

## Antimicrobial Activity and Spectral Chemical Analysis of Methanolic Leaves Extract of *Adiantum Capillus-Veneris* Using GC-MS and FT-IR Spectroscopy

Haider Mashkooor Hussein<sup>1</sup>, Imad Hadi Hameed<sup>2\*</sup>, Omar Ali Ibraheem<sup>3</sup>

<sup>1</sup>College of Science, Al-Qadisiya University, Iraq

<sup>2</sup>Faculty of Nursing, Babylon University

<sup>3</sup>Institute of medico-legal

Available Online: 7<sup>th</sup> February, 2016

### ABSTRACT

The objective of this research was study the phytochemical composition of *Adiantum capillus-veneris* and to evaluate the isolates for possible in vitro antifungal and antibacterial activities. The compound obtained were screened by GC-MS method. While agar-well diffusion method was employed to measure antimicrobial activity against five bacteria and fourteen fungi and yeast. Thirtyone bioactive phytochemical compounds were identified in the methanolic extract of *Adiantum capillus-veneris*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS Fragment- ions and Pharmacological actions. GC-MS analysis of *Adiantum capillus-veneris* revealed the existence of the  $\alpha$ -D-Glucopyranoside, O- $\alpha$ -D-glucopyranosyl-(1.fwdarw.3)- $\beta$ -D-fruc, d-Mannose, 5,7-Dodecadiyn -1,12-diol, 3-Trifluoroacetoxypentadecane, 3-Trifluoroacetoxypentadecane, Pterin-6-carboxylic acid, Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-,ethyl ester, D-Carvone, Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester), D-Glucose, 6-O- $\alpha$ -D-galactopyranosyl, Estragole, Phenol, 2-methyl-5-(1-methylethyl), 3-Allyl-6-methoxyphenol, Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl), 7-epi-trans-sesquisabinene hydrate, Tetraacetyl-d-xylonic nitrile,  $\gamma$ -Sitosterol, Ergosta-5,22-dien-3-ol, acetate, (3 $\beta$ ,22E), Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo,methyl ester, 9,10-Secocholesta -5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl)oxy], Cis-Vaccenic acid, L-Ascorbic acid, 6-octadecanoate, L-Ascorbic acid, 6-octadecanoate, Deoxyspergualin, Tributyl acetylcitrate, 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid, 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradecahydro-16, 9-Octadecenamide, (Z), Olean-12-ene-3,15,16,21,22,28,-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ), (22S)-21-Acetoxy-6 $\alpha$ ,11 $\beta$ -dihydroxy-16 $\alpha$ ,17apropylmethylenedioxy, Ethyl iso-allocholate, Olean-12-ene-3,15,16,21,22,28-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ) and Olean -13(18)-ene. The FTIR analysis of *Adiantum capillus-veneris* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxylic acids, Esters, Nitro Compounds, Hydrogen bonded Alcohols and Phenols. *Adiantum capillus-veneris* was highly active against *Aspergillus terreus* (7.09 $\pm$ 0.32). Methanolic extract of bioactive compounds of *Adiantum capillus-veneris* was assayed for in vitro antibacterial activity against *Bacillus subtilis*, *Pseudomonas euorgenosa*, *Streptococcus faecalis*, *Salmonella typhi* and *Staphylococcus aureus* by using the diffusion method in agar. The zone of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 3.07 $\pm$ 0.21 to 7.09 $\pm$ 0.32 mm for all treatments.

**Keywords:** GC/MS, Bioactive compounds, FT-IR, *Adiantum capillus-veneris*.

### INTRODUCTION

*Adiantum capillus-veneris* is a wooden herb with a height of about 35 centimeters, with crowning rhizome. *Adiantum capillus-veneris* (Family: Adiantaceae) is one of the most common pteridophyte species with potential importance for medicinal and nutritive purpose. Its a common fern widely distributed throughout the world. Adiantaceae generally occur in the mountainous region of throughout India; in plains they grow on rocks, inhabiting in shady places near swamps and on slopes of lower hills<sup>1</sup>, India is profusely rich in the history of medicinal plants and its 75% folk population is still using herbal preparations in the form of powder, extracts and decoctions because these are easily available in nature and the natives have stronger

faith on traditional knowledge<sup>2</sup>. The synonyms of the plant include *Adiantum capillus*, *A. michelii*, *A. modestum*, *A. schaffneri*, and *A. tenerum*. Its most common names are avenca and maidenhair fern. *Adiantum capillus-veneris*. *L* is cultivated as an ornamental plant in Japan and Europe because of its beautiful evergreen frond. The plant is best used fresh, though it can also be harvested in the summer and dried for later use<sup>3,4</sup>. In traditional herbal medicinal system, *Adiantum capillus-veneris* is used as expectorant, diuretic, febrifuge, as hair tonic, in chest diseases, in catarrhal infection, to treat hard tumours in spleen, antimicrobial and anticancerous<sup>3,5,6</sup>. The dried whole plant is used as an antipyretic and diuretic, and also in the treatment of bronchitis in folklore medicine in China<sup>7</sup>. It is

also used as detoxicant in alcoholism and to expel worms from the body<sup>3</sup>. Externally, it is used as a poultice on snake bites, bee stings etc.<sup>8</sup>. It is effective with female conditions and is used to regulate menstruation, dysmenorrhoea, and facilitate childbirth by speeding up the labor. It seems most effective for young women and those having trouble

getting back on cycle after birthing, nursing, or coming off birth control pills<sup>9,10</sup>. The aims of this study were chemical analysis of methanolic leaves extract of *Adiantum capillus-veneris* and evaluation of antimicrobial activity.

Table 1: Major phytochemical compounds identified in methanolic extract of *Adiantum capillus-veneris*.

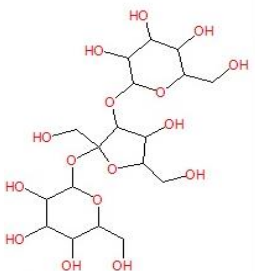
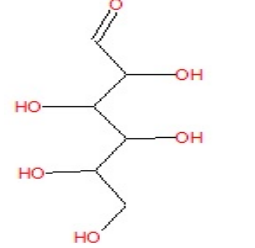

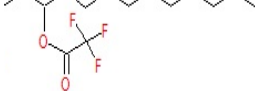
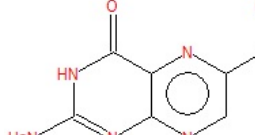
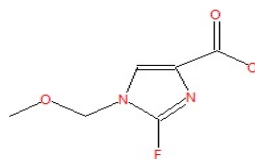
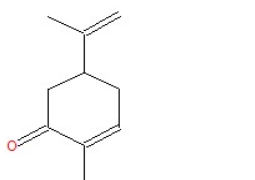
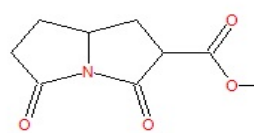
S.No	Phytochemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacologic al actions
1.	$\alpha$ -D-Glucopyranoside, O- $\alpha$ -D-glucopyranosyl-(1.fwdarw.3)- $\beta$ -D-fruc	3.693	504	504.169035		60,73,85,97,113,126,145,163,181,199	Anti-diabetic activity
2.	d-Mannose	3.722	180	180.063388		60,73,85,103,131,149,158,179	Ani-bacterial activity
3.	5,7-Dodecadiyn - 1,12-diol	4.466	194	194.13068		51,55,65,79,91,105,115,124,148,163,179,193	New chemical compound
4.	3-Trifluoroacetoxy pentadecane	4.626	324	324.227615		55,69,83,97,111,125,163,210,233	Anti-nephrotoxic and antioxidant activities
5.	Pterin-6-carboxylic acid	4.712	207	207.039239		57,69,73,93,105,122,149,163,177,189,207	Anti-tumour
6.	Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-, ethyl ester	4.987	202	202.075371		56,72,85,100,114,127,139,157,182,202	Anti-cancer, anti-viral, anti-HIV, anti-protozoal and anti-mycobacterial
7.	D-Carvone	6.961	150	150.1044655		54,82,93,108,135,150	Antimicrobial and anti-diabetic effect
8.	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	7.207	197	197.068808		55,69,84,98,110,142,166,197	Anti-viral and anti-Tumor activity

Table 1: Major phytochemical compounds identified in methanolic extract of *Adiantum capillus-veneris*.

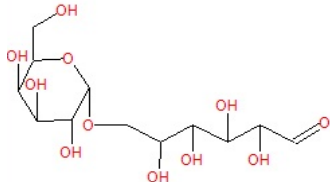
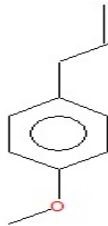
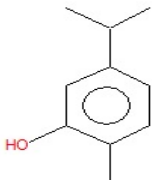
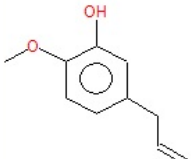
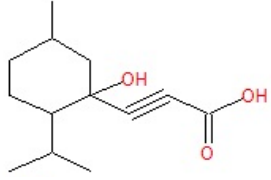
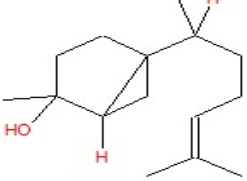
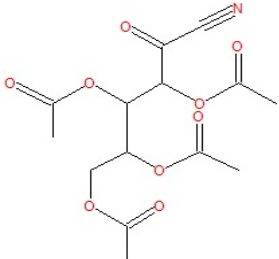
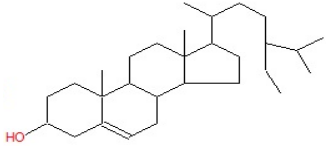
S.No	Phytochemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacologic al actions
9.	D-Glucose ,6-O- $\alpha$ -D-galactopyranosyl -	7.344	342	342.1 1621		60,73,85,1 10,126,14 4,164,182, 212,261	Anti-trypanosomal activity
10.	Estragole	7.481	148	148.0 88815		51,63,77,9 1,105,121, 133,148	Estragole has many biological effects, including antioxidant and anti-inflammatory activity
11.	Phenol,2-methyl-5-(1-methylethyl)-	7.727	150	150.1 04465 5		51,77,91,1 35,150	analgesic, antiviral and anti-diabetic
12.	3-Allyl-6-methoxyphenol	8.466	164	164.0 8373		55,65,77,9 1,103,131, 149,164	Antihistaminic, anti-inflammatory and antioxidant activities
13.	Ppropionic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-	8.986	224	224.1 41245		55,81,95,1 09,135,16 3,178,191, 206	Anti-angiogenic activity against solid tumor growth.
14.	7-epi-trans-sesquisabinene hydrate	9.290	222	222.1 98365		55,69,82,9 3,105,119, 133,147,1 61,179,18 9,204,223	Anti-cancer
15.	Tetraacetyl-d-xylonic nitrile	10.960	343	343.0 90332		60,73,112, 133,245,2 81	Anti-oxidative activities and anti-viral effects
16.	y-Sitosterol	13.816	414	414.3 86166		55,69,81,1 45,161,21 3,255,303, 329,381,3 96,414	Anti-inflammatory effect

Table 1: Major phytochemical compounds identified in methanolic extract of *Adiantum capillus-veneris*.

