IJBPAS, June, 2013, 2(6): 1282-1306

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL COMPOSITION OF *Solanum elaeagnifolium cav.* AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Solanum elaeagnifolium (family *Solanaceae*) is among the underutilized nine wild solanum species in Egypt. The present study was carried out to investigate the antibacterial activity of the petroleum ether, chloroform, methanol and water extracts of this plant against antibiotic resistant bacteria. 114 bacterial isolates from 94 hospitalized patients (male & female) were collected and subjected to this study. From the collected 114 isolates, 25 were antibiotic resistant bacteria namely: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*. Different extracts of *S. elaeagnifolium* were tested for its antibacterial activity. The lipid fraction (ether extract) showed the highest antibacterial activity. Its fatty acids and hydrocarbons composition was identified using GC/Mass. 13 fatty acids were identified among them: palmitic (38%), 9,12-octadecadienoic acid (18%) and stearic acid (16%) were the major fatty acids detected**.** While 12 hydrocarbons and 2 sterols were identified from them: octacosane (36%), heptacosane (13%) and triacontane (12.7%) comprised the major hydrocarbons fraction of *S. elaeagnifolium.* This work through light on the potential value of underutilized *S. elaeagnifolium* as a future source of antibacterial agent for the antibiotic resistant bacteria.

Keywords: *Solanum elaeagnifolium,* antibacterial activity, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*

INTRODUCTION

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller **[1]**.

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal plants still play a vital role to cover the basic health needs in the developing countries. The most important chemical bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds **[2]**. Consumers are also seeking natural foods and natural preservatives for healthier lifestyles and natural ways of preventing ailments. So, medicinal plants are also being sought for their medicinal value, as antioxidants and as antimicrobials **[3]**.

Solanaceae family is almost worldwide in distribution, however, the majority of genera and species are neotropical. *Solanaceae* family according to **Cuevas-Arias *et al.*, 2008**, **[4]** comprises 96 genera and almost species are *Solanum* L. (1,000 spp.), *Lycianthes* (Dunal) Hassl. (200 spp.), *Cestrum* L. (175 spp.), *Nicotiana* L. (95 spp.), *Physalis* L. (80 spp.), and *Lycium* L. (75 spp.).

Hence, these studies are very important in discovering effective but at low cost antimicrobial compounds. Although antimicrobial activities of genus *Solanum* were studied, there is little information about antimicrobial activity of some of the *Solanum* sp. Among them *Solanum melogena* **[5]**.

The methanol and aqueous extracts of leaves of five different medicinal plants, *Solanum nigrum* L., *S. torvum* Sw., *S. trilobatum* L., *S. surattense* Burm. and *S. melongena* L. are belonging to Solanaceae family were used for the investigation of antibacterial studies. In antibacterial screening performed by disc diffusion method against two gram negative bacteria namely *Xanthomonas campestris* (plant pathogen) and *Aeromonas hydrophila* (animal pathogen), it was found that the methanol extracts of all the plant samples showed significant activity against the two tested bacteria **[6, 7]**. The extracts of *Solanum xanthocarpum* showed high sensitivity to *Kiebsiella pneumoniae* and *Salmonella typhi*, moderate sensitivity to *Escherichia coli* and less sensitivity and resistant to *Bacillus cereus* **[8]** While, *Solanum palinacanthum* Dunal, which presented activity against *Aeromonas hydrophila*, *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus*

ochraceus. *Solanum palinacanthum* is a perennial herb or sub-shrub **[9]**.

Family Solanaceae is represented in Egyptian flora with ten genera out of the 94 worldwide genera. Egyptian flora comprises of genus *Solanum* is including 9 species out of 1700 species worldwide **[10]**. *S. elaeagnifolium* Cav. will be subjected to phytochemical screening. The bioactive fraction against the antibiotic resistant bacteria will be identified. This study aimed to utilize the wild Egyptian genetic resources to obtain cheap antimicrobial drugs.

MATERIAL AND METHODS

Plant Material and Extraction

Fresh plant material of *Solanum elaeagnifolium* Cav. (Family: Solanaceae) was collected at Burg el Arab (western Mediterranean coast), canal banks. Leafy branches of *S. elaeagnifolium* were air-dried in shade, and then subjected to drying oven at 40°C to constant weight. The dried material was powdered and kept in plastic bags, and subjected later to extraction.

Fifty grams of air-dried powder plant material was extracted successively using the following solvents: petroleum ether, chloroform, methanol and water by using a soxhlet extractor until colorless extract obtained on the top of the extractor. Extract of each solvent was concentrated under reduced pressure using rotary evaporator and dissolved in dimethyl sulfoxide (DMSO), and then subjected to antimicrobial activity assay according to **[11]**.

Susceptibility of bacterial isolates to antibiotics

Bacterial isolates (114 isolates), were isolated from 94 patients (56 males and 58 females) hospitalized in El-Demerdash hospital during three months period. Of the 114 isolates, 22 were isolated from urine; 13 from blood; 57 from different wounds discharge; 12 from sputum; and 4 from throat. The bacteria were identified by traditional biochemical tests **[12]**. The used reference bacterial strains were: *Staphylococcus aureus* ATCC 29737, *Esherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATTC 25619. The test organisms were sub-cultured at 37°C and maintained on nutrient agar media.

The bacterial isolates were tested for its bacterial resistance using disk diffusion method. Antibiotic disks (Oxoid) used were cefaclor (30 µg), tobramycin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), cefadroxil (30 µg) and sulphamthazole trimethoprim (25 µg). The diameters of inhibition zones were measured. Zones of inhibition were determined according to **CLSI M100-S21 (2011**) **[13]**, isolates were

categorized as susceptible and resistant while intermediate were considered as resistant. The experiment was done three times and the mean values were presented.

Antibacterial Activity

Petri plates containing 20 ml of Muller Hinton agar medium were seeded with a 24 h culture of the bacterial strains. Wells of 6mm diameter each were cut into the agar; to each well 50 µl (concentration of 100 mg/ml) of the investigated plant extracts were tested added. The inocula size was adjusted so as to deliver final inocula of approximately 108 colony-forming units (CFU)/ml. Incubation was performed at 37°C for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone around the wells after 24 h.

Determination of Minimum Inhibitory Concentration (MIC) and Antimicrobial Combination

The Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method in Mueller Hinton agar medium (**Oxoid)** according to **EUCAST (2000) [14]**. The extracts were dissolved in DMSO, and diluted to give serial two-fold dilutions ranging from 0.05 to 5 mg/ml (The final concentration of DMSO in the assay did not exceed 2%). Before gelling, 20 ml of agar medium were added to each of the Petri dishes containing the plant extract, 2 µl of each bacterial strain (104 CFU/ml) were inoculated on the Mueller Hinton agar surface. MIC was defined as the lowest extract concentration, showing no visible bacterial growth after incubation time (37℃ for 24h). The experiment was done three times and the mean values were presented.

Antimicrobial combination of ciprofloxacin and the *Solanum elaeagnifolium* carried out according to **Eliopoulos (1996) [15],** Interactions between ciprofloxacin and the *S. elaeagnifolium* on sabroad Agar - 1199B and the survivors of the time-kill experiments were evaluated by a checkerboard titration assay in tubes. The bacterial inocula were prepared, Ciprofloxacin was tested at nine concentrations (1024, 256, 512, 128, 64, 32, 16, 8 and 4 µg/ml), and *S. elaeagnifolium* was tested at nine concentrations (1024 to 4 µg/ml). Rows of tubes containing each drug alone at the same concentrations were also included. Tubes were assessed visually for growth after an 18 h incubation period at 37°C. The effect of drug combinations was estimated at the point of maximal effectiveness by the fractional inhibitory concentration index (ΣFIC), *i.e*., the sum of the fractional inhibitory concentration of each drug, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone.

