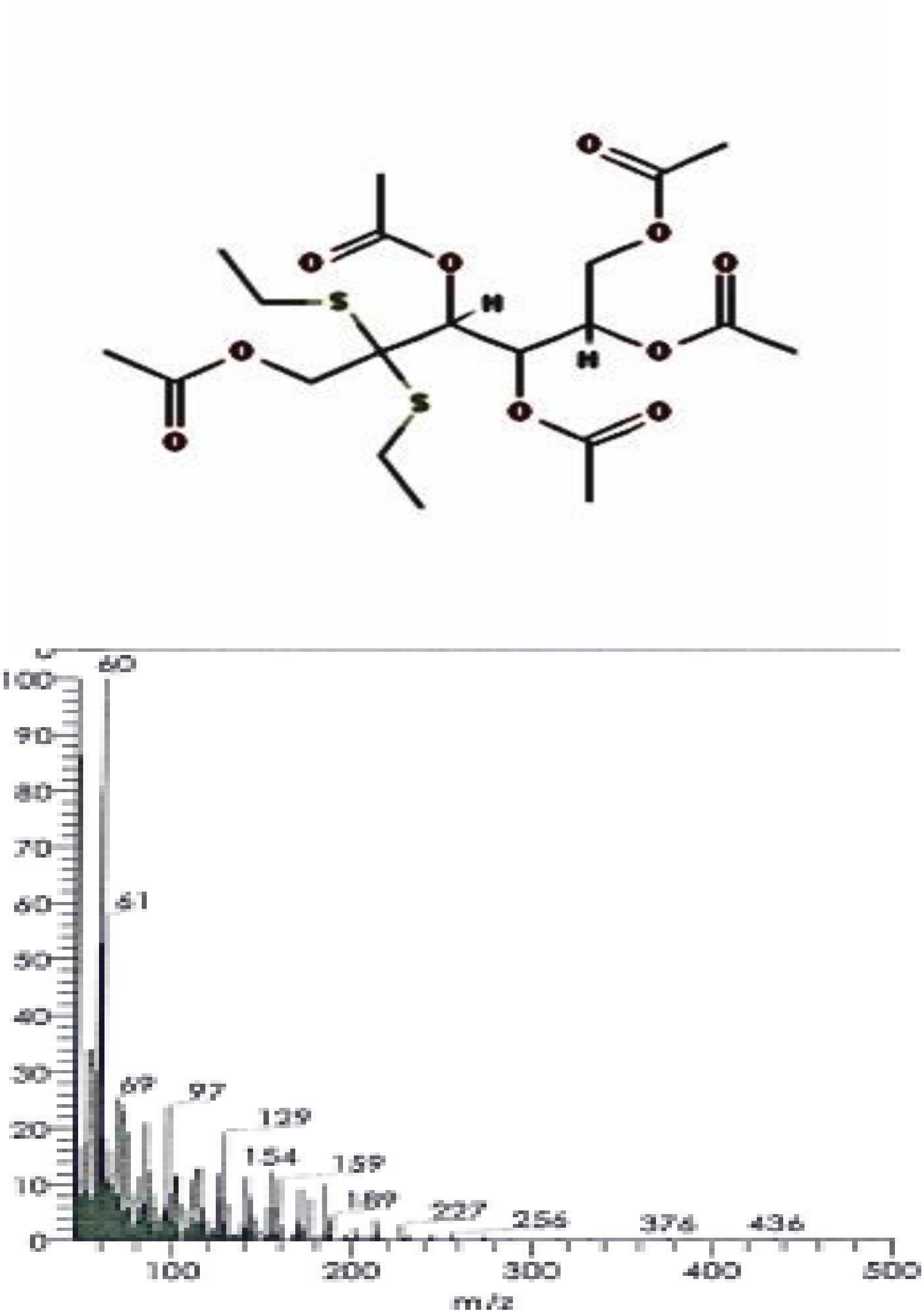
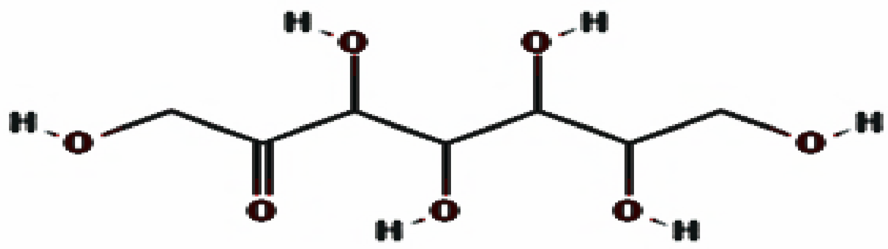


Figure (3. 9): mass spectrum of compound number (IX)



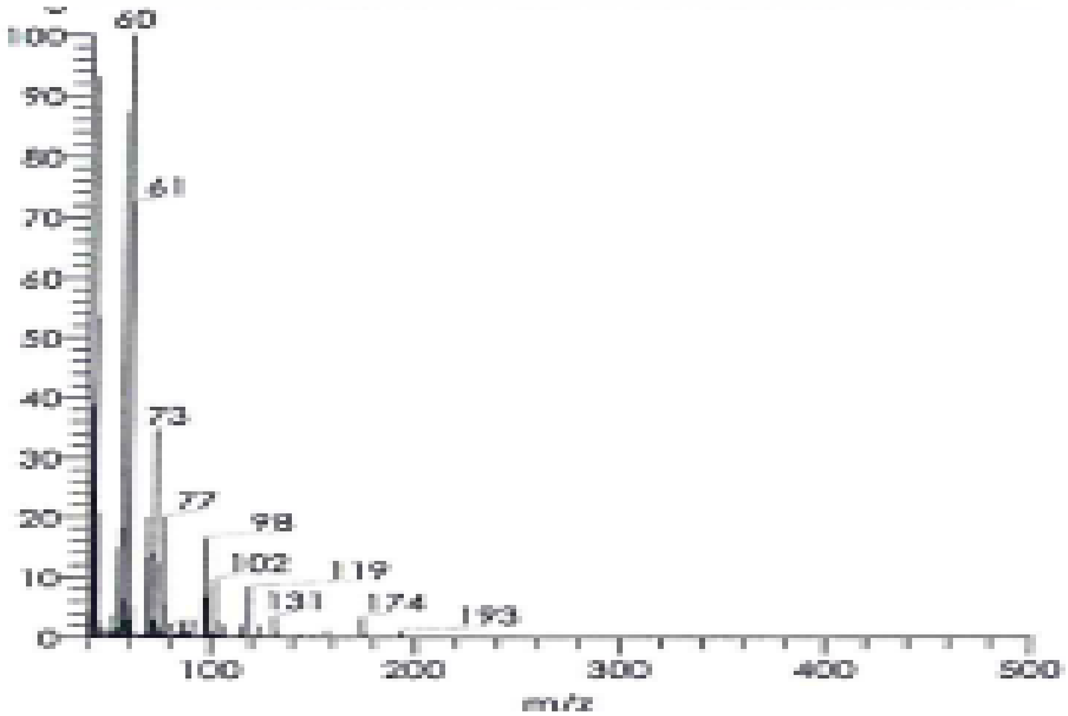
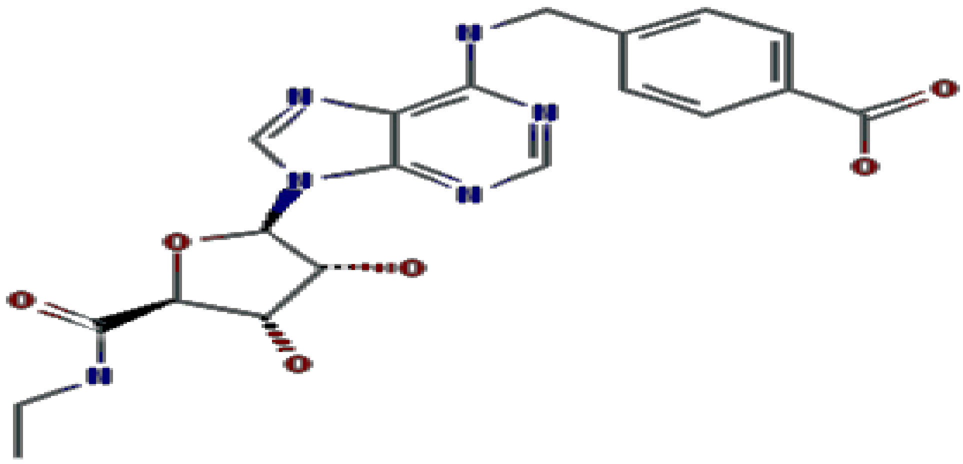