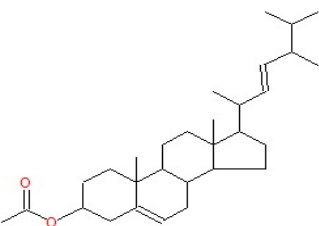
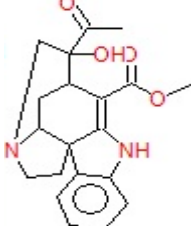
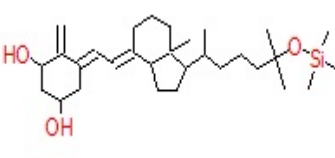
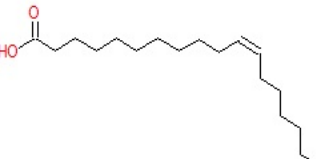
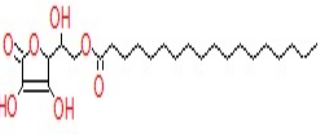
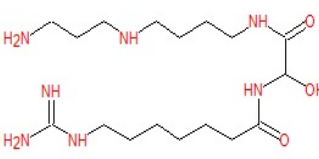
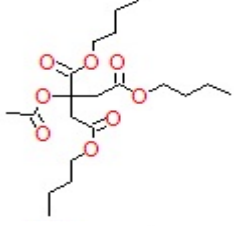
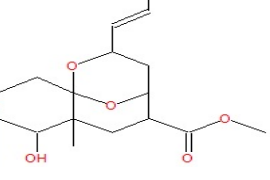
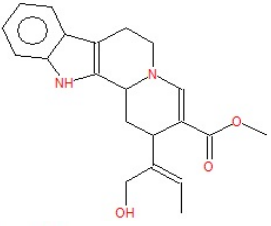
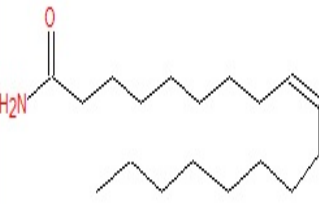
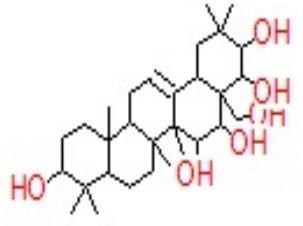
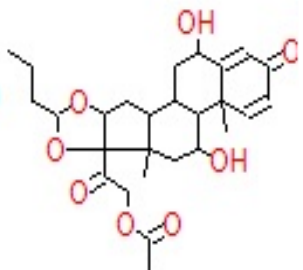
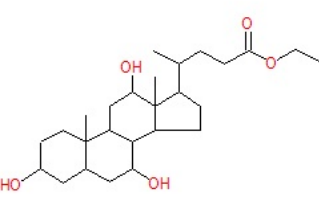
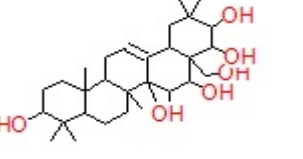
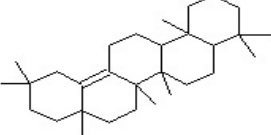
S.No	Phytochemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacologic al actions
17.	Ergosta-5,22-dien-3-ol, acetate , (3 $\beta$ ,22E)-	15.978	440	440.3 6543		55,67,91,1 05,145,15 9,213,227, 255,281,3 27,365,38 0	Anti-tumor activity and immunomodulatory activity
18.	Curan-17-oic acid ,2,16-didehydro-20-hydroxy-19-oxo,methyl ester	16.007	354	354.1 57957		69,83,97,1 11,129,16 7,180,194, 209,226,2 68,283,35 4	Anti-yeast activity.
19.	9,10-Secocholesta - 5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl)oxy]	16.665	488	488.3 68572		55,81,95,1 31,152,21 1,251,340, 398,412	New chemical compound
20.	Cis-Vaccenic acid	16.940	282	282.2 5588		55,69,83,9 7,111,123, 180,222,2 64,282	Anti-inflammatory effects
21.	L-Ascorbic acid , 6-octadecanoate	17.134	442	442.2 93055		57,69,85,9 7,111,129, 143,171,1 85,199,22 7,241,267, 284,327,3 68,424	Antioxidant and antiinflammatory activity
22.	Deoxyspergualin	17.295	387	387.2 95788		59,72,86,1 28,187,21 6,252	Anti-angiogenic action
23.	Tributyl acetylcitrate	17.953	402	402.2 25368		57,112,12 9,157,185, 213,231,2 59,273,32 9	Anticancer activity and Antimicrobial activity
24.	10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid	18.502	310	310.1 78024		55,69,81,9 3,152,179, 211,250,2 78,310	Antimicrobial activity

Table 1: Major phytochemical compounds identified in methanolic extract of *Adiantum capillus-veneris*.

S.No	Phytochemical compound	RT (min)	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacologic al actions
25.	18,19-Secoyohimban-19-oic acid , 16,17,20,21-tetradehydro-16	18.725	352	352.178692		57,69,85,9 5,126,149, 221,256,2 79,352	Anti-inflammatory activity
26.	9-Octadecenamide ,(Z)-	18.857	281	281.271864		59,72,83,1 14,184,21 2,264,281	Anti-inflammatory activity and antibacterial activity
27.	Olean-12-ene-3,15,16,21,22,28, -hexol,(3β,15α,16α,21β,22α)-	19.601	506	506.360739		107,135,1 90,207,23 1,249,280, 298,334,3 52,381,43 9	Anti-inflammatory
28.	(22S)-21-Acetoxy-6α,11β-dihydroxy-16α,17α-propylm ethylenedioxy	20.287	488	488.241018		55,91,121, 149,223,2 79,297,35 1,387,416, 445,488	New chemical compound
29.	Ethyl iso-allocholate	20.859	436	436.318874		55,69,81,9 5,213,253, 400,418	Anti-inflammatory activity
30.	Olean-12-ene-3,15,16,21,22,28 -hexol,(3β,15α,16α,21β,22α)-	25.631	506	506.360739		135,190,2 07,231,24 9,280,298, 334,352,3 86,439,48 8	Anti-inflammatory
31.	Olean -13(18)-ene	27.113	410	410.391253		109,135,2 05,257,27 2,395,410	Significant anti-proliferation effect and anti-inflammatory

## MATERIALS AND METHODS

### Extraction and isolation

*Adiantum capillus-veneris* were purchased from local market in Hilla city, middle of Iraq. After thorough

cleaning and removal of foreign materials, the *Adiantum*

Table 2. FT-IR peak values of *Adiantum capillus-veneris*

No.	Peak (Wave number cm <sup>-1</sup> )	Intensity	Bond	Functional group assignment	Group frequency
1.	896.90	70.886	C-H	Alkenes	675-995
2.	921.97	70.297	C-H	Alkenes	675-995
3.	1024.20	51.450	C-F stretch	Aliphatic fluoro compounds	1000-10150
4.	1205.51	75.588	C-O	Alcohols, Ethers, Carboxylic acids, Esters	1050-1300
5.	1232.51	75.058	C-O	Alcohols, Ethers, Carboxylic acids, Esters	1050-1300
6.	1261.45	76.466	C-O	Alcohols, Ethers, Carboxylic acids, Esters	1050-1300
7.	1315.45	77.468	NO <sub>2</sub>	Nitro Compounds	1300-1370
8.	1373.32	76.507	C-H	Alkanes	1340-1470
9.	1516.05	80.267	-	Unknown	-
10.	1606.70	75.878	-	Unknown	-
11.	1732.08	86.700	-	Unknown	-
12.	2735.06	92.036	-	Unknown	-
13.	2850.79	84.751	H-O	H-bonded H-X group	2500-3500
14.	2920.23	80.581	C-H	Alkanes	2850-2970
15.	3271.27	82.637	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

Table 3a: Antibacterial activity of *Adiantum capillus-veneris*

Leaf extract / Antibiotics	Bacteria				
	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus faecalis</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
Streptomycin	2.09±0.11 <sup>a</sup>	2.00±0.11	1.02±0.21	1.77±0.10	2.00±0.19
Rifamycin	2.11±0.12	3.00±0.10	2.00±0.13	2.04±0.11	1.57±0.10
Kanamycin	1.79±0.10	1.09±0.07	2.06±0.11	0.99±0.10	0.69±0.07
Cefotaxime	1.84±0.01	2.00±0.01	3.03±0.16	2.08±0.20	2.07±0.04
Chloramphenicol	2.00±0.13	2.85±0.12	3.00±0.16	2.87±0.20	2.98±0.14
Leaf extract	5.35±0.22	6.00±0.25	7.77±0.38	6.29±0.29	6.86±0.26

<sup>a</sup> The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract, 30 µg/mL of antibiotics (Streptomycin; Rifamycin; Kanamycin; Cefotaxime and chloramphenicol).

Table 3b: Antifungal activity of *Adiantum capillus-veneris*.

Bacteria	Antibiotics /Plant extract			
	Plant	Amphotericin B	Fluconazol	Miconazole nitrate
<i>Aspergillus niger</i>	6.07±0.22 <sup>a</sup>	2.00±0.10	2.99±0.21	2.08±0.10
<i>Aspergillus terreus</i>	7.09±0.32	3.07±0.13	2.03±0.18	3.00±0.14
<i>Aspergillus flavus</i>	6.07±0.27	2.06±0.20	3.77±0.26	2.95±0.16
<i>Aspergillus fumigatus</i>	5.99±0.30	1.99±0.08	3.00±0.17	2.00±0.16
<i>Candida albicans</i>	6.03±0.21	2.88±0.17	2.85±0.11	2.09±0.19
<i>Saccharomyces cerevisiae</i>	4.00±0.19	2.00±0.16	2.00±0.10	3.14±0.16
<i>Fusarium sp.</i>	4.81±0.20	3.00±0.15	3.24±0.15	2.01±0.12
<i>Microsporum canis</i>	3.98±0.18	2.37±0.19	2.02±0.10	2.18±0.13
<i>Streptococcus faecalis</i>	3.07±0.21	3.08±0.14	2.80±0.10	2.09±0.11
<i>Mucor sp.</i>	4.05±0.17	2.02±0.17	1.99±0.12	1.00±0.10
<i>Penicillium expansum</i>	3.89±0.19	3.00±0.19	2.82±0.16	1.97±0.13
<i>Trichoderma viride</i>	4.66±0.25	2.09±0.13	2.22±0.13	2.41±0.18
<i>Trichoderma horzianum</i>	4.00±0.20	1.00±0.02	3.05±0.17	2.96±0.19
<i>Trichophyton mentagrophytes</i>	4.09±0.19	2.08±0.11	1.76±0.10	1.03±0.10

<sup>a</sup> The values ( average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract and 30 µg/mL of (Amphotericin B; Fluconazol and Miconazole nitrate).