Preparation and Identification of the Lipids Material

A. Separation of Unsaponifiable Lipid Fraction

The extracted lipids (Petroleum ether extracts) of the studied plant was saponified with alcoholic potassium hydroxide by dissolving about five grams of lipid from each plant in 480ml ethanol. This ethanolic solution was mixed with solution of 40 gram of potassium hydroxide in 100 ml distilled water and the mixture was refluxed for about three hours. The solution was concentrated to two third of its volume, excess water was added and the soap solution was shaken in a separating funnel for several times with fresh portion of peroxide free ether until complete extraction was obtained. The combined ether extracts were washed with water until free from alkalinity as indicated by litmus paper, dried over anhydrous sodium sulfate then filtered. The filtrate was evaporated to dryness under vacuum. Weigh the residue and this represents the quality of hydrocarbons and sterols obtained and this converted to the methyl ester with ethereal diazomethane as following: Methyl esters were obtained by trans-methylation of the lipids by refluxing them for 90 min with methanol – benzene – sulfuric acid (20:10:1) according to **Harborne (1973) [16] and Vogel's (2000) [17],** the solution was concentrated to two third of its volume, excess water was added for washings until free from acidity as indicated by litmus paper, dried over anhydrous sodium sulfate and filtered, the filtrate was subjected to analysis using GC/Mass.

B. Separation of Saponifiable Lipid Fraction

After removal of unsaponifiable fraction with ether, soapy solution was converted into the corresponding free fatty acid by means of 2.5% sulfuric acid, and the librated free fatty acids were extracted with ether. The ether extract was washed several times with distilled water until free from acids. The ether extract was dried over anhydrous Na_2SO_4 and filtered, followed by distillation and the last traces of ether were removed under vacuum at 60 °C, and kept in desiccators. Weigh the residue and this represents the quality of fatty acids obtained and this converted to

the corresponding methyl ester which was analyzed by GC/Mass.

GC/Mass of Unsaponifiable and Saponifiable Lipid Fractions [18]

The investigation carried out by GC/Mass (HP5890), oven program (initial temp: 50 °C, initial time: 2 min, rate 1: 10°C/min, final temp.: 200, final time: 5min), injection temp. : 220, injection volume 1µl, injection mode: splitless, carrier gas: N.gas and Detector temps: 300 °C.

RESULTS

The studied 114 bacterial isolates obtained from hospitalized patients in Egypt were identified by traditional biochemical tests **[12].** Among 114 of the studied isolates, 6 were Gram positive which is identified as *Staphylococcus aureus* and 19 were Gram negative (14 isolates were *Escherichia coli* and 5 isolates were *Pseudomonas aeruginosa*).

The studied bacterial isolates were tested for its susceptibility to different antibiotics **Table 2.** using disk diffusion method. The results presented in **Table 2**, indicating that these bacterial isolates are antibiotic resistant to studied antimicrobial agents **(Table 1)** (cefaclor, tobramycin, chloromphenicol, erythromycin, sulphamthazole trimethoprim, levofloxacin, ciprofoxacin and cefadroxil).

Extracts of *Solanum elaeagnifolium* (ether, chloroform, methanol and water) were screened for their antimicrobial activity against 25 bacterial isolates and the results are presented in **Table 2**. On application of the ether extract, the inhibition zone ranged from 52mm to 29mm **(Table 2)**. The highest inhibition zone was against *E. coli* isolates followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Concerning of chloroform extract, the inhibition zone was lower than ether extract, it ranged from 21mm to zero mm as shown in **Table 3**. The higher inhibition zone was against Staphylococcus *aureus* followed by *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*.

Methanol and water extracts of the studied plant showed negative results (no inhibition zone was detected) against the all studied bacterial isolates.

MIC of Ciprofloxacin Compared to Ether Extract Against the Studied Bacterial Isolates

However the commercial antibiotic "Ciprofloxacin" gave different MIC values **(Table 4)** against the studied 25 antibiotic resistant bacterial strains. These values ranging from 16-64 µg/ml in case of *S. aureus;* 8-512 µg/ml in case of *E. coli* and 32-256 µg/ml in case of *Ps. Aeruginosa*.

The studied ether extract of *S. elaeagnifolium* against some bacterial strains showed constant inhibition activity, (MIC 3000 µg/ml)

Antimicrobial Combination

The Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method in Mueller Hinton agar medium (Oxoid) according to **[14]**. The extracts were dissolved in DMSO, and diluted to give serial two-fold dilutions ranging from 0.05 to 5 mg/ml (The final concentration of DMSO in the assay did not exceed 2%). The effect of drug combinations was estimated at the point of maximal effectiveness by the fractional inhibitory concentration index (ΣFIC), *i.e.*, the sum of the fractional inhibitory concentration of each drug, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone.

(ΣFIC), *i.e.*, the sum of the fractional inhibitory concentration of each drug, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone. ΣFIC = ((MIC of Cipro. In combination/MIC of Cipro alone) + (MIC of *S. elaeagnifolium* in combination / MIC of *S. elaeagnifolium* alone).

The ΣFIC data were interpreted according to the following criteria: **synergy** was defined as an ΣFIC of < or = 0.5, **addition** was defined as 0.5 > ΣFIC = or < 1, **indifference** was defined as 1 > ΣFIC < or = 2, and **antagonism** was defined as an ΣFIC of > or = 2. All experiments were carried out at least three times, and results are expressed as the mode values.

When using different concentrations of Ciprofloxacin ranges from (4-1024) µg/ml and combined to similar ranges of *S. elaeagnifolium* ether extract **(Table 5)**, the MICs of different concentrations of ciprofloxacin were changed.

In vitro interactions between antimicrobial agents and plant extract using the previous method increased the antimicrobial agent effect of antibiotics against bacterial isolates. This comination showed Antagonism against six isolates (four of them are *Escherichia coli* and two are *Staphylococcus aureus*), and additive against three isolates (two of them *Pseudomonas aeruginosa* and one *Escherichia coli*). While the synergistic effects between combination of antibiotic and plant extracts with significant reduction in the MICs of the test antibiotics against nineteen isolates as shown in **Table 6**.

GC/Mass

The saponified matter of the lipid material extracted from the studied plant was applied to GC/Mass. The resulted are outlined in **Table 7**, 13 fatty acids were detected. The major fatty acids detected from *Solanum elaeagnifolium* are Palmitic acid (35%), 9, 12-Octadecadienoic acid (18%) and Stearic acid (16%). Total ionic chromatogram is outlined in **Figure 1**, while the

fragmentation pattern of the major fatty acid are outlined in **Figures 2-5**.

The unsaponified matter of the lipid material extracted from studied specimen were applied to GC/Mass. 14 hydrocarbons were detected **(Table 8)**. The major hydrocarbons in *Solanum elaeagnifolium* are: octacosane (36%), heptacosane (13%) and triacontane (12.7%). Total ionic chromatogram is outlined in **Figure 6**, while the fragmentation pattern of the major fatty acid are outlined in **Figures (7-9)**.