Figure (3. 10): mass spectrum of compound number (X)



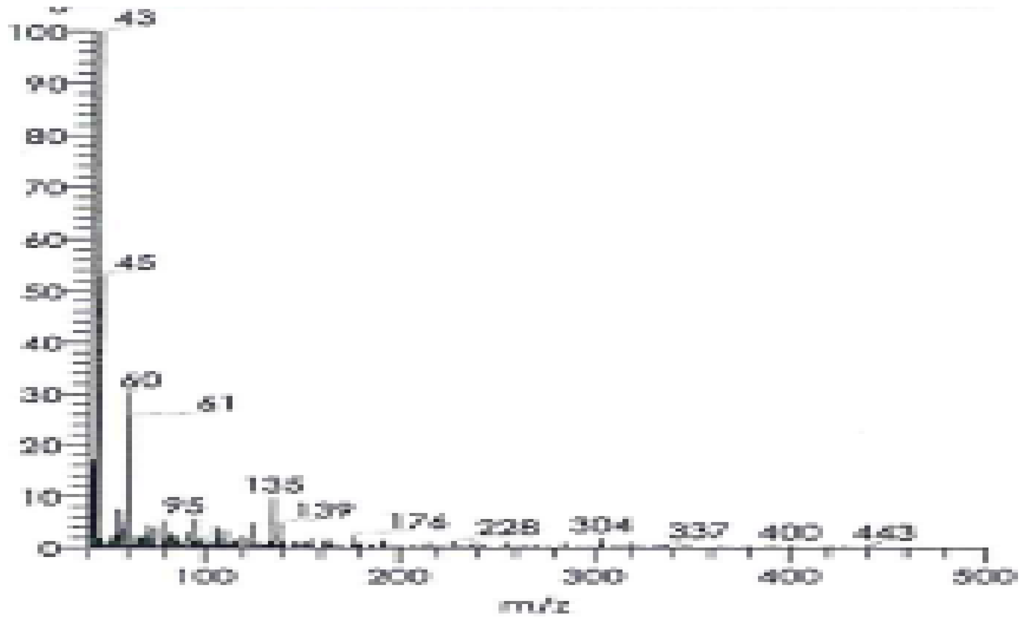


Figure (3.11.a): mass spectrum of compound number (XI)

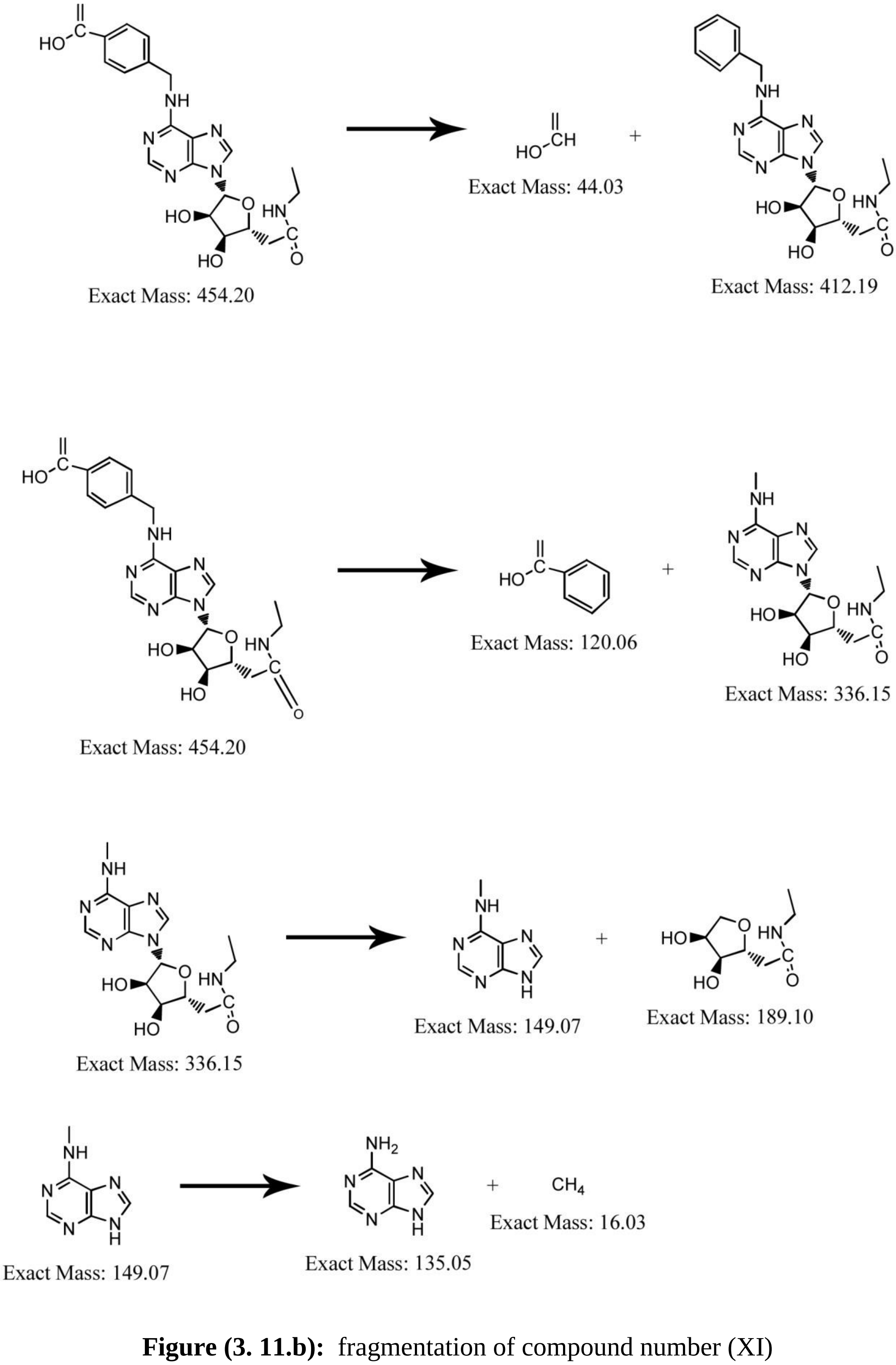


Figure (3. 11.b): fragmentation of compound number (XI)