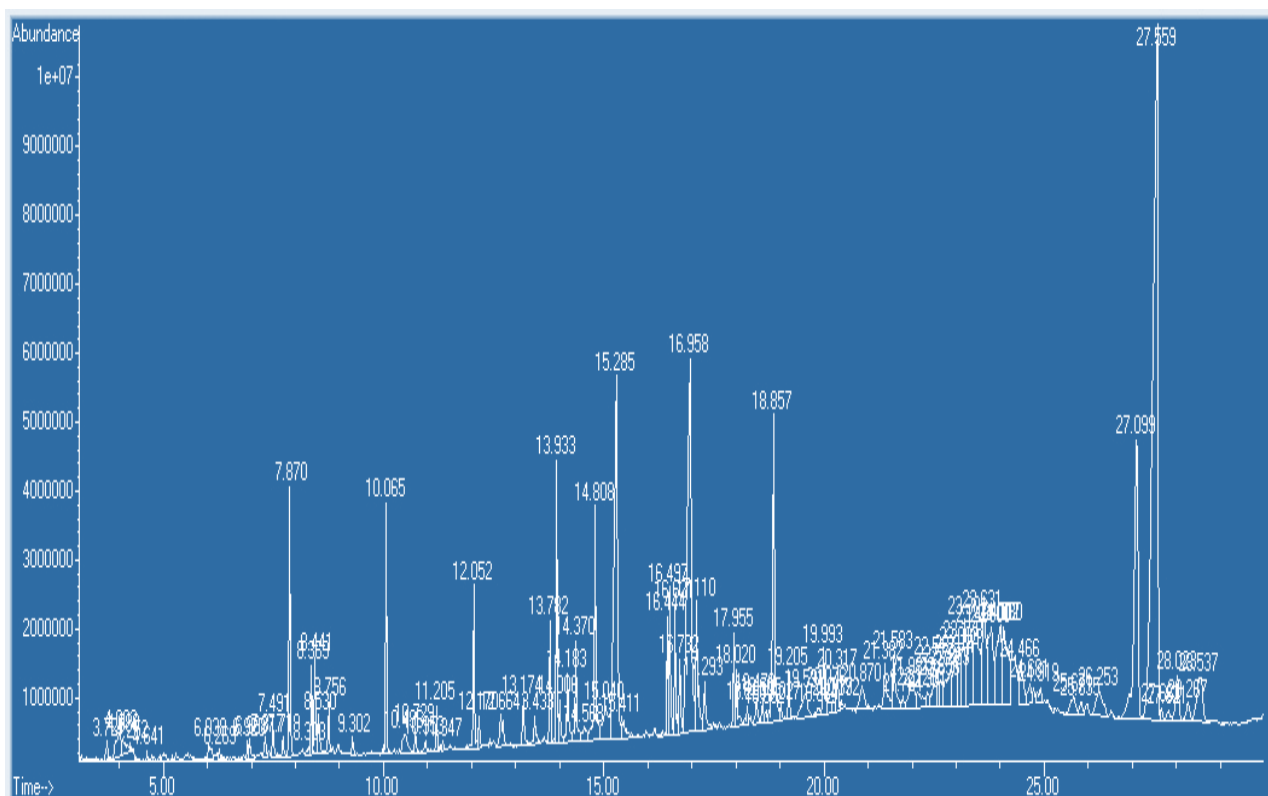


Figure 1: GC-MS chromatogram of methanolic extract of *Adiantum capillus-veneris*.

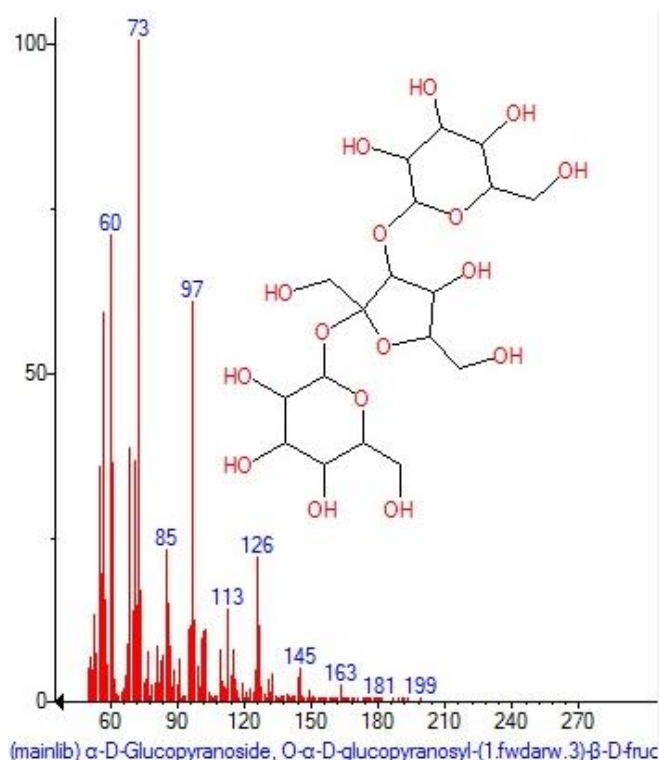


Figure 2: Structure of α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fruc with 3.693 (RT) present in *Adiantum capillus-veneris*.

#### Determination of antibacterial activity of crude bioactive compounds of *Adiantum capillus-veneris*

The anti-bacterial activity was evaluated using Mueller-Hinton agar. The bacterial plates were incubated at 37 °C

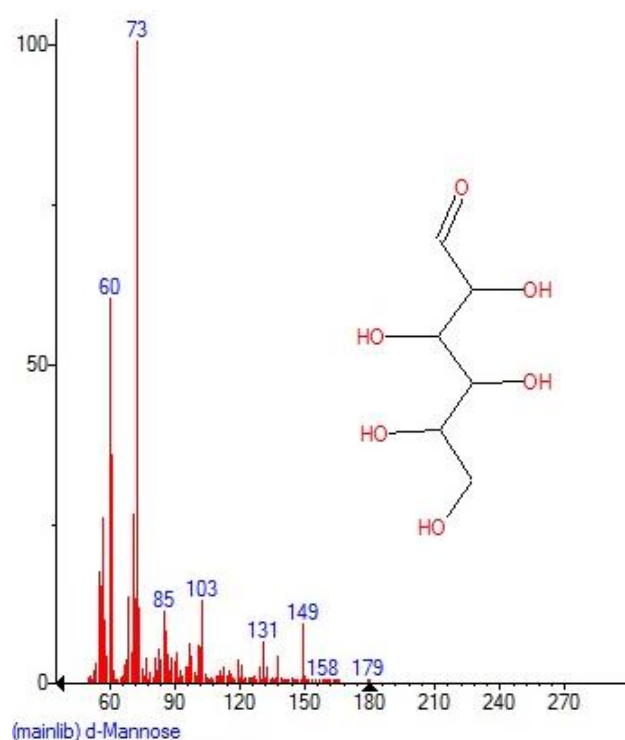


Figure 3: Structure of d-Mannose with 3.722 (RT) present in *Adiantum capillus-veneris*.

for 24 h. After incubation, the diameter of the inhibition zone was measured to evaluate the antimicrobial activity. Each test was performed twice and the average of the results was calculated. The extraction solvents were used

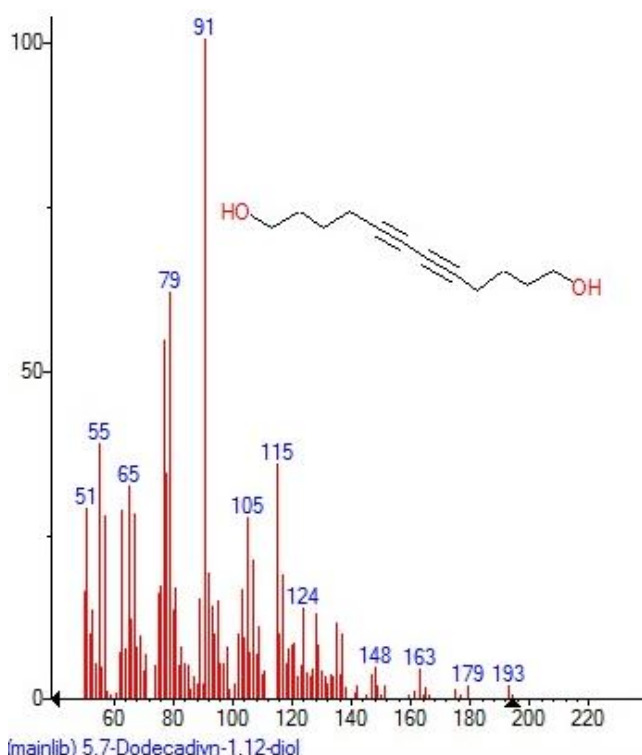


Figure 4: Structure of 5,7-Dodecadiyn-1,12-diol with 4.466 (RT) present in *Adiantum capillus-veneris*.

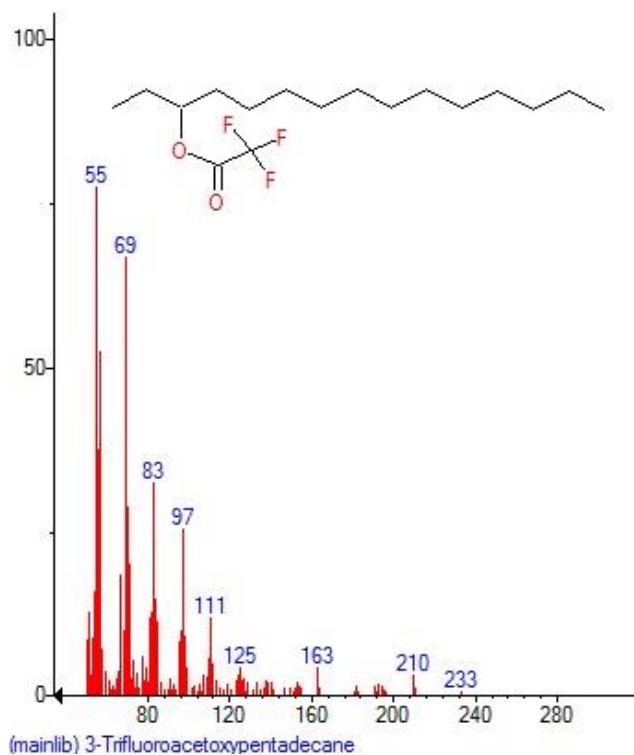


Figure 5: Structure of 3-Trifluoroacetoxy-pentadecane with 4.626 (RT) present in *Adiantum capillus-veneris*.