Table 1: Antimicrobial Agents Used in this Study

Antibiotic Class	Antibiotic Name & Symbol	Disc Conc.	Diameter of the inhibition zone in millimeter (mm)		
			Resistant (R) = or <	Intermediate (I) From- To	Sensitive (S) = or ›
Amino glycosides	Tobramycin TOB	10 µg	12	13-14	15
Cephalosporin's	Cefadroxile CRF	30 µg	14	15-17	18
Macrolides	Erythromycin E	15 µg	15	16-20	21
Quinolones	Ciprofloxacin CiP	5 µg	15	16-20	21
	Levofloxacin Levo	5 µg	13	14-16	17
Sulfonamides	Sulfamethazol Trimethoprime SXT	10 µg	10	11-15	16
B- lactams	Cefaclor CEC	30 µg	14	15-17	18
Miscellineous	Chloramphenicol C	30 µg	17	18-20	21

Table 2: Susceptibility Test of Six *Staphylococcus aureus*, Forteen *Escherichia coli* and Five *Pseudomonas aeruginosa* to Standard Antibiotics

Antibiotics	CRF	C 30	E 15	TOB 10	SXT 25	LEV 5	CIP 5	CEC 30
A 1	0 R	10 R	0 r	7 r	20 S	10 r	0 r	0 r
A 2	12 R	8 R	1 r	15 s	25 S	15 i	12 r	12 r
A 8	0 R	10 R	0 r	0 r	20 S	10 r	0 r	0 r
A 10	0 R	25 S	0 r	0 r	22 S	6 r	0 r	0 r
A 15	0 R	25 S	0 r	0 r	20 S	10 r	0 r	0 r
A 30	0 R	23 S	0 r	10 r	18 S	18 s	0 r	0 r
G 1	0 R	10 R	10 r	21 s	0 R	27 s	30 s	0 r
G 2	0 R	0 R	12 r	0 r	0 R	10 r	21 s	0 r

G 3	**0 R**	**0 R**	**0 r**	**0 r**	**0 R**	**0 r**	**0 r**	**0 r**
G 4	**0 R**	**0 R**	**0 r**	**0 r**	**0 R**	**0 r**	**0 r**	**0 r**
G 5	**0 R**	**13 R**	**0 r**	**0 r**	**0 R**	**20 s**	**29 s**	**0 r**
G 32	**0 R**	**0 R**	**0 r**	**0 r**	**0 R**	**0 r**	**0 r**	**0 R**
C 3	**0 R**	**0 R**	**0 r**	**8 r**	**0 R**	**7 r**	**8 r**	**0 R**
C 4	**0 R**	**22 S**	**0 r**	**11 r**	**15 S**	**8 r**	**0 r**	**0 R**
C 8	**6 R**	**25 S**	**0 r**	**2 s**	**0 R**	**1.2 r**	**9 r**	**0 R**
C 10	**0 R**	**25 S**	**0 r**	**11 r**	**0 R**	**9 r**	**0 r**	**0 R**
C 13	**0 R**	**2 I**	**0 r**	**8 r**	**0 R**	**6 r**	**0 r**	**0 R**
C 15	**0 R**	**1 R**	**0 r**	**0 r**	**0 R**	**7 r**	**0 r**	**0 R**
C 18	**0 R**	**0 R**	**0 r**	**18 s**	**13 I**	**0 r**	**0 r**	**0 r**
C 21	**0 R**	**22 S**	**0 r**	**9 r**	**0 R**	**0 r**	**0 r**	**0 r**
C 22	**0 R**	**22 S**	**0 r**	**6 r**	**0 R**	**7 r**	**0 r**	**0 r**
C 24	**0 R**	**2 I**	**0 r**	**8 r**	**0 R**	**11 r**	**0 r**	**0 r**
C 26	**0 R**	**22 S**	**0 r**	**12 r**	**0 R**	**1 r**	**0 r**	**0 r**
C 27	**0 R**	**19 I**	**0 r**	**11 r**	**13 I**	**8 r**	**0 I**	**0 r**
C 28	**0 R**	**0 R**	**0 r**	**0 r**	**0 R**	**0 r**	**0 r**	**0 r**
C 30	**0 R**	**12 R**	**0 r**	**0 r**	**0 R**	**0 r**	**0 r**	**1 r**
C 34	**0 R**	**0 R**	**0 r**	**0 r**	**2 S**	**24 s**	**0 r**	**18 s**

NOTE: (r: Resistant; i:Intermediate; s: Sensitive).

Table 3: Inhibition Zone of Ether and Chloroform Extracts of *Solanum elaeagnifolium* Against Studied 25 Bacterial Isolates in mm (A: *Staphylococcus aureus, C: Escherichia coli and G: Pseudomonas aeruginosa)*

Tested organisms		**Mean Diameter of the studied extracts inhibition zone (mm)**	
		Petroleum ether	**Chloroform**
St ap hy loc oc cu s	**Control**	**52**	**21**
	A1	**30**	**13**

	A2	**35**	**12**
	A8	**40**	**20**
	A10	**40**	**17**
	A15	**40**	**18**
	A30	**40**	**15**
Escherichia coli	**Control**	**52**	**15**
	C3	**52**	**__**
	C3	**41**	**12**
	C8	**40**	**15**
	C10	**40**	**12**
	C13	**50**	**12**
	C18	**40**	**__**
	C21	**40**	**__**
	C22	**42**	**12**
	C24	**50**	**12**
	C26	**40**	**___**
	C27	**42**	**___**
	C28	**50**	**___**
	C30	**35**	**__**
	C43	**40**	**12**
Pseudomonas aeruginosa	**Control**	**41**	**20**
	G2	**39**	**17**
	G3	**40**	**___**
	G4	**30**	**15**
	G5	**29**	**19**
	G32	**39**	**17**

Table 4: The MIC of Ciprofloxacin Against the Studied Bacterial Isolates

		Different ciprofloxacin antibiotic concentration (µg/ml)								
	Bacterial isolates	**512**	**256**	**128**	**64**	**32**	**16**	**8**	**4**	**MIC**
Staphylococcus aureus	**Control**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**16**
	A1	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**64**
	A2	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**32**
	A8	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**64**
	A10	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**64**
	A15	**-**	**-**	**-**	**-**	**-**		**+**	**+**	**16**
	A30	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**64**
Escherichia coli	***E. coli***	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**32**
	C3	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**32**
	C4	**-**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**256**
	C8	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C10	**-**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**256**
	C13	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C18	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**64**
	C21	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C22	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C23	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C26	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**32**
	C27	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C28	**-**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**256**
	C30	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**512**
	C34	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**8**

Pseudomonas aeruginosa	**Ps.**	-	-	-	-	-	+	+	+	**32**
	G2	-	-	-	-	+	+	+	+	**64**
	G3	-	-	+	+	+	+	+	+	**256**
	G4	-	-	-	-	+	+	+	+	**64**
	G5	-	-	+	-	+	+	+	+	**64**
	G32	-	-	-	-	+	+	+	+	**64**

Table 5: Combination Between Ciprofloxacin and the Ether Extract of *Solanum elaeagnifolium* Against Studied Bacterial Isolates

Conc. of Ether extract	Concentration of Ciprofloxacin								
	1024	**512**	**256**	**128**	**64**	**32**	**16**	**8**	**4**
1024									
512									
256									
128				**C4,C28**				**C4,C28, C8**	
64			**C27,C13 C18,C30**	**C34,C10 C21,G2**		**A1**	**G32**		
32									
16									
8									**A2**
4			**C22**	**A30,C3 A8,G3**	**G4,C23 C26,G5**	**A10**		**A15**	***St, E. coli, Ps.***

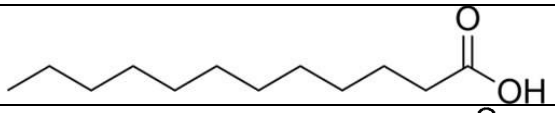
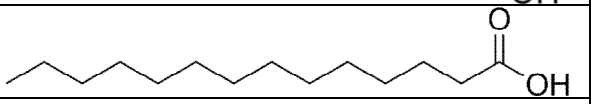
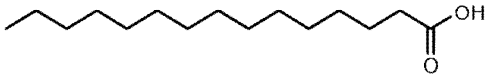
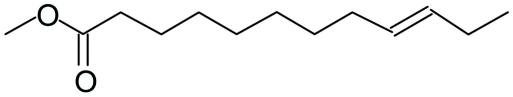
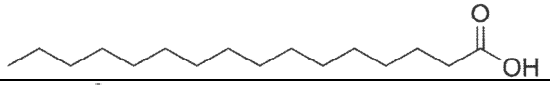
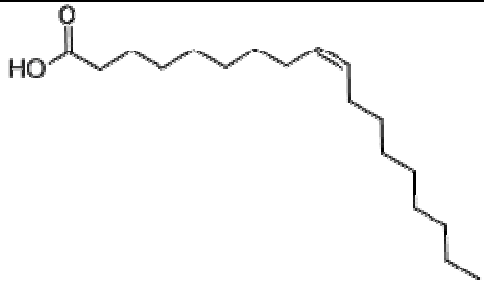
NOTE: The combination between Ciprofloxacin and ether extract of *Solanum elaeagnifolium* showed that: The combination between the concentrations 1024-512-256 of Ciprofloxacin and the concentrations 1024-512-256-128 of the ether extract killed all bacterial isolates. Then the bacterial isolates started to resist this combination beginning of the concentrations 128 of Ciprofloxacin and 128 of ether extract. Each bacterial isolates can resist according to it's MIC