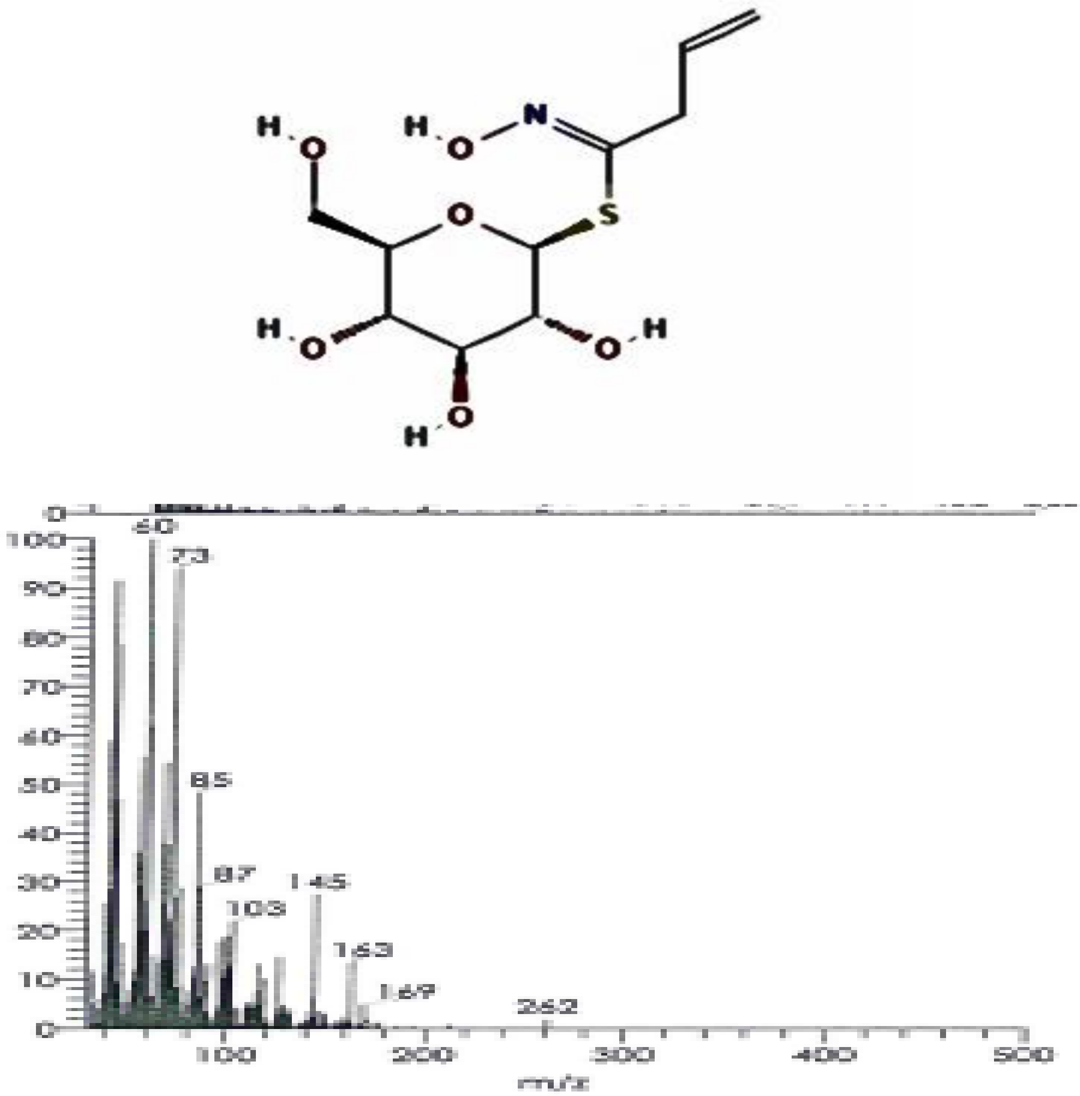
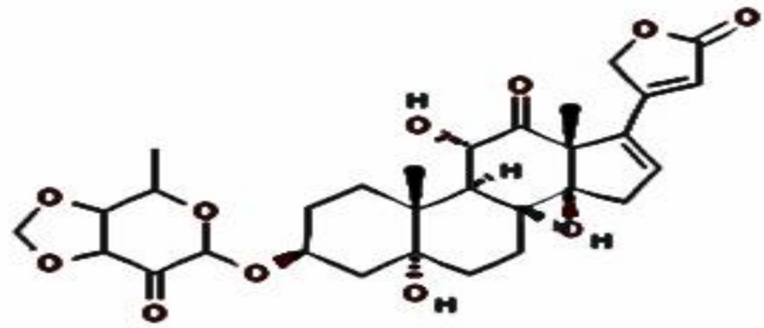


Figure (3. 12): mass spectrum of compound number (XII)



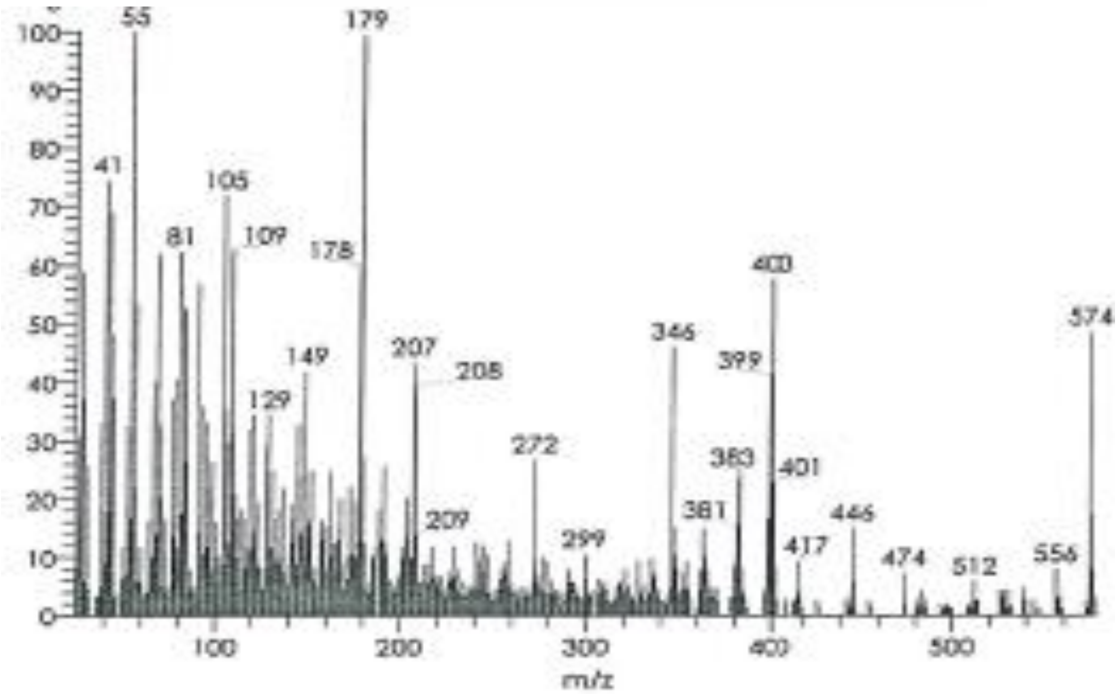


Figure (3. 13): mass spectrum of compound number (XIII)