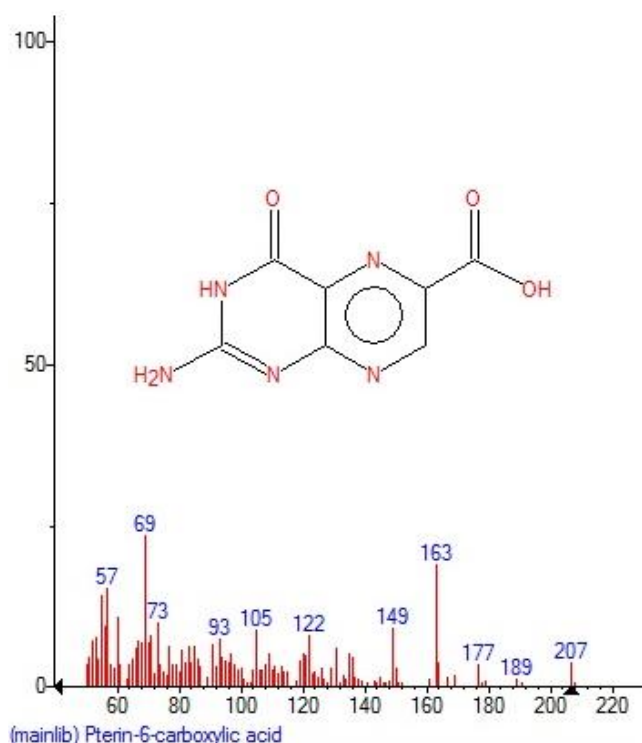


Figure 6: Structure of Pterin-6-carboxylic acid with 4.712 (RT) present in *Adiantum capillus-veneris*.

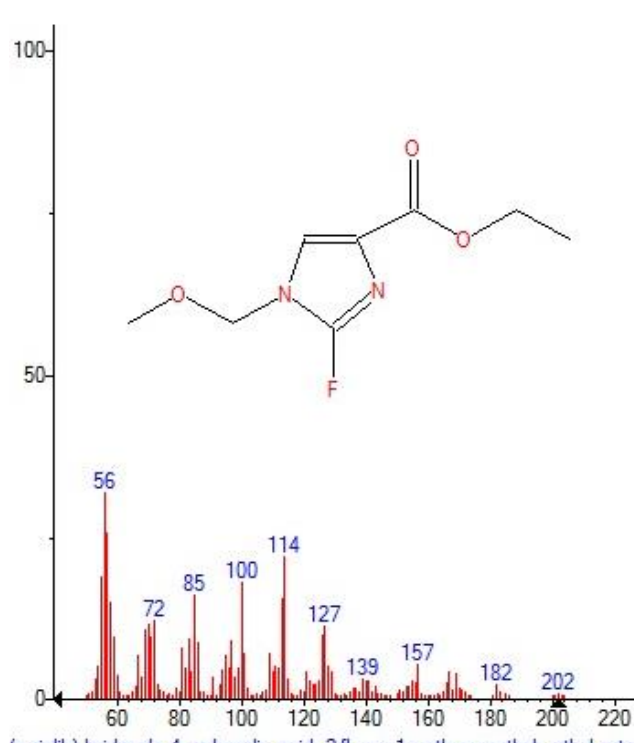


Figure 7: Structure of Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-, ethyl ester with 4.987 (RT) present in *Adiantum capillus-veneris*.

as negative control<sup>17</sup>. The test pathogens were swabbed in Muller Hinton agar plates. 60µl of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in

diameter. The plates were incubated at 37C° for 24 hours and examined. After the incubation the diameter of inhibition zones around the discs was measured<sup>18</sup>.



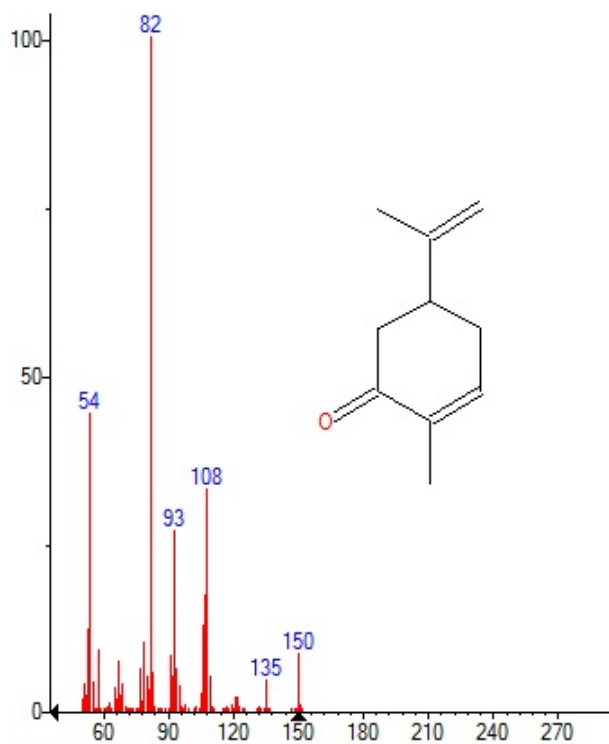


Figure 8: Structure of D-Carvone with 6.961 (RT) present in *Adiantum capillus-veneris*.

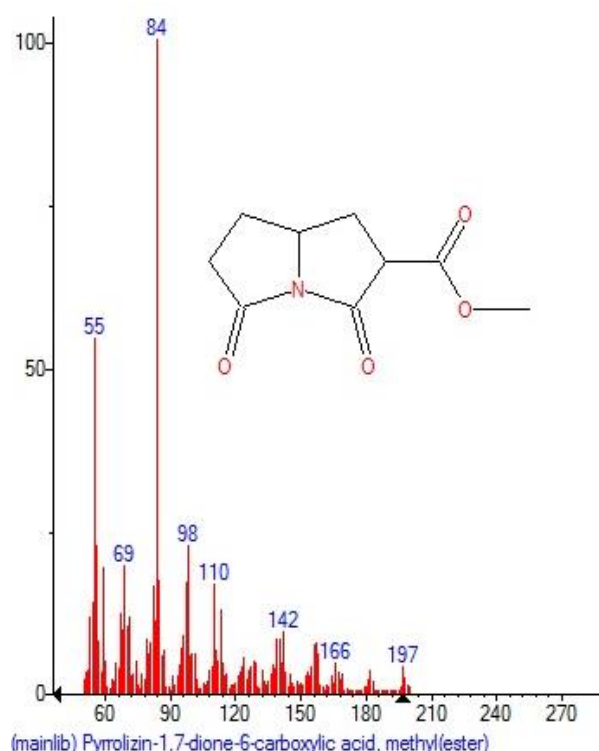


Figure 9: Structure of Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester) with 7.207 (RT) present in *Adiantum capillus-veneris*.

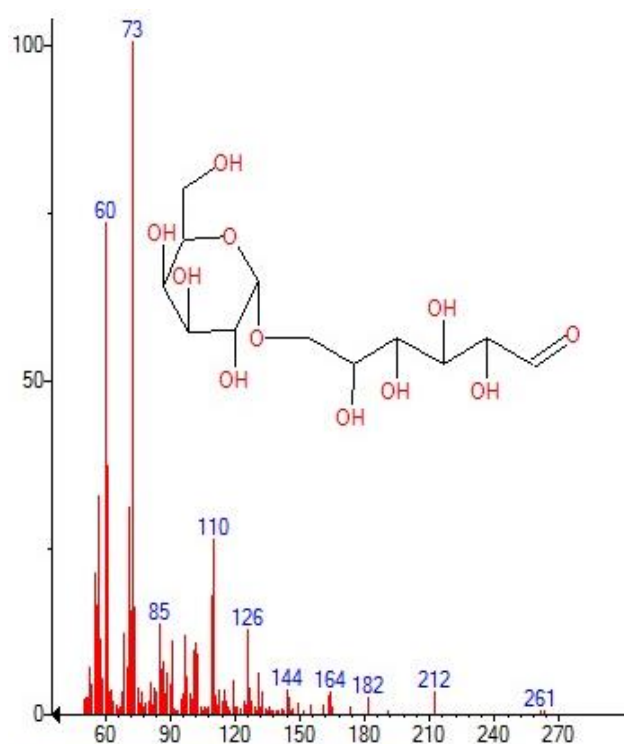


Figure 10: Structure of D-Glucose, 6-O-α-D-galactopyranosyl with 7.344 (RT) present in *Adiantum capillus-veneris*.

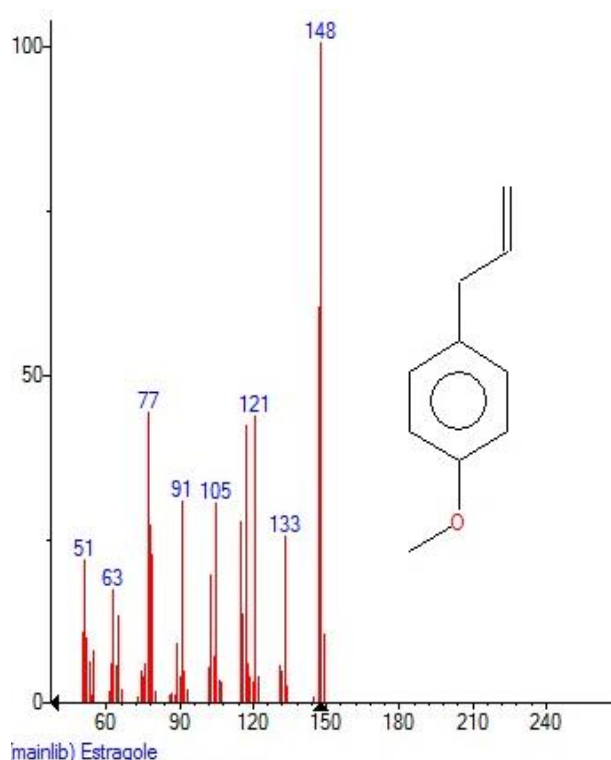


Figure 11: Structure of Estragole with 7.481 (RT) present in *Adiantum capillus-veneris*.

#### Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the samples

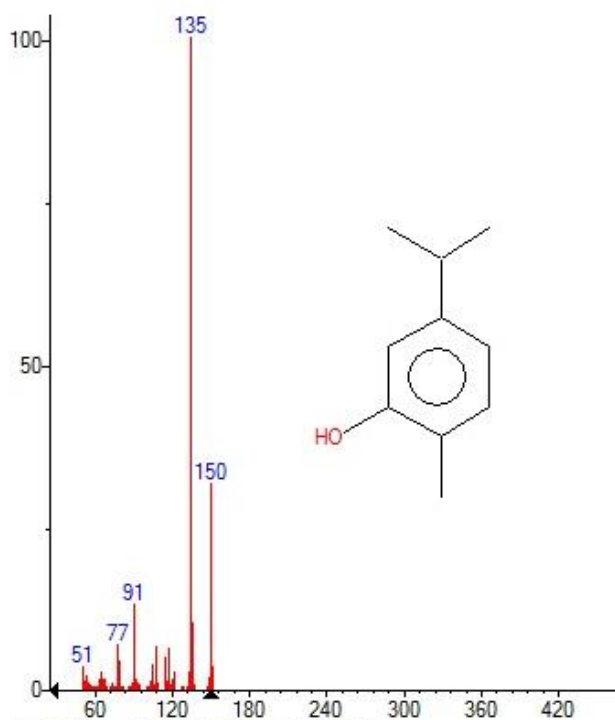


Figure 12: Structure of Phenol, 2-methyl-5-(1-methylethyl) with 7.727 (RT) present in *Adiantum capillus-veneris*.

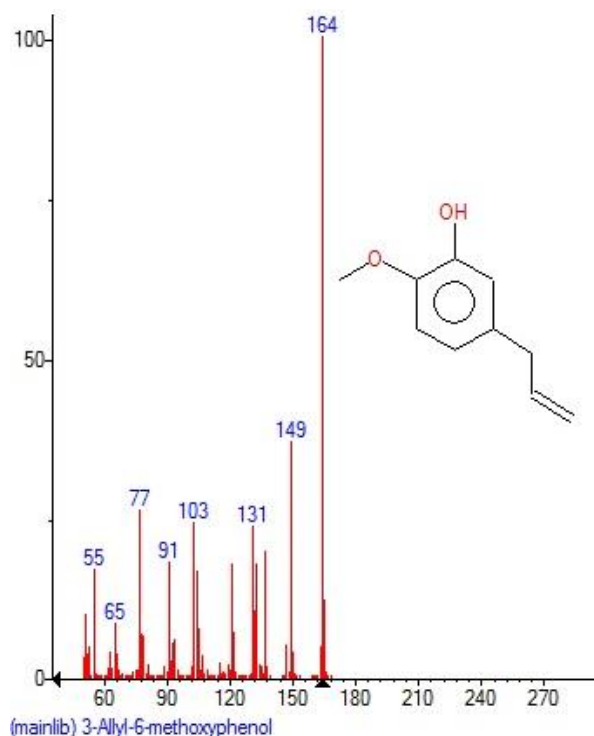


Figure 13: Structure of 3-Allyl-6-methoxyphenol with 8.466 (RT) present in *Adiantum capillus-veneris*.

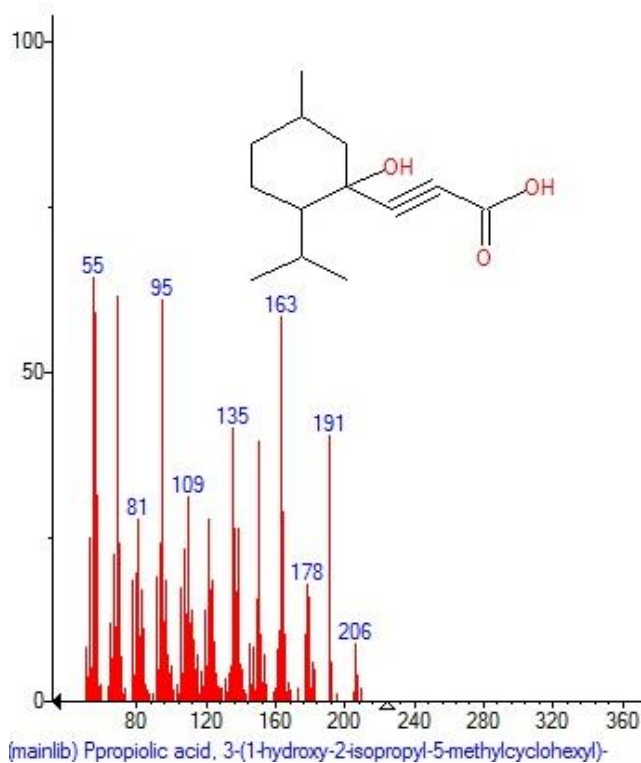


Figure 14: Structure of Propionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl) with 8.986 (RT) present in *Adiantum capillus-veneris*.

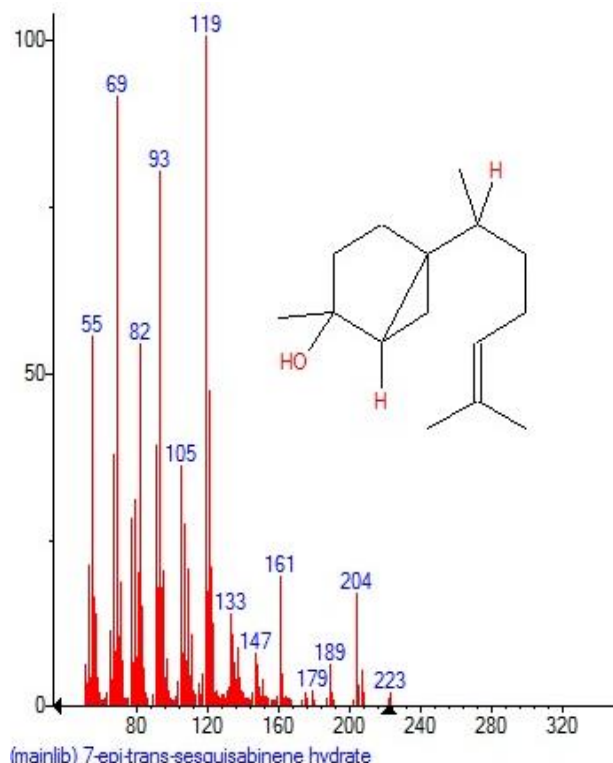


Figure 15: Structure of 7-epi-trans-sesquisabinene hydrate with 9.290 (RT) present in *Adiantum capillus-veneris*.

solutions *Adiantum capillus-veneris* was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms.

Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity

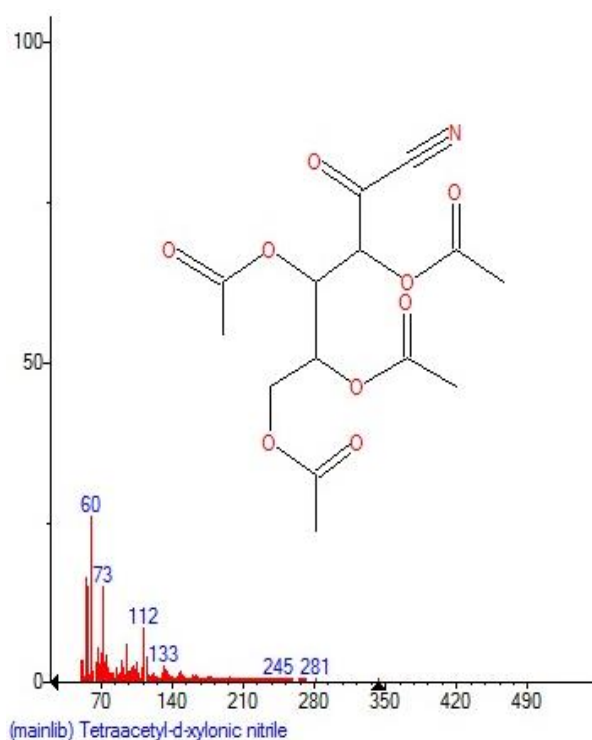


Figure 16: Structure of Tetraacetyl-d-xylosyl nitrile with 10.960 (RT) present in *Adiantum capillus-veneris*.

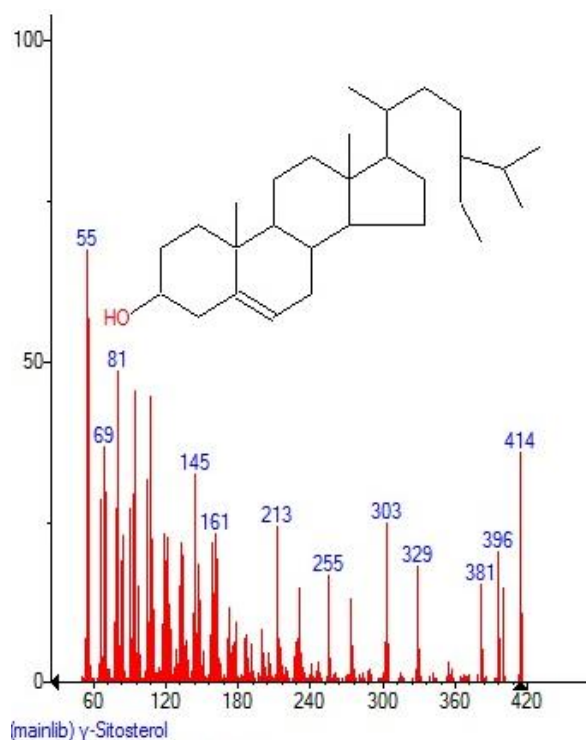


Figure 17: Structure of γ-Sitosterol with 13.816 (RT) present in *Adiantum capillus-veneris*.

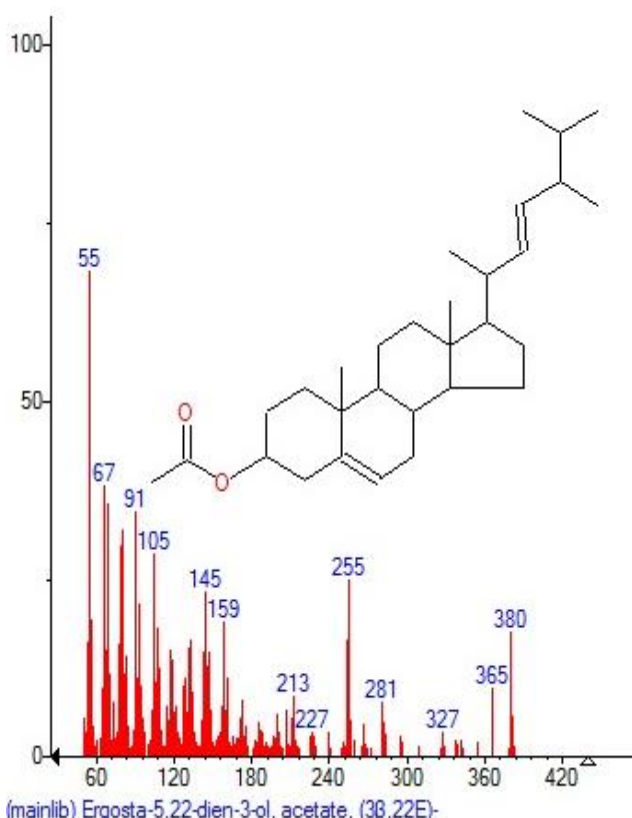


Figure 18: Structure of Ergosta-5,22-dien-3-ol, acetate, (3β,22E) with 15.978 (RT) present in *Adiantum capillus-veneris*.