Table 6: The ΣFIC of Studied Bacterial Isolates

	Bacterial isolates	The ΣFIC	The ΣFIC criteria
Staphylococcus aureus	**Control**	**0.2**	**Synergy**
	A1	**0.5**	**Synergy**
	A2	**0.1**	**Synergy**
	A8	**2**	**Antagonism**
	A10	**0.5**	**Synergy**
	A15	**0.1**	**Synergy**
	A30	**2**	**Antagonism**
Escherichia coli	**E. coli**	**0.1**	**Synergy**
	C3	**4**	**Antagonism**
	C4	**0.5**	**Synergy**
	C8	**0.05**	**Synergy**
	C10	**0.5**	**Synergy**
	C13	**0.5**	**Synergy**
	C18	**4**	**Antagonism**

	C21	**0.1**	**Synergy**
	C22	**0.5**	**Synergy**
	C23	**1**	**Addition**
	C26	**2**	**Antagonism**
	C27	**0.5**	**Synergy**
	C28	**0.5**	**Synergy**
	C30	**0.5**	**Synergy**
	C34	**16**	**Antagonism**
Pseudomonas aeruginosa	**Ps**	**0.1**	**Synergy**
	G2	**0.5**	**Synergy**
	G3	**0.5**	**Synergy**
	G4	**1**	**Addition**
	G5	**1**	**Addition**
	G32	**0.2**	**Synergy**

Table 7: Fatty Acids of *Solanum elaeagnifolium* Detected and Identified by GC- Mass; the Ionic Chromatogram Outlined in Figure 1

Compounds	**R_t.**	**Conc. %**	**Formula**	***Structure***
lauric acid	**13.2**	**0.5**	**$C_{12}H_{24}O_2$**	
tetradecanoic acid	**15.5**	**4.**	**$C_{14}H_{28}O_2$**	
pentadecanoic acid	**16.4**	**2**	**$C_{15}H_{30}O_2$**	
9-dodeconoic acid	**16.5**	**1**	**$C_{13}H_{24}O_2$**	
palmitic acid	**17.5**	**35**	**$C_{16}H_{32}O_2$**	
9-hexadecenoic acid	**17.7**	**1.5**	**$C_{17}H_{32}O_2$**	

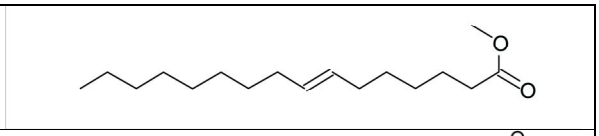
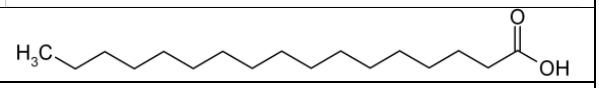
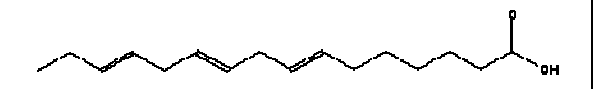
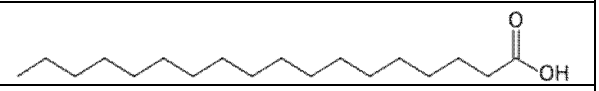
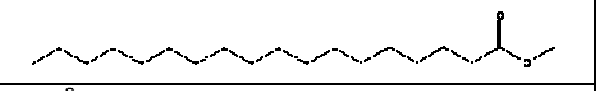
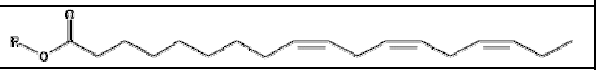
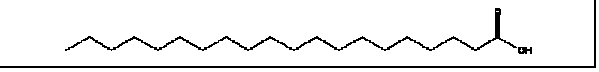
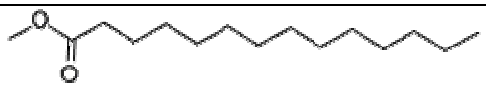
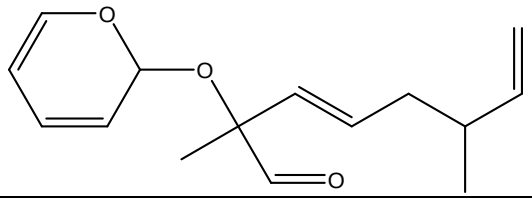
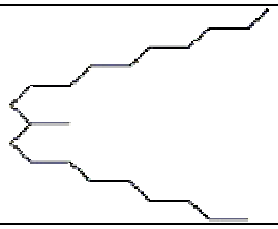
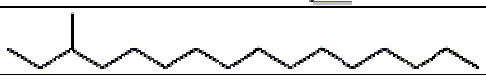
7- hexadecenoic acid	**17.9**	**4**	**$C_{16}H_{30}O_2$**	
heptadecenoic acid	**18.5**	**2**	**$C_{17}H_{34}O_2$**	
7,10,13-hexadecatrienoic acid	**18.8**	**3**	**$C_{16}H_{26}O_2$**	
stearic acid	**20.0**	**16**	**$C_{18}H_{36}O_2$**	
9,12-octadecadienoic acid	**21.0**	**18**	**$C_{18}H_{32}O_2$**	
linolenic acid	**22.3**	**11%**	**$C_{18}H_{30}O_2$**	
eicosanoic acid	**23.5**	**2 %**	**$C_{20}H_{40}O_2$**	

Table 8: Hydrocarbons Detected by GC-Mass; the Ionic Chromatogram is Outlined in Figure 6

Compounds	Rt.	Conc. %	Formula	*Structure*
methyl tetradecanoate	**10.2**	**0.7**	**$C_{15}H_{30}O_2$**	
2,6-octadienal, 2,6-dimethyl-8-(tetrahydro-2H-2 pyranyloxy)	**13.6**	**1.5**	**$C_{15}H_{20}O_3$**	
17-Pentatriacontene	**17.9**	**0.9**	**$C_{35}H_{70}$**	
hexatriacontane	**19.4**	**1.9**	**$C_{36}H_{74}$**	
10-methyl- eicosane	**20**	**4.6**	**$C_{21}H_{44}$**	
3-methylhexadecane	**20.6**	**4**	**$C_{17}H_{36}$**	
heptacosane	**21.8**	**13**	**$C_{27}H_{56}$**	

triacontane	**22.5**	**12.7**	**$C_{30}H_{62}$**	
n-eicosane	**23.4**	**6.6**	**$C_{20}H_{42}$**	
octacosane	**24**	**36**	**$C_{28}H_{58}$**	
heptadecane	**24.8**	**8**	**$C_{17}H_{36}$**	
hexadecane	**25.3**	**2.1**	**$C_{16}H_{34}$**	
pentadecane	**26.2**	**1**	**$C_{15}H_{32}$**	
n-heneicosane	**26.9**	**5.5**	**$C_{21}H_{44}$**	