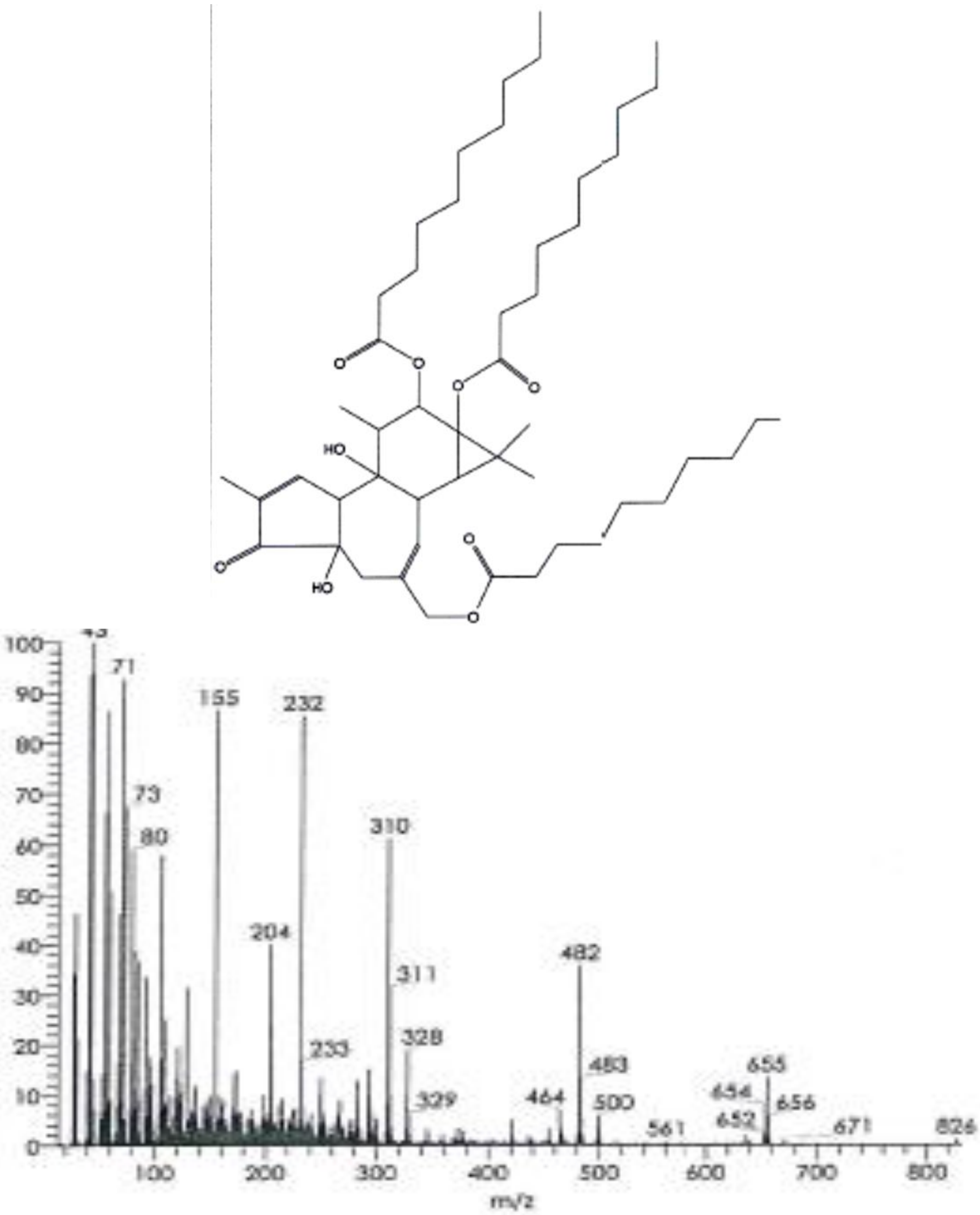


Figure (3. 14): mass spectrum of compound number (XIV)

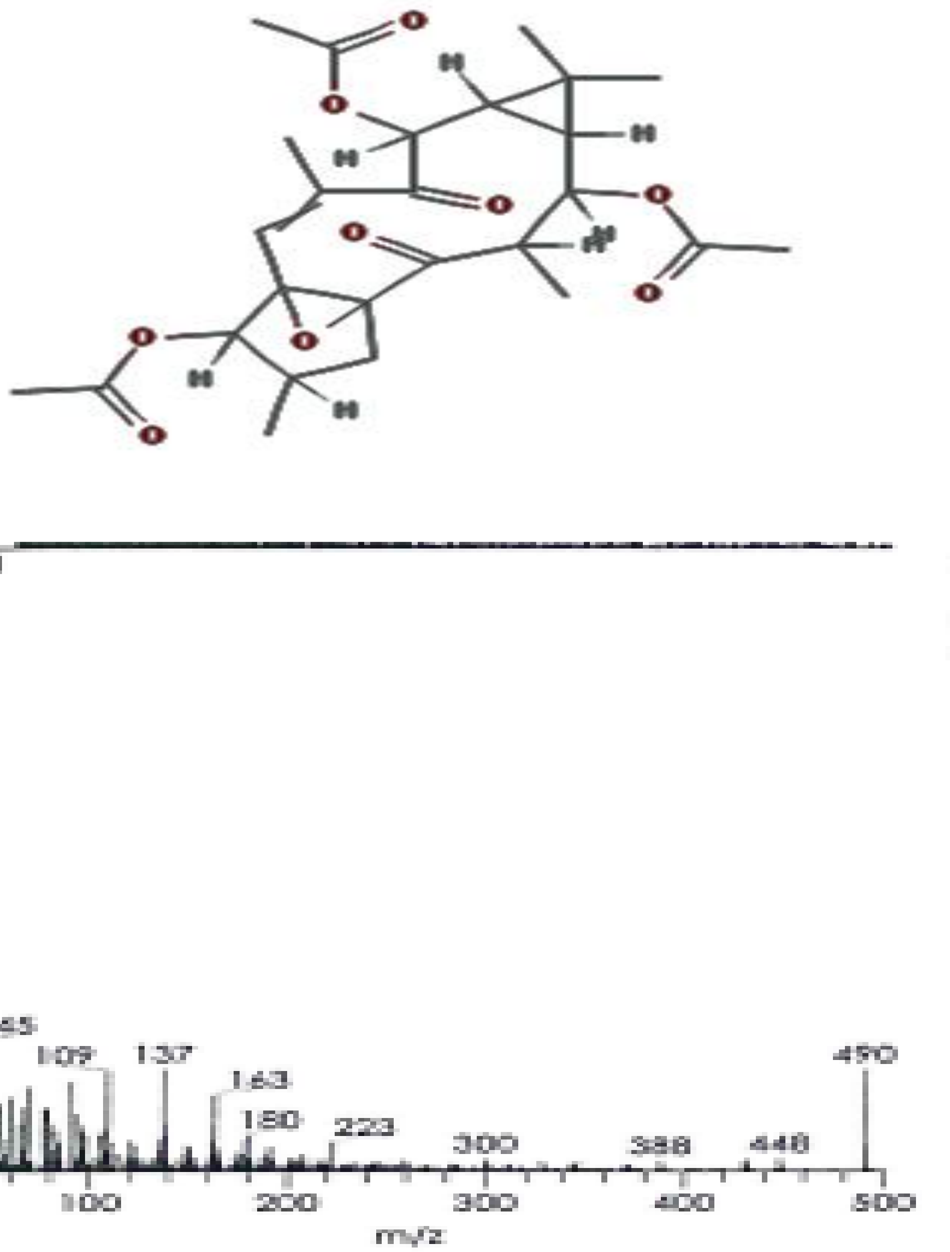


Figure (3.15): mass spectrum of compound number (XV)