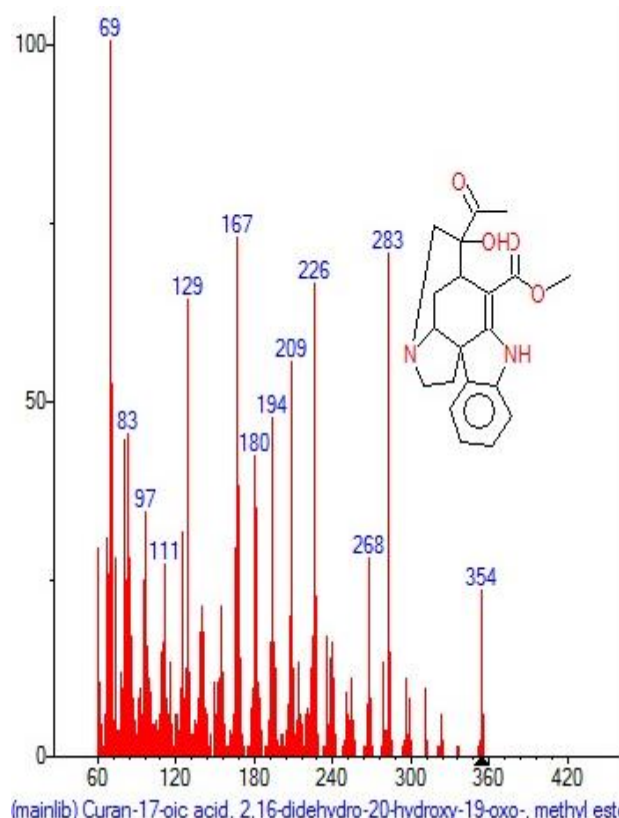


Figure 19: Structure of Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester with 16.007 (RT) present in *Adiantum capillus-veneris*.

was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation<sup>25,26</sup>.  
Statistical analysis

Results of the study were based on analysis of variance (ANOVA) and Differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Identification of phytochemical compounds

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Adiantum capillus-veneris*, shown in Table 1. The GC-MS chromatogram of the 31 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Adiantum capillus-veneris* showed the presence of thirtyone major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 1,7-Dioxaspiro[5,5]undec-2-ene Figure 2. The second peak indicated to be 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one Figure 3. The next peaks considered to be  $\alpha$ -D-Glucopyranoside, O- $\alpha$ -D-glucopyranosyl-(1.fwdarw.3)- $\beta$ -D-fruc, d-Mannose, 5,7-Dodecadiyn-1,12-diol, 3-Trifluoroacetoxypentadecane, 3-Trifluoroacetoxypentadecane, Pterin-6-carboxylic acid, Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-ethyl ester, D-Carvone, Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester), D-Glucose, 6-O- $\alpha$ -D-galactopyranosyl, Estragole, Phenol,2-methyl-5-(1-methylethyl), 3-Allyl-6-methoxyphenol, Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl), 7-epi-trans-sesquibinene hydrate, Tetraacetyl-d-xylonic nitrile,  $\gamma$ -Sitosterol, Ergosta-5,22-dien-3-ol, acetate, (3 $\beta$ ,22E), Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo,methyl ester, 9,10-Secocholesta-5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl)oxy], Cis-Vaccenic acid, L-Ascorbic acid, 6-octadecanoate, Deoxyspergualin, Tributyl acetyl citrate, 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid, 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradecahydro-16, 9-Octadecenamide, (Z), Olean-12-ene-3,15,16,21,22,28,-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ), (22S)-21-Acetoxy-6 $\alpha$ ,11 $\beta$ -dihydroxy-16 $\alpha$ ,17apropylmethylenedioxy, Ethyl iso-allochololate, Olean-12-ene-3,15,16,21,22,28,-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ) and Olean-13(18)-ene (Figure 3-32). The FTIR analysis of *Adiantum capillus-veneris* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxylic acids, Esters, Nitro Compounds, Hydrogen bonded Alcohols and Phenols which shows major peaks at 896.90, 1024.20, 1205.51, 1261.45, 1315.45, 1373.32, 1606.70, 2735.06, 2850.79, 2920.23 and 3271.27 (Table 2; Figure 33). The present study has been found useful in the identification of several constituents present in the methanolic extract of the plants. Many medicinal plants are rich source of secondary metabolites such as alkaloids, phenol, cardiac glycosides, flavonoids, tannins and terpenoids determined by gas chromatography and mass spectrum<sup>21</sup>. In Nepal, a paste made from the fronds is applied to the forehead to relieve headaches and to the chest to relieve chest pains<sup>27,28</sup>. Olagunju et al. (2006)<sup>9</sup> revealed that secondary plant metabolites exert a wide range of biological activities on physiological systems. Kumar et al. (2010)<sup>10</sup> also reported that the activities of some plant constituents with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and nhexadecaonic acid),

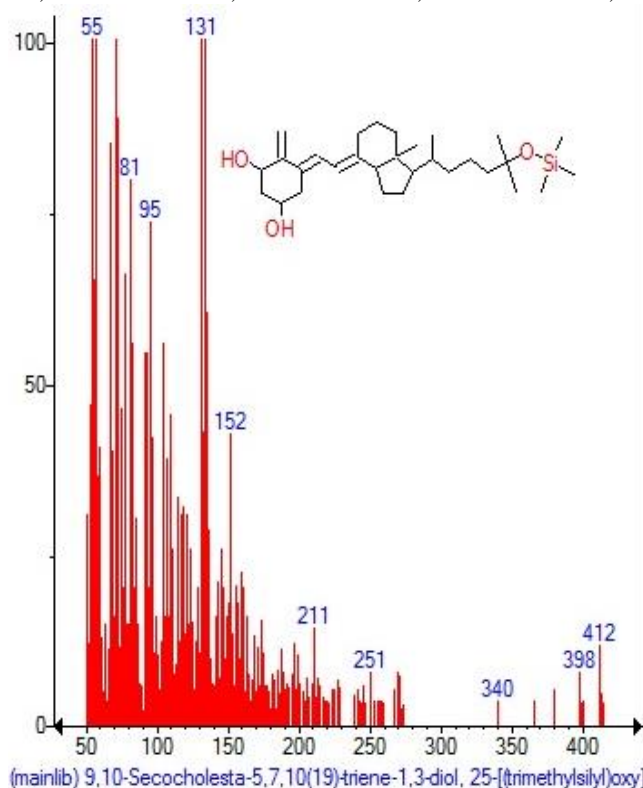


Figure 20: Structure of 9,10-Secocholesta-5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl)oxy] with 16.665 (RT) present in *Adiantum capillus-veneris*.

6-octadecanoate, Deoxyspergualin, Tributyl acetyl citrate, 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid, 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradecahydro-16, 9-Octadecenamide, (Z), Olean-12-ene-3,15,16,21,22,28,-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ), (22S)-21-Acetoxy-6 $\alpha$ ,11 $\beta$ -dihydroxy-16 $\alpha$ ,17apropylmethylenedioxy, Ethyl iso-allochololate, Olean-12-ene-3,15,16,21,22,28,-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ) and Olean-13(18)-ene (Figure 3-32). The FTIR analysis of *Adiantum capillus-veneris* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxylic acids, Esters, Nitro Compounds, Hydrogen bonded Alcohols and Phenols which shows major peaks at 896.90, 1024.20, 1205.51, 1261.45, 1315.45, 1373.32, 1606.70, 2735.06, 2850.79, 2920.23 and 3271.27 (Table 2; Figure 33). The present study has been found useful in the identification of several constituents present in the methanolic extract of the plants. Many medicinal plants are rich source of secondary metabolites such as alkaloids, phenol, cardiac glycosides, flavonoids, tannins and terpenoids determined by gas chromatography and mass spectrum<sup>21</sup>. In Nepal, a paste made from the fronds is applied to the forehead to relieve headaches and to the chest to relieve chest pains<sup>27,28</sup>. Olagunju et al. (2006)<sup>9</sup> revealed that secondary plant metabolites exert a wide range of biological activities on physiological systems. Kumar et al. (2010)<sup>10</sup> also reported that the activities of some plant constituents with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and nhexadecaonic acid),

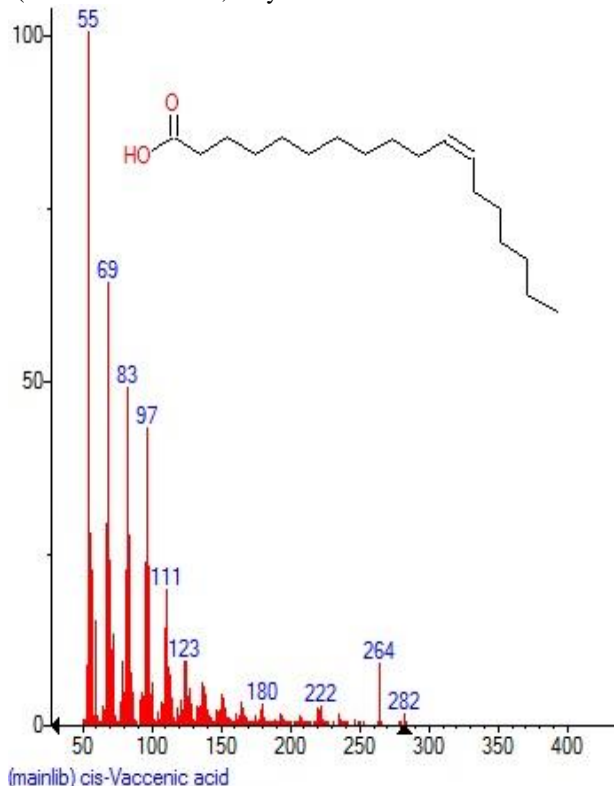
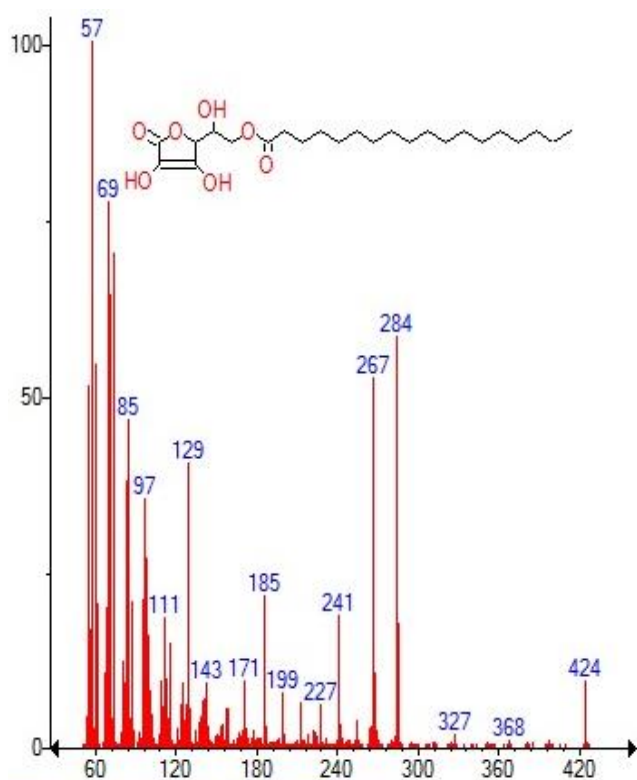
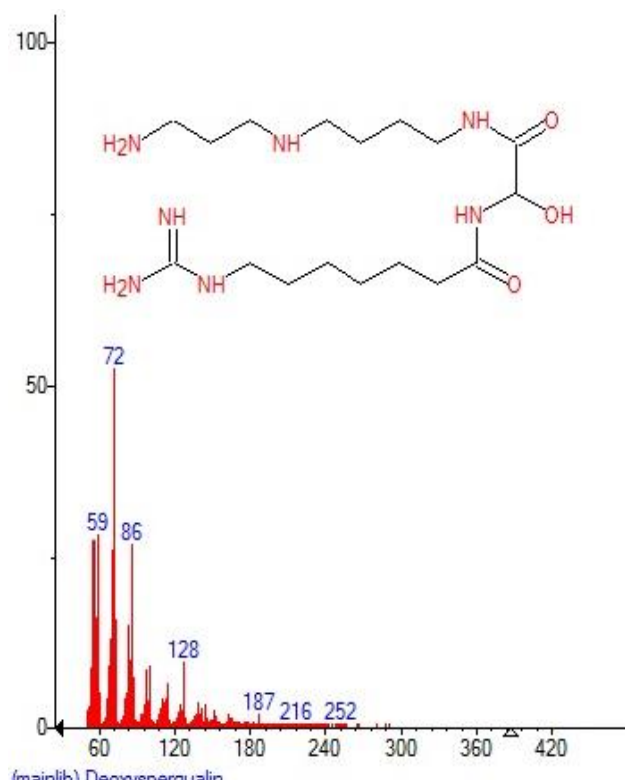