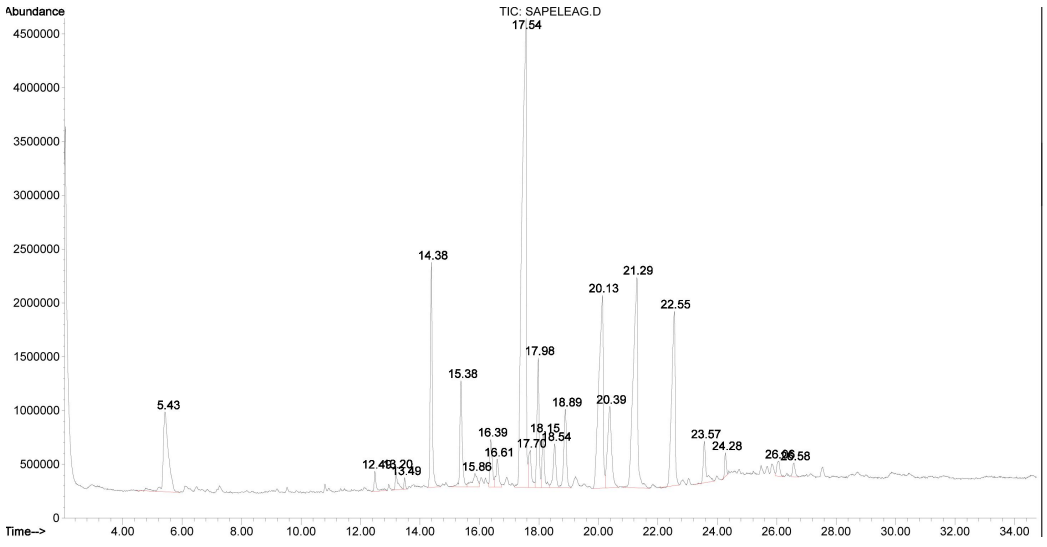


Figure 1: Toltal Ionic Chromatogram (TIC) of *S. elaeagnifolium* Fatty Acids Identified by GC/Mass