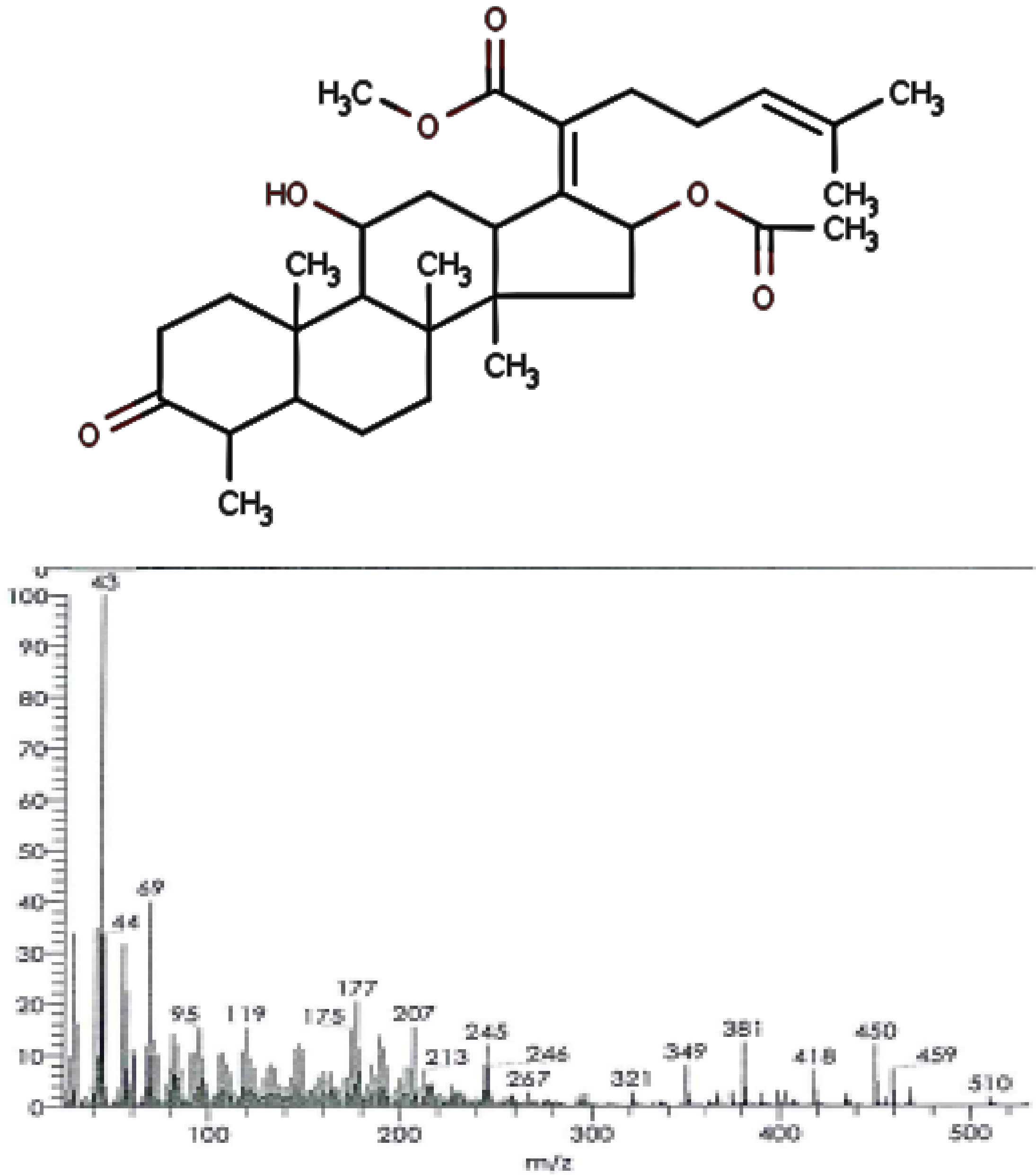


Figure (3.16): mass spectrum of compound number (XVI)

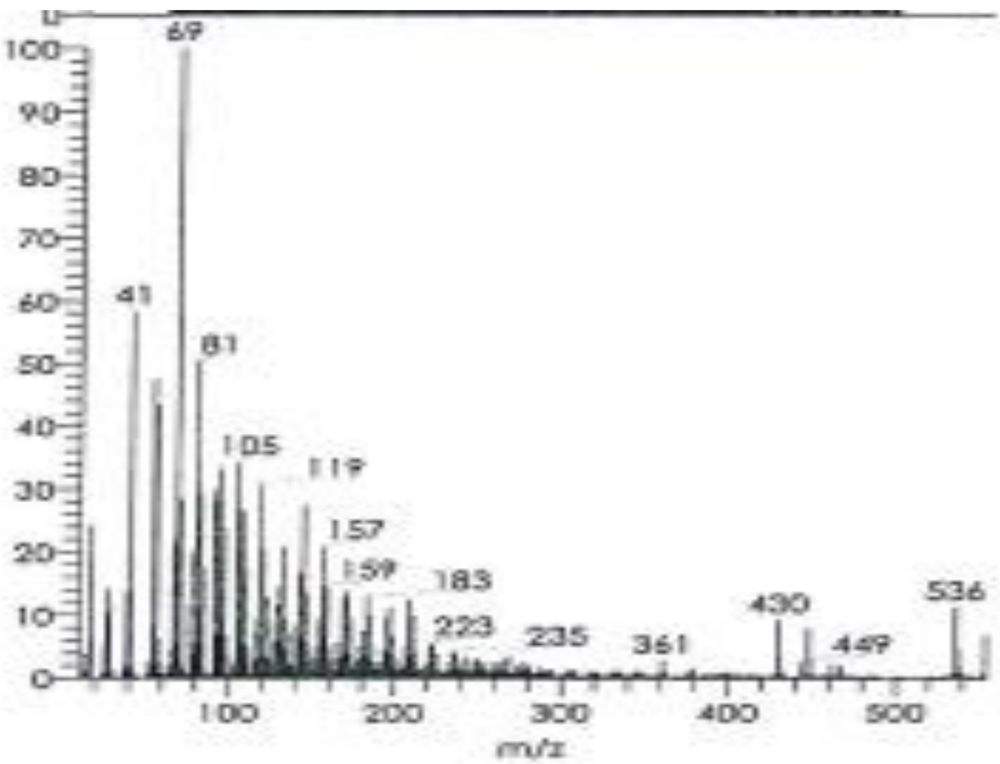
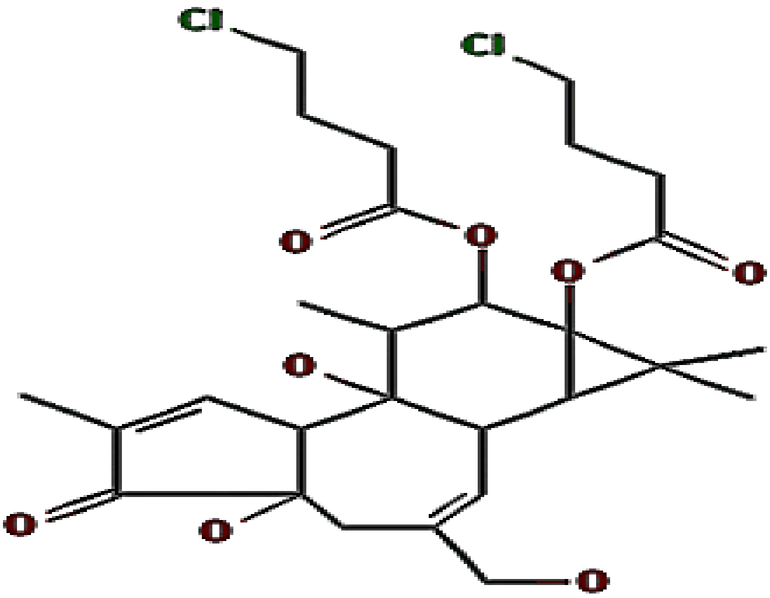