Figure 21: Structure of Cis-Vaccenic acid with 16.940 (RT) present in *Adiantum capillus-veneris*.



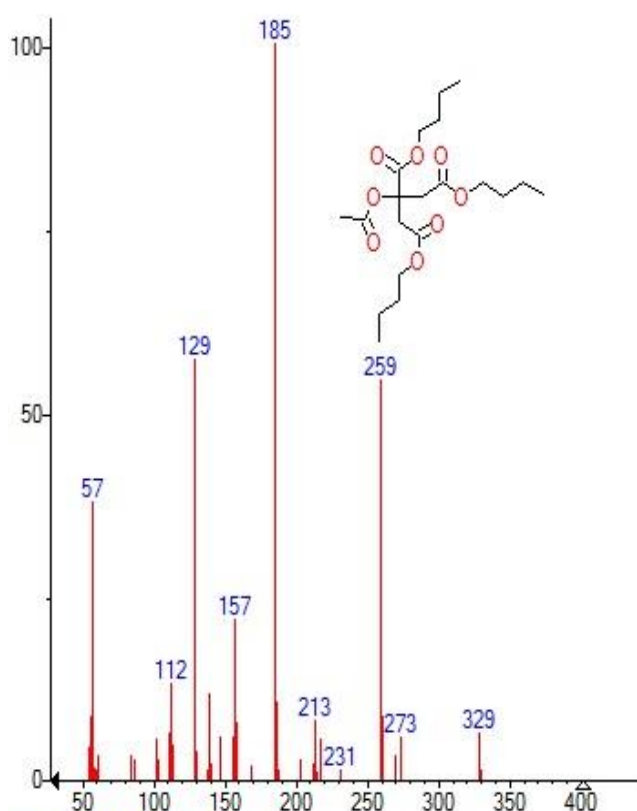
(mainlib) L-Ascorbic acid, 6-octadecanoate

Figure 22: Structure of L-Ascorbic acid, 6-octadecanoate with 17.134 (RT) present in *Adiantum capillus-veneris*.



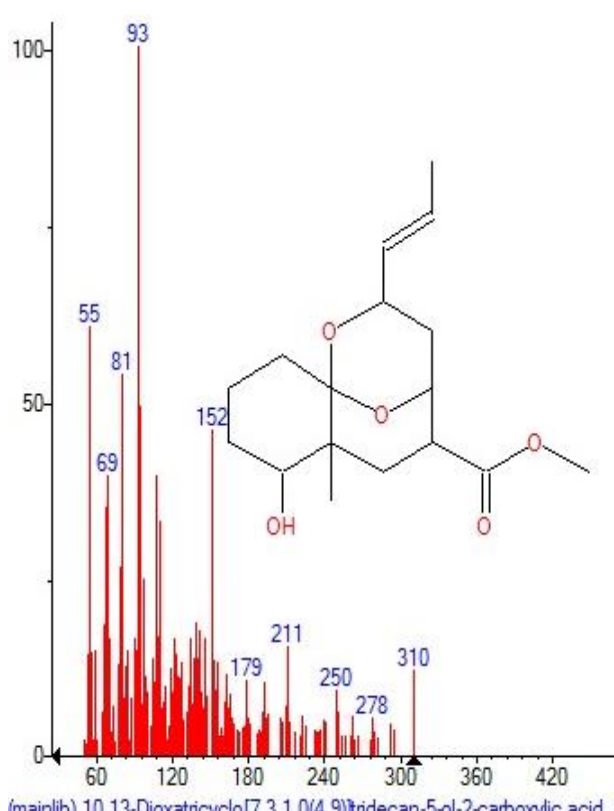
(mainlib) Deoxyspergualin

Figure 23: Structure of Deoxyspergualin with 17.295 (RT) present in *Adiantum capillus-veneris*.



(mainlib) Tributyl acetylcitrate

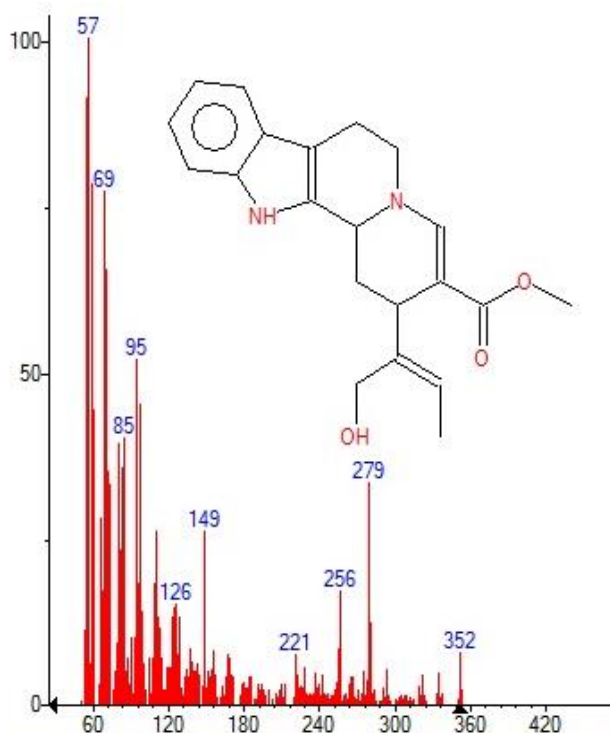
Figure 24: Structure of Tributyl acetylcitrate with 17.953 (RT) present in *Adiantum capillus-veneris*.



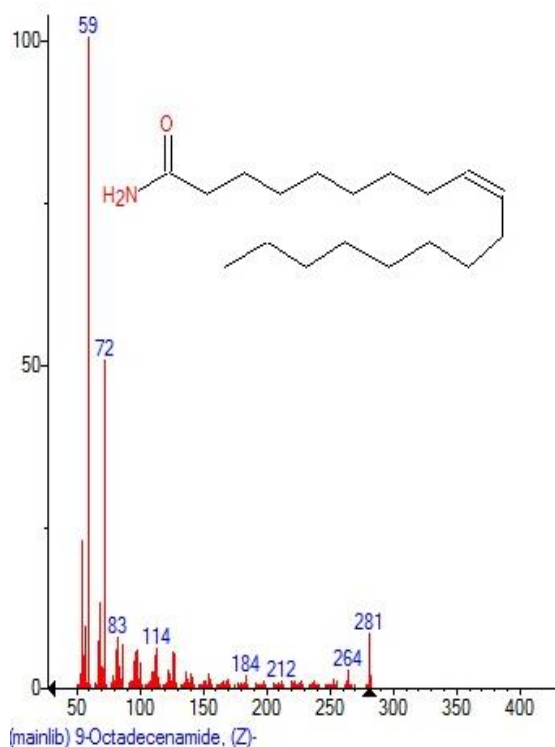
(mainlib) 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid, 4

Figure 25: Structure of 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid with 18.502 (RT) present in *Adiantum capillus-veneris*.