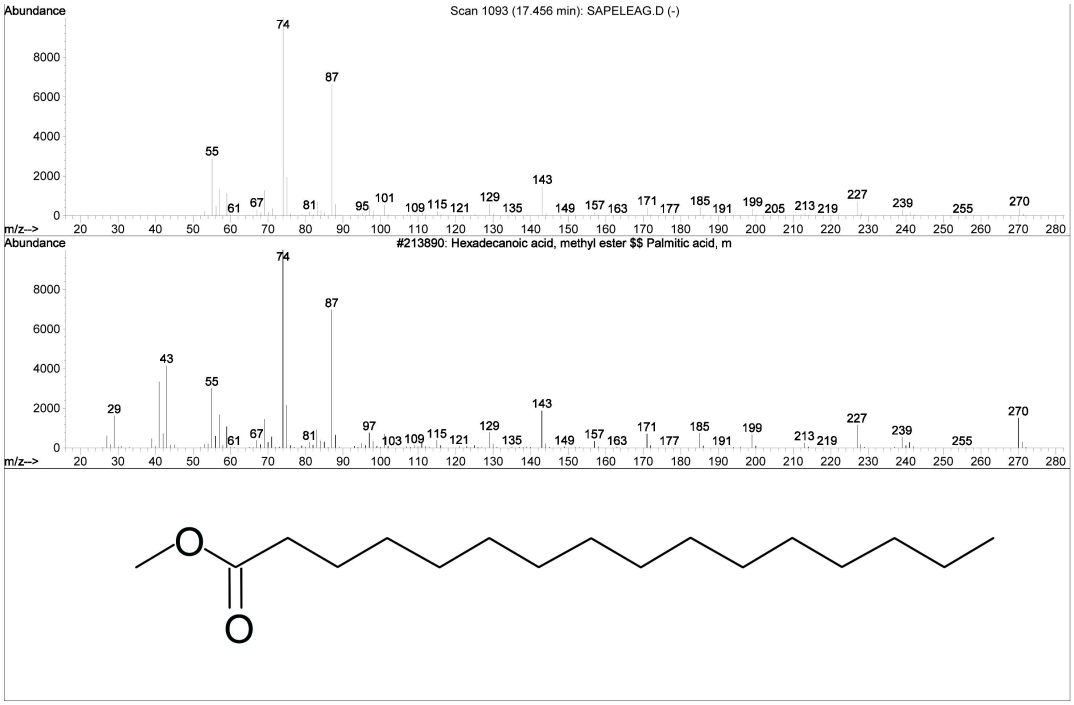


Figure 2: Fragmentation Pattern of Palmitic Acid Using GC/Mass

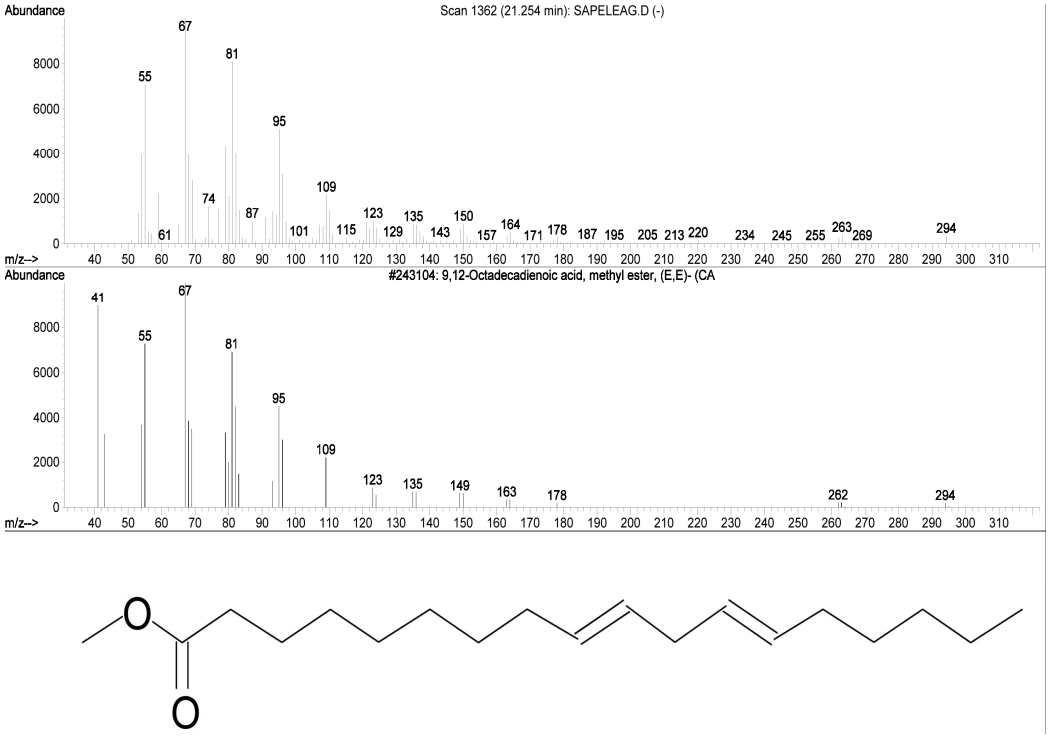


Figure 3: Fragmentation Pattern of 9,12- octadecadienoic Acid Using GC/Mass

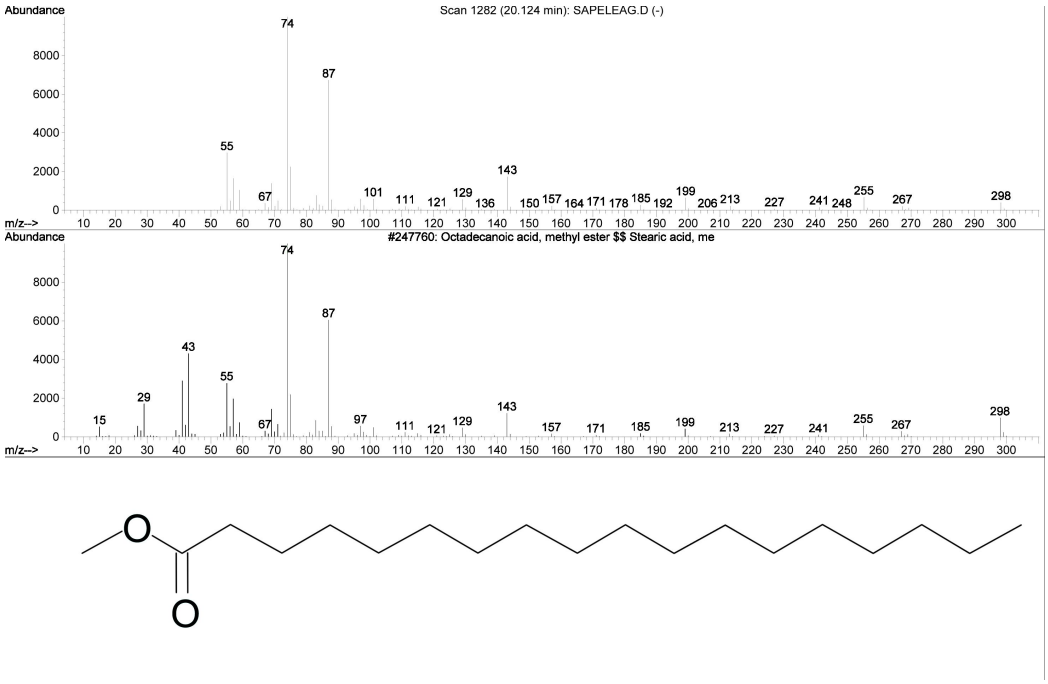


Figure 4: Fragmentation Pattern of Stearic Acid Using GC/Mass

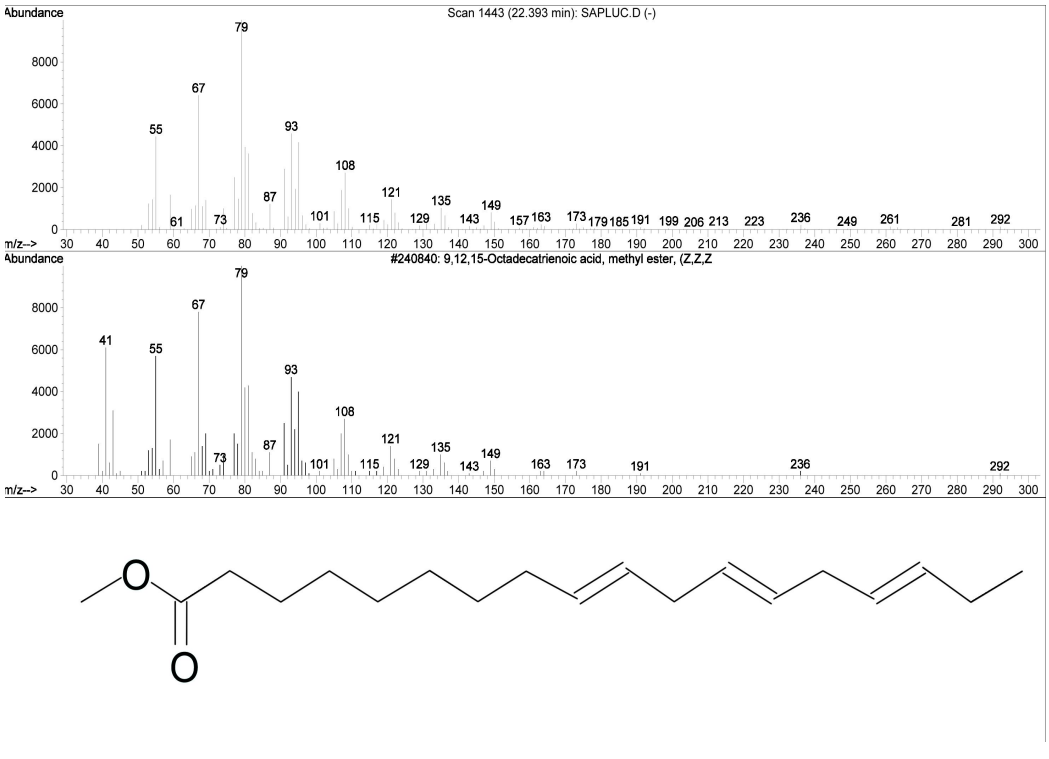


Figure 5: Fragmentation Pattern of Linolenic Acid Using GC/Mass

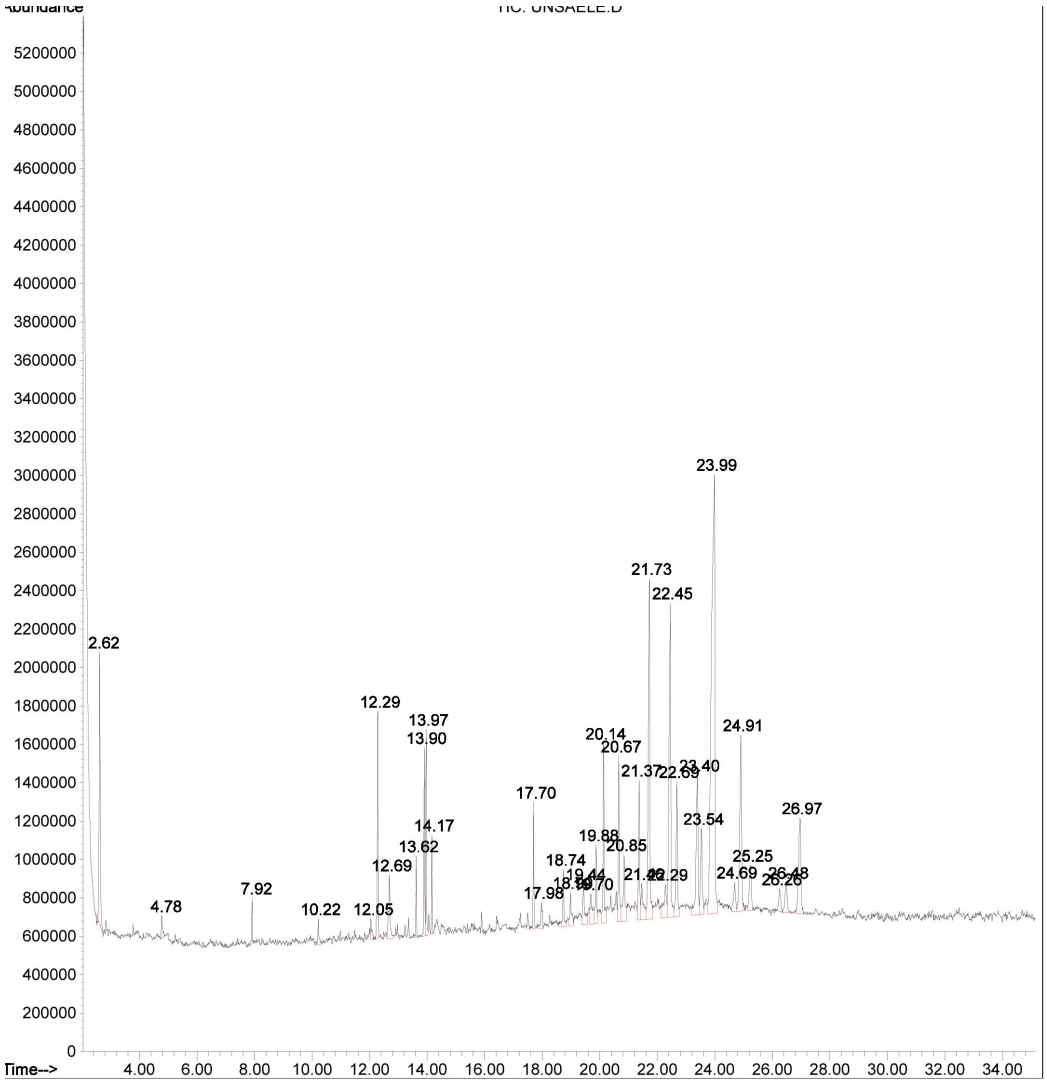


Figure 6: Toltal ionic chromatogram (TIC) of *S. elaeagnifolium* Unsaponifiable Matter Identified by GC/Mass