Figure (3.17): mass spectrum of compound number (XVII)



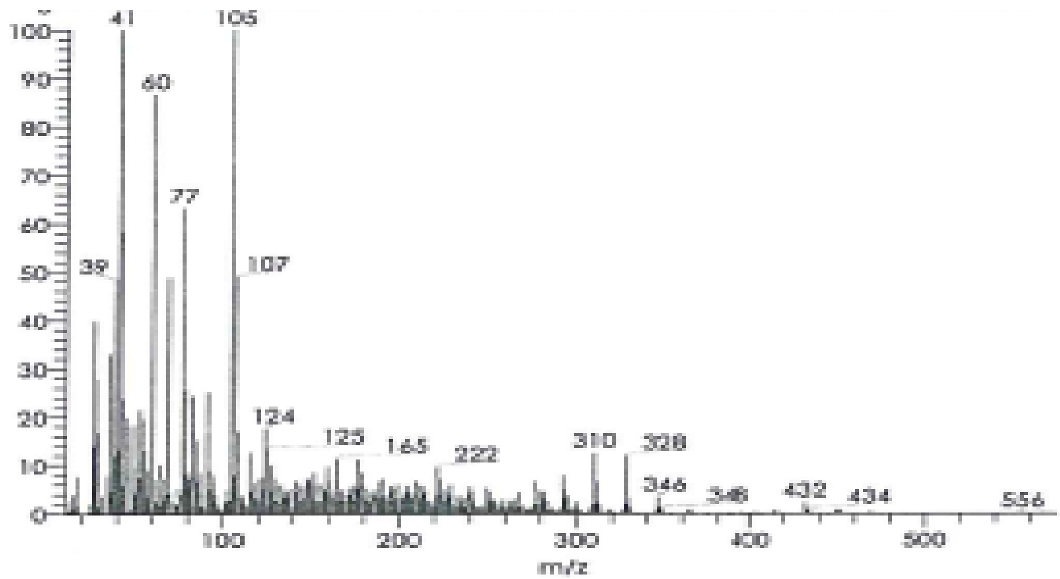


Figure (3.18): mass spectrum of compound number (XVIII)

3. 2 Application to insects :

1.) Data in Table(3.3) demonstrate the repellency effect of the essential oil on *Sitophilus granaria* after 30 minutes of exposure in the area preference bioassay[20].

Table (3. 3):Repellent activity of plants oil of Nerium Oleander L, Ruta graveolens and Basil (Ocimum spp.) aginst Sitophilus granaria after 30 minutes

No	Essential oil extract	Mean % Repellency
1	***Nerium Oleander*** **L**	**100%**
2	***Ruta graveolens***	**98%**
3	**Basil (Ocimum spp.)**	**95%**

2)

3) Acute toxicity of essential oil extract of *N. oleander*, *Ruta graveolens* and *Basil (Ocimum spp.)* was determined on the stored grain pest. granarium and *Culex pipie's Mosquitoes*, following exposing them to (5ml /50ml air)from extracts of plants for 5, 10 and 24 hours in different experiments.

Throughout this study, it was detected the mortality, Fumigant toxicity of essential oils of *N. oleander*, *Ruta graveolens* and *Basil (Ocimum spp.)* on adults of insects noted after 5, 10 and 24 h of exposure, as shown in (Table 3.4, 3.4, 3.5)

Statistical analysis showed that mortality rate is proportional to the type and time of exposure.The outcomes indicated that when applying essential oil extract on insects, they died due to some toxic materials found out in the essential oil extraction . Likewise the results showed that the mortality rate was higher in the insects exposed to the essential oil *N. oleander* and *Ruta graveolens* extracts than in the those insects exposed to essential oil basil extract.

Table(3.4): Mortality percent of plants oil (*N. olender, R. graveolens, Basil*) against selected insect at 5h exposure time.

S.#	**Insect name**	Mortality (%) after 5h		
		N. oleander	*R. graveolens*	*Basil*
1	*Varroa destructor*	50	60	10
2	*Culex pipie's Mosquitoes*	70	50	20
3	*Sitophilus granaria*	20	30	10

Table(3.5): Mortality percent of plants oil (*N. olender, R. graveolens, Basil*) against selected insect at 10 h exposure time.

S.#	**Insect name**	Mortality (%) after 10 h		
		N. oleander	*R. graveolens*	*Basil*
1	*Varroa destructor*	60	80	30
2	*Culex pipie's Mosquitoes*	90	80	40
3	*Sitophilus granaria*	60	50	20

Table(3.6): Mortality percent of plants oil (*N. olender, R. graveolens, Basil*) against selected insect at 24h exposure time.