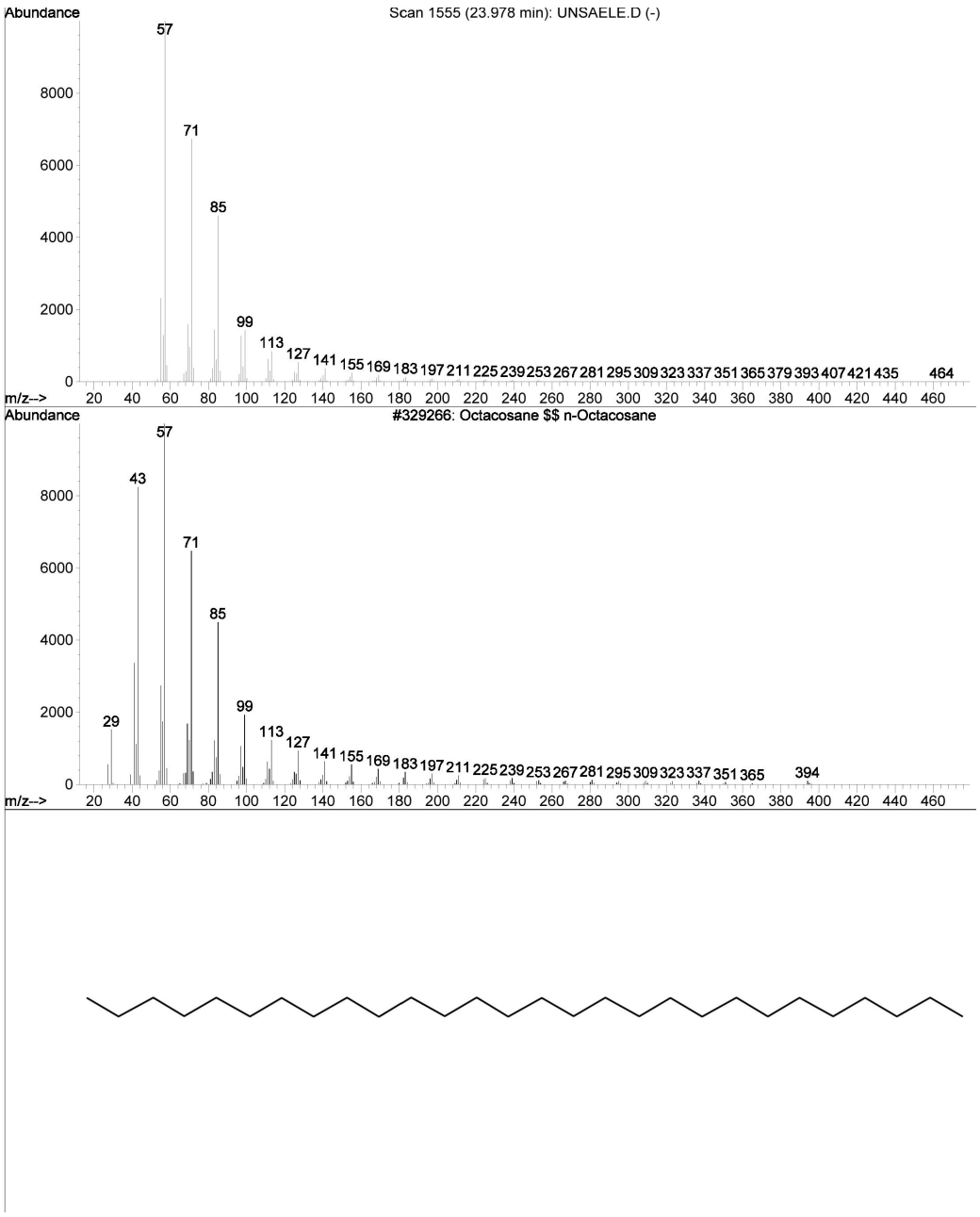


Figure 7: Fragmentation Pattern of Octacosane Using GC/Mass

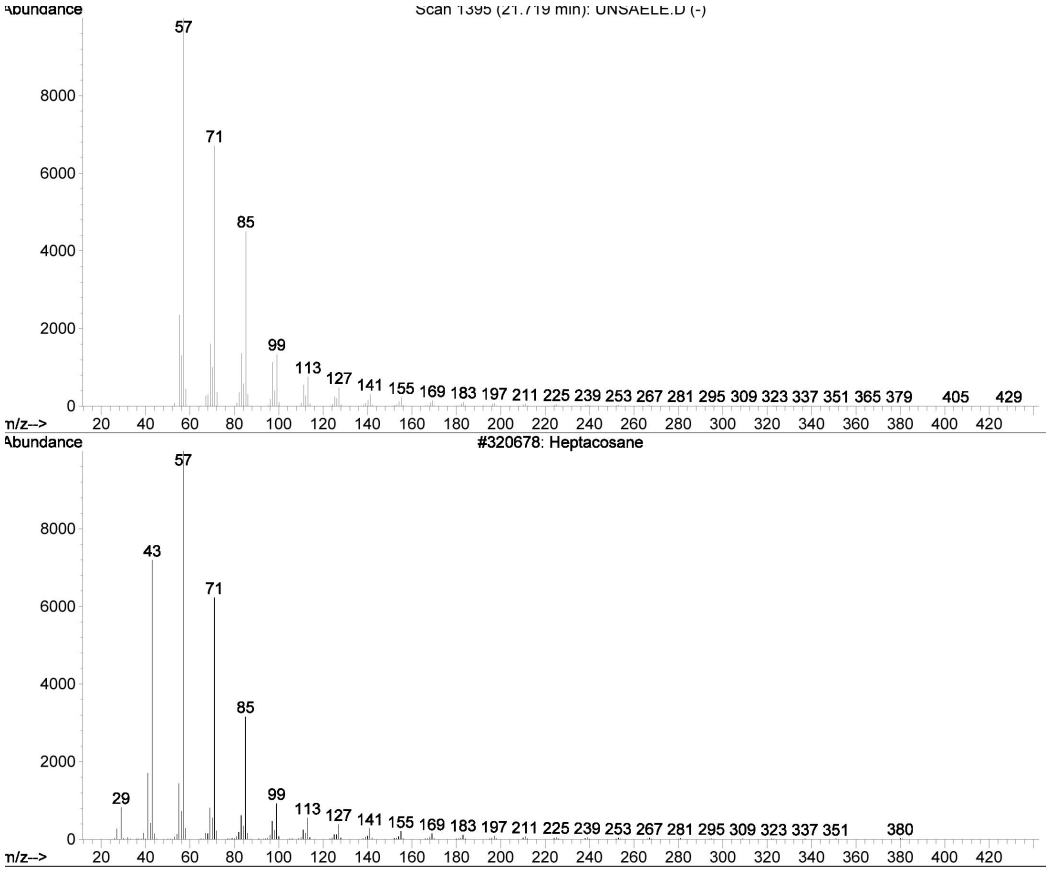


Figure 8: Fragmentation Pattern of Heptacosane Using GC/Mass

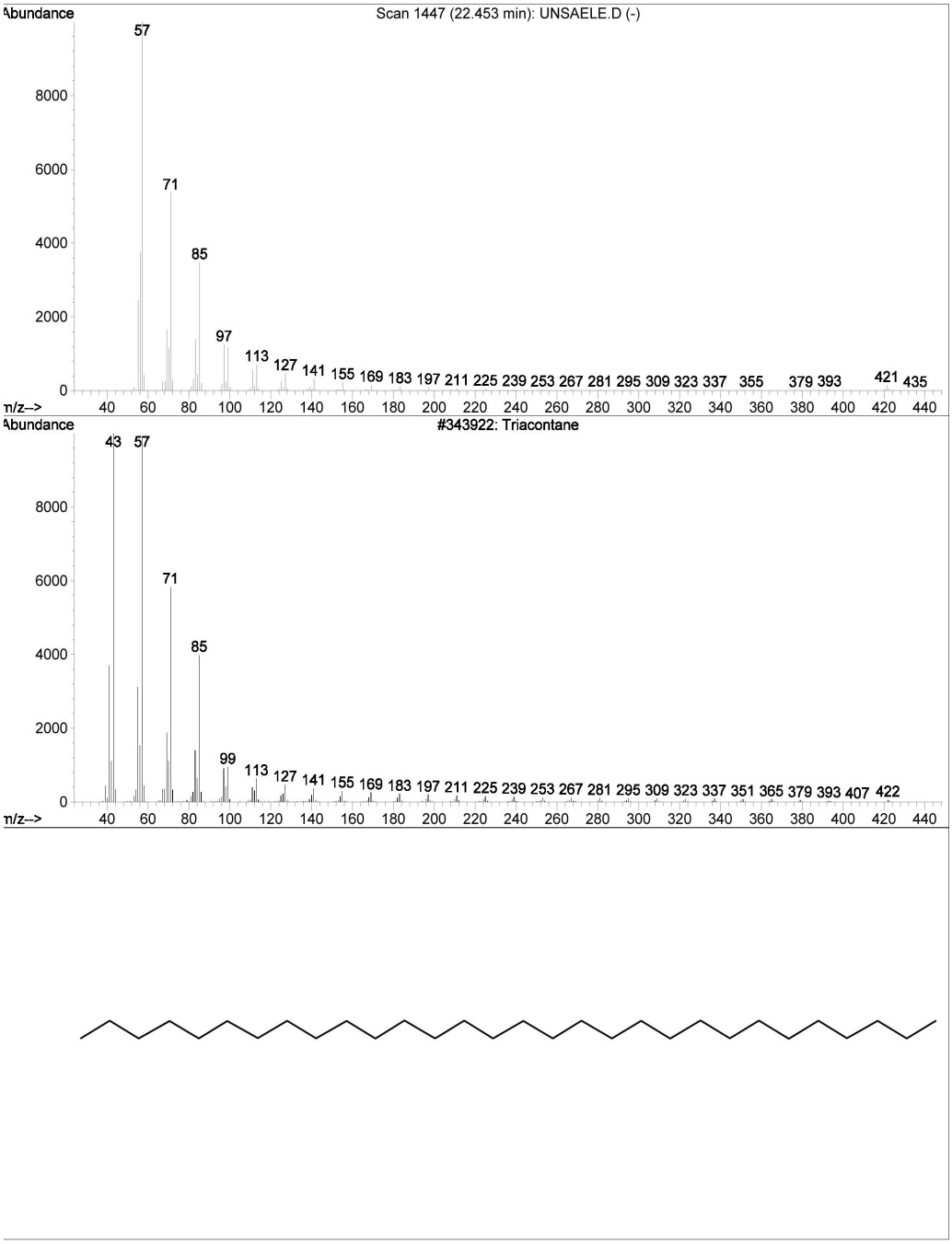


Figure 9: Fragmentation Pattern of Triacontane Using GC/Mass

DISCUSSION

Medicinal plants continued to be an important therapeutic aid for the treatment of various diseases. Over the last decades there has been much interest in the discovery of natural antibacterial agents from plants **[19].** The recent researches applied worldwide on *Solanum* species encourage the authors to trace the antibacterial activity of some Egyptian

Solanum species which still grow as weed in cultivated fields and underutilized species among others in Egyptian flora.

The fatty acids identified by GC-Mass of *S. elaeagnifolium* revealed that palmitic (38%), 9,12-octadecandienoic (18%) and stearic (16%) were the major fatty acids. Similar results were reported by **[20]**, in other *Solanacous* species such as *Cestrum diurnum* ether extract which showed the fatty acids composition were : palmitic (27.6 %), stearic (2.6 %) and oleaic (3.06 %).

The antimicrobial activities of fatty acids have been well known for many years **[21, 22]**. Some fatty acids have been demonstrated to be bactericidal to important pathogenic microorganisms including antibiotic resistant staphylococcus aureus **[23-26]**.

In our study the lipid (fatty acids and hydrocarbons) fraction of *S. elaeagnifolium* showed antibacterial effect against 25 bacterial isolates of antibiotic resistant strains namely: *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. This result was supported by **Bhattacharjee *et al.*, 2005, [20]**, who clamied that the fatty acids of *Cestrum diurnum* (*Solanceae*) with its main constituents as Palmitic, Stearic and Oleic showed antibacterial activity against the pathogenic strains of *Staphylococcus aureus, Bacillus subtilis, E- coli and Pseudomonus aeruginosa*.

The ealier work of **Gandhiappan & Rengasamy, 2012, [19]**, reported that the chloroform and Methanolic extract of leaves from 6 *Solanum* species (*S. anguivi, S. nigrum, S. pubescens, S. surratense, S. torvum, S. Swartz, S. trilobatum*) showed moderate activity against human pathogenic bacteria such as *Staphylococcus aureus* MTCC 96, *Micoccus luteus* ATCC 4698, *Vibro cholerae* ATCC 14035 and *Klebsiella pneumoniae* MTCC 109.

Often, the methanol extract of some *Solanum* species showed high antibacterial activity, this phenomomena was reported by **De Britto *et al.*, 2011, [27].** The auther observed highly significant antibacterial activity of methanol extracts of *S. surattense*; followed by *S. nigrum* then *S. torvum* against *Xanthomonas campestries* (plant pathogen). This data were supported by **Parameswari *et al.*, 2012, [28]** who mentioned that the methanolic extracts of *Solanum nigrum* showed highest antibacterial activity (against *Bacillius subtilis, Escherichia coli, Klebsiella pneumoniae and Pseudomonus aeruginosa*), compared to ethanol extract. In our study Methanol and water extracts of *S. elaeagnifolium* showed negative results on its application to the studied antibiotic resistant bacteria. On the other hand,

acetone and methanol extracts from *Solanum tomentosum* showed antibacterial activity at 5 mg/ml; but non of these extracts inhibited the growth of *Staphylococcus aureus, Echerichia coli* and *Klebsiella pneumonae* **[29]**.

The bacteriostatic effect was detected using ethanolic extract of *Solanum surrattense* **[30].**

Mc Gaw *et al*., 2002, [31] and **Seidel & Talylor, 2004, [30],** found that the fatty acid (lauric, palmitic, linolenic, linoleic, oleic, stearic, myristic acids) have potential antibacterial and antifungal values.

However, the studied lipid material (fatty acids and hydrocarbons) of *S. elaeagnifolium* gave an application to the studied 25 antibiotic resistant bacterial strains **(Table 2)**. The application of each of the pure fatty acid fraction and hydrocarbon fraction gave negative antibacterial effect to the same tested bacterial strains. This result was explained by **Nair & Chanda, 2007, [32]** who claimed that the therapeutic benefits of specific plant are related to the presence of dozens of active constituents that combine together and then have more effective healing properties than their isolated constituents.

The combination of the antimicrobial therapy is usually applied to expand antibacterial spectrum and reduce the selection of resistant mutants during patient treatment. In addition, the combinations that exhibit synergy could improve the healing outcome for patients **[33]**. In the present study when combining the *S. elaeagnifolium* lipid fraction with the ciprofloxacin antibiotic, a synergic effect appear in the antibacterial inhibition activity for *Staphylococcus aureus, E. coli* and *Pseudomonus aeruginosa* **(Table 4 & 5)**. The combination protocols are of potential approach either to show synergistic interaction with antibiotics or enhance the susceptibility level of the resistant strains to antibiotics [34].

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