S.#	**Insect name**	Mortality (%) after 24h		
		N. oleander	*R. graveolens*	*Basil*
1	*Varroa destructor*	80	90	40
2	*Culex pipie's Mosquitoes*	100	100	80
3	*Sitophilus granaria*	80	70	50

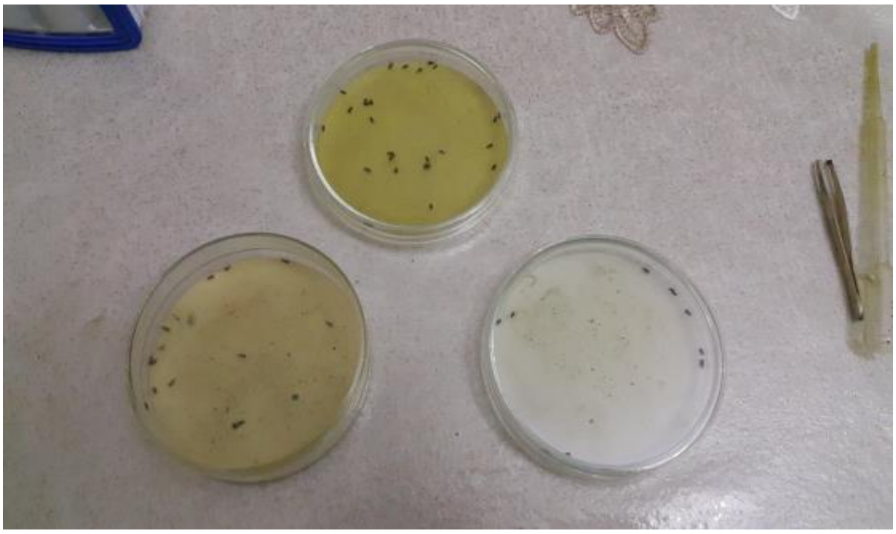


Figure (3.19): Mortality rate of adults of *Sitophilus granaria* noted depending on essential oil extract of *Basil (Ocimum spp.), Ruta graveolens* and *N. oleander*

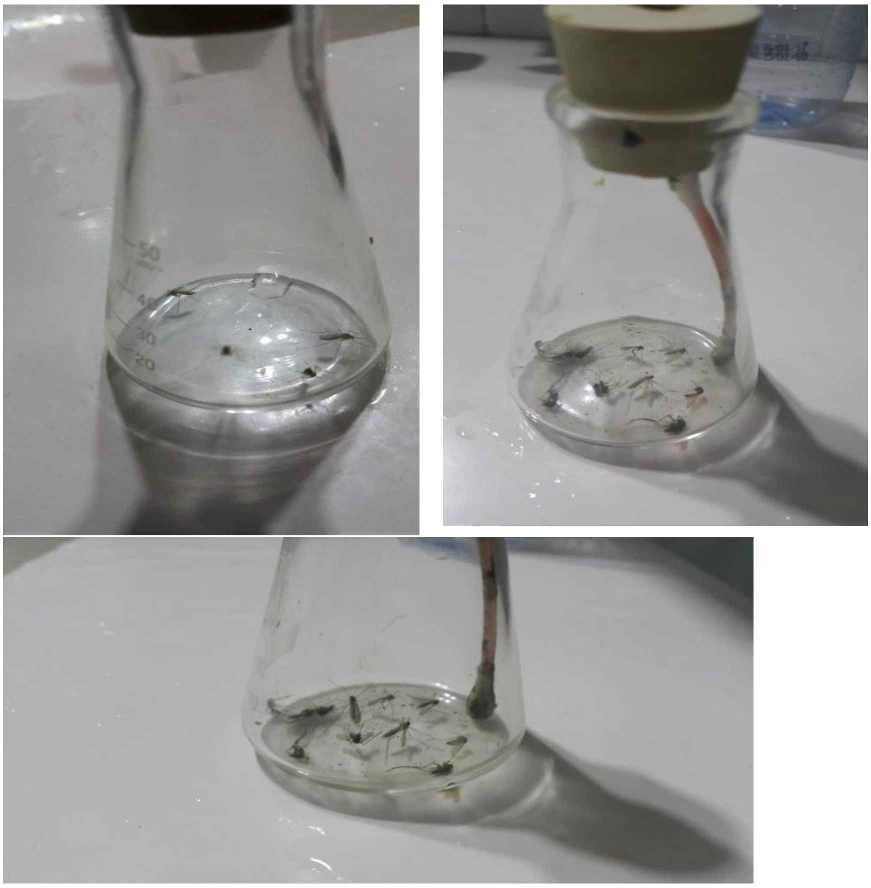


Figure (3.20): Mortality rate of adults of *Culex pipie's Mosquitoes* noted depending on essential oil extract of *N. oleander*

3.3 Discussion and explanation :

Widespread use of chemicals is posing severe hazards to our environment including animal and plant life. For decades, scientists are working for the development of relatively harmless and non-persistent chemicals for the control of contagions and pests. To achieve this end, investigators are looking for plant metabolites, which have been displaying any possible as biocidal agents. It is a notable fact that plants have been evolving for over 400 million years and have established protection mechanisms, such as repellents and even insecticidal effects to protect themselves against insect attack. Different studies have been performed of plant extracts and oils against insects [24-26].

In the present study *N. oleander*, *R. graveolens* and *Basil* (*Ocimum spp*.) extracts were appraised for insecticidal potential against *Varroa* destructor, *Culex pipie's Mosquitoes and Sitophilus granaria* after 5, 10 and 24 hours

of contact.

This result can be clarified by the association that may occur between the insect physiology and the manner of action of essential oils. In fact, these volatile compounds act directly on the nerve structure of the insect which is primitive for larvae and clarifies their resistance to plants essential oil extracts.[27]

It' important to note that the essential oil of *N- olander and Ruta graveolens* have a clear effect as a repellent against the *Sitophilus granaria.*

In addition to this repellent capacity ,there is another effect which can be described as a toxic against the three types of insects.(shown in tables 3.4, 3.5, and 3.6). tables show the % mortality of plants oils against the three types of insects.

In table(3.4), it was noticed that when increasing exposure time the percent mortality goes up. This table revealed that *Culex pipie's Mosquitoes* were affected more than *Varroa* destructor and *Sitophilus* when exposed *to N-oleander* and *Ruta* for 5 hours.

Basil oil had the least effect against all the three insects at the same exposure time. It showed 20% mortality against *Culex pipie's Mosquitoes* and less than 20% against other insects.

In table (3.5), the same results have been recorded as in table 2. When increasing exposure time, the percent of mortality increases.

This table showed that *Culex pipie's Mosquitos* was affected more than *Varroa* and *Sitoghilus* when exposed to *N-oleander* and *Ruta* for 10 hours.

Varroa and *Sitohplilus* had the same mortality percent when exposed to *N-oleander* for the same exposure time.

In contrast, Basil oil had the least effect against all the three insects at the same exposure time. It showed less than 50% mortality against all the three types of insects.

Generally speaking, when exposure time increases, the percent of mortality increases. (Table 3.6) revealed that *Culex pipie's Mosquitoes* was affected more than *Varroa* destructor and *Sitophilus granaria* when exposed *to N. olender and R. graveolens* for 24h. The mortality was 100% and all insects were died. Basil oil was least effect against all insects at the same exposure time. It showed 80% mortality against *Culex pipie's Mosquitoes* and less than 50% against the other insects.

The consequences of phytochemical screening of essential oil extract of *N. oleander*, which were established by us, are in agreement with other studies associated with the *Nerium* family. The type of this family produce flavonoids, coumarins, and triterpenes [28, 29]. The larvicidal activity observed amongst extracts of *N. oleander* could be clarified by the action or effect of phytochemical mechanisms: flavonoids, sterols, terpenes, triterpenes, and coumarins.

Flavonoids have important roles in stress response mechanisms in plants. The adaptive part of flavonoids in plant self-protection against bacterial, fungal, and viral infections as well as insects starts to gain significance in the understanding of plant protection. Flavonoids, which act as antioxidants or enzyme inhibitors, are involved in photosynthesis and cellular energy transmission processes and may serve as predecessors of toxic materials [30, 31] or have a pharmacological activity [30].

Preceding studies showed that the larvicidal activity of *N. oleander* was assessed against *Culex* larvae; 43% of death was found from the concentration of (30 mg/mL) throughout the 24-hour contact [25]. In this the same percent of mortality was observed for a concentration of 60 mg/mL (which is the double of concentration found by the earlier study). In alternative work, Madhuri et al., in 2013 [32], found that *N. oleander* did not display any larvae mortality of *Culex* in 1% aqueous extract, while 100 ppm of oil solution (0.1 mg/mL) of *N. oleander* had 74% mortality in 72 hours.

The results acquired by El-Sayed and Ghada in 2014 [33] showed that diethyl ether extract of *N. oleander* leaves would decrease the population dynamics of *C. pipiens*, with LC50 (10.5 mg/mL). In this study, we found 57.57 mg/mL for the LC50, which is five times more than the concentration obtained by El-Sayed and Ghada.

In the North West of Morocco, one initial assessment study of the larvicidal activity of the *N. oleander* plant on fourth-stage larvae (L4) of the species *C. pipiens* was conducted. In this study, Aouinty et al. in 2006 [34] showed that the aqueous extract of this plant were unsuccessful in terms of toxicity with a calculated LC50 of about (3.13 mg/mL).

The differences between our result and other result may be due to numerous causes such as the ecological circumstances, extraction method, drying, period and gathering sites, agricultural practices, plant age [35–38], concentration of the extract, concentration of its active components, or even factors concerning mosquito. These can impact the performance, the physicochemical features, and the chemical conformation of the extract.

Thus, Fakoorziba et al. in 2015 [39], in southern Iran, determined that there was a high to low fatal effect of extracts of *N. oleander* leaves against mosquito larvae (Diptera: Culicidae): *Anopheles stephensi* depending on the solvent used: chloroform, petroleum, benzene, water, and acetone.

Conversely, numerous studies have reported that all parts of the *N. oleander* plant, including sap, dried or boiled, are toxic to humans, animals, fish, birds, and in specific certain insects [40–43]. Certainly, the leaves contain a blend of a very toxic cardiac glycosides of cardenolides like oleandrin, oleandrigenin, digoxin, digitonin, digitoxigenin, nerizoside, neritaloside, and odoroside [44, 45].

The usage of synthetic insecticides against insects harm human products.

There are substantial interests in the usage of natural products as alternatives of synthetic insecticides. There is a great awareness for a need for new pesticides with a new mode of action to improve efficacy and safety over those chemicals presently in use. Nonetheless, chemical control has been related with hazards like environmental pollution, human toxicity, development of insecticides resistance and adverse effects on non-target organisms [1]. Several plant extracts have been revealed to be active against insects. Amongst these are extracts of neem tree [2]

3.4 Conclusion:

The results of this study indicate that all essential oils extracted from N. oleander, Ruta and Basil have got anti-insect activity and can be exploited to reduce the use of manufactured pesticides and protect the environment from their harm.

These results were compatible with the results of previous studies. Based on this, it is possible to manufacture insecticides from the oils extracted from these plants commercially and have a wide use and less harmful effects on human health and the ecosystem.

This may help future studies isolate the active ingredients and compounds from the oils extracted from these plants and apply them to other types of harmful insects to see the effectiveness and manufacturing of environmentally friendly insecticides.

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جامعة النجاح الطنية
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استخراج وتحديد هوية الزيوت العطرية من النباتات المحلية الفلسطينية ، واستخدامها كمبيد حشري او كطاردة للحشرات

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إشراف
د. وحيد الجندي
د. عروة حوشية

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس – فلسطين
2017

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الملخص

في هذا البحث تم دراسة تأثير الزيوت المستخلصة من ثلاثة نباتات مختلفة (الدفلة، الفيجن والريحان) على الحشرات وهي سوسة القمح، البعوض و فاروا، حيث لوحظ مؤخرا أن هناك زيادة في استخدام المبيدات الحشرية المصنعة كيميائيا، والتي ساهمت في تلوث البيئة وصحة الإنسان. من المهم أن نقول إن هذه الزيوت المستخرجة لديها بعض الفعالية كمبيدات حشرية. وقد تم اختيار هذه النباتات (الدفلة، الفيجن و الريحان) لأن هناك بعض الدراسات السابقة أظهرت فعالية مستخلصات هذه النباتات ضد بعض الأمراض، وأظهرت فعالية كمضادات للميكروبات والفطريات. لذلك، في هذه الدراسة، تم اختبار الزيوت المستخرجة منها كمبيدات حشرية وطاردة للحشرات. وقد تم تطبيقها على سوسة القمح ، البعوض، وعث الفاروا لما لهذه الحشرات آثار ضارة على صحة الإنسان والاقتصاد.

تم جمع النباتات من مركز البحوث الزراعية (نارك) حيث تم جمعها من خلال فترة الازهار ثم أخذت إلى المختبر لاستخراج الزيوت منها. تم أخذ 800 جرام من كل نبتة حيث تم تجفيفها وتقطيعها إلى قطع صغيرة وتم نقعها في الإيثانول لمدة 48 ساعة، ثم تم تصفية المذيب وتبخيره للحصول على الزيوت المستخلصة. تم إجراء كل من التحاليل التالية (TLC و GC-MS) من أجل تحديد المركبات الأساسية التي تشكل الزيوت المستخلصة .

بعد ذلك، تم اختبار الزيوت المستخلصة من هذه النباتات على الحشرات المذكورة أعلاه، والتي تم الحصول عليها من كلية الزراعة في جامعة النجاح حيث تم اختبار الزيوت المستخرجة كطارد

لسوسة القمح عن طريق وضع كمية من الزيت المستخلص من كل نبتة على ورقة ترشيح و وتركها قليلا في الهواء لتبخير المذيب ومن ثم وضع الحشرات عليها. ثم تم اختبار هذه الزيوت إذا كان لديها فعالية كمبيد حشري من خلال عملية التبخر.

وأظهر تحليل GC-MS أن هناك مركب مشترك بين الفيجن والريحان (IV) * كان له فعالية كمبيد للحشرات. وعلاوة على ذلك، كان هناك مركب مشترك اخر بين الفيجن والدفلة مشترك (I) *. من الاختبار الذي تم إجراؤه على الحشرات ، لوحظ أن الزيت المستخلص من الدفلة كانت فعاليته 100٪ كطارد لحشرة سوسة القمح بينما نسبة فعالية الزيت المستخلص من الفيجن والريحان كطارد لهذه الحشرة فكانت (98٪) و (95٪) على التوالي.

من ناحية أخرى، لوحظ أن نسبة الوفيات الحشرات التي تعرضت الى الزيوت العطرية المستخلصة من الدفلة والفيجن هي أعلى نسبة حيث كان معدل الوفيات في حشرة البعوض ما يقرب 100٪. في حين كان أعلى معدل وفيات (80٪) للبعوض عند تعرضه للزيت المستخلص من الريحان . وكان معدل الوفيات للحشرات الاخرى أقل مقارنة بالنتائج السابقة.

ينبغي إجراء المزيد من الدراسات من أجل عزل وتوصيف فعالية السمية وكفاءتها الفعالة كمصدر طبيعي لمبيدات الحشرات.