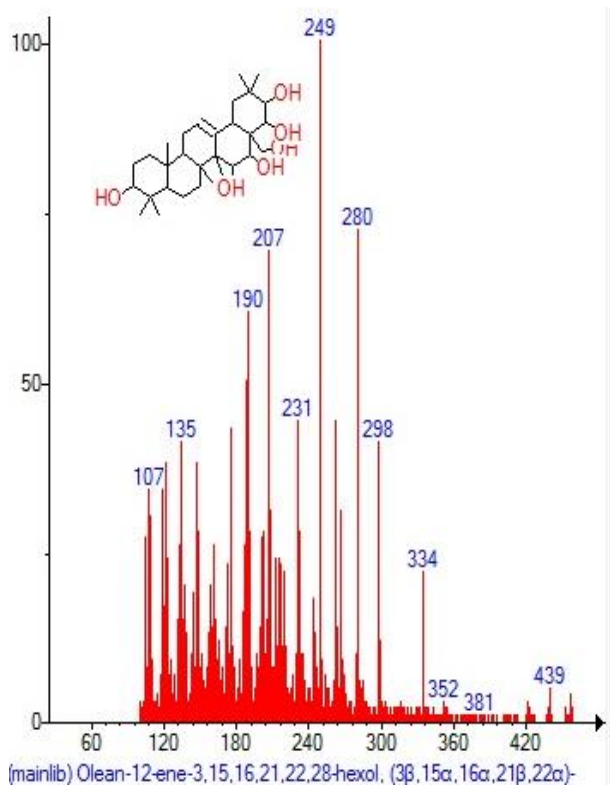




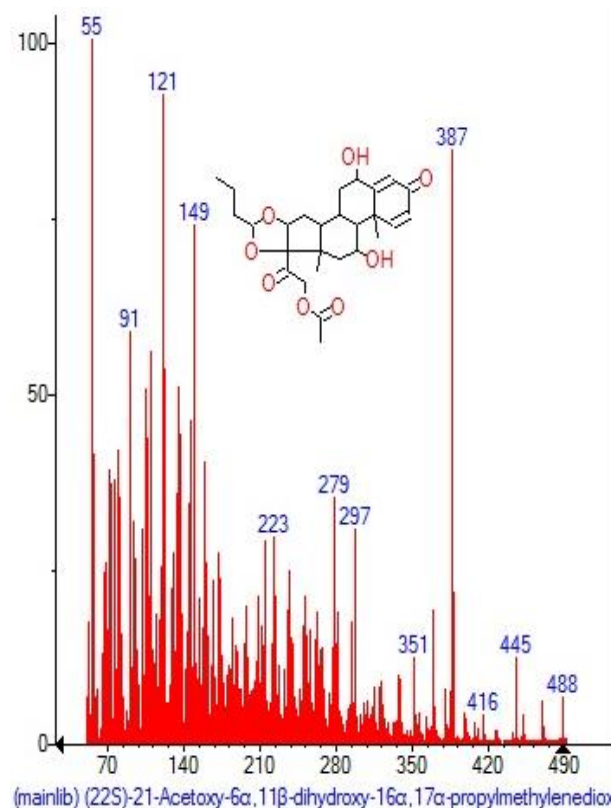
(mainlib) 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-  
Figure 26: Structure of 18,19-Secoyohimban-19-oic acid ,  
16,17,20,21-tetrahydro-16 with 18.725 (RT) present in  
*Adiantum capillus-veneris*.



(mainlib) 9-Octadecenamide, (Z)-  
Figure 27: Structure of 9-Octadecenamide ,(Z) with  
18.857 (RT) present in *Adiantum capillus-veneris*.



(mainlib) Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)-  
Figure 28: Structure of Olean-12-ene-3,15,16,21,22,28,-  
hexol,(3β,15α,16α,21β,22α) with 19.601 (RT) present in  
*Adiantum capillus-veneris*.



(mainlib) (22S)-21-Acetoxy-6α,11β-dihydroxy-16α,17α-propylmethylenedioxy  
Figure 29: Structure of (22S)-21-Acetoxy-6α,11β-  
dihydroxy-16α,17αpropylmethylenedioxy with 20.287  
(RT) present in *Adiantum capillus-veneris*.



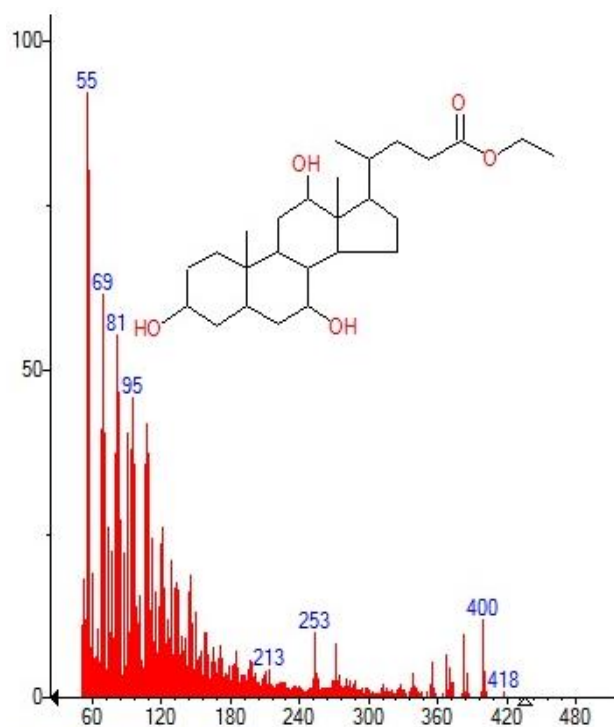


Figure 30: Structure of Ethyl iso-allocholate with 20.859 (RT) present in *Adiantum capillus-veneris*.

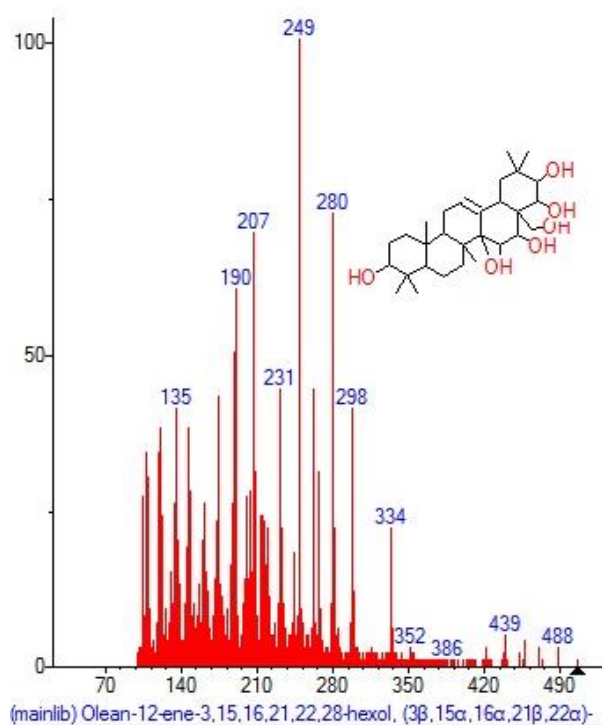


Figure 31: Structure of Olean-12-ene-3,15,16,21,22,28-hexol,(3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )- with 25.631 (RT) present in *Adiantum capillus-veneris*.

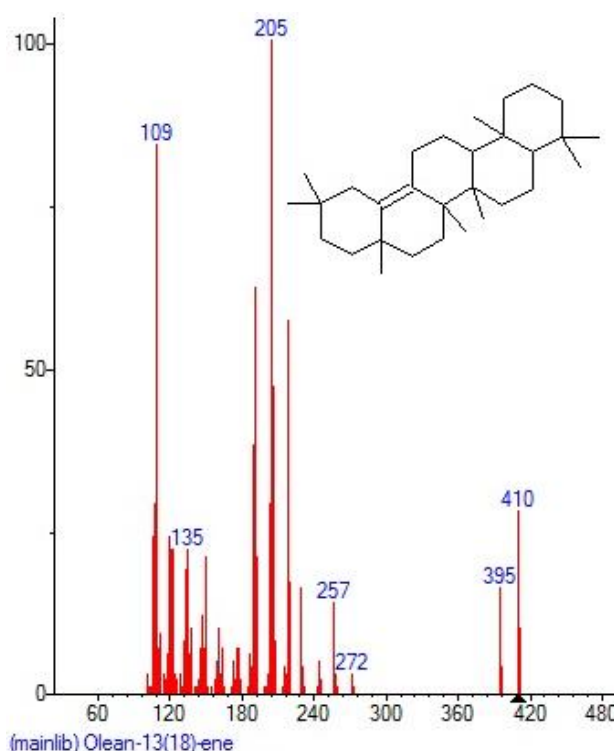


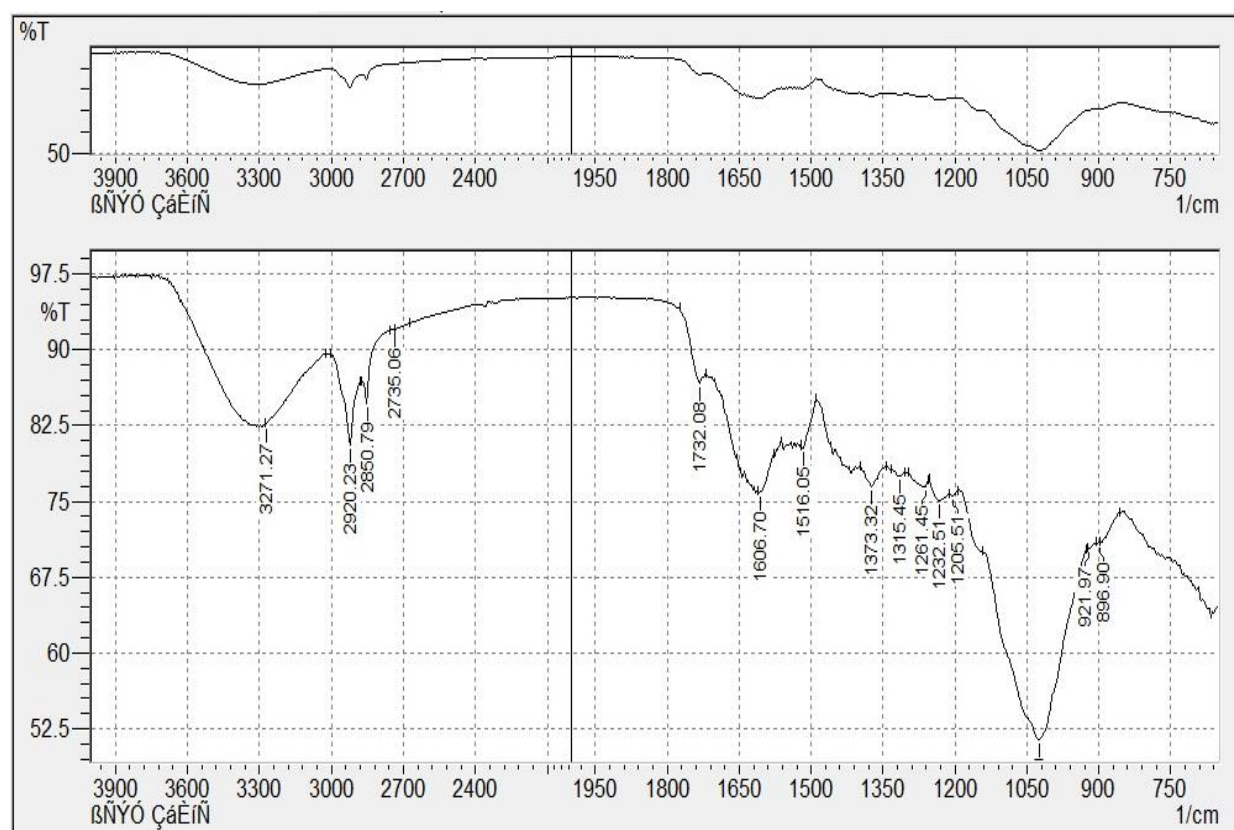
Figure 32: Structure of Olean-13(18)-ene with 27.113 (RT) present in *Adiantum capillus-veneris*.

unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic,

antieczemic and anticoronary. Bharathy et al. (2012)<sup>29</sup> analyzed that the Phytol is a diterpene with antimicrobial properties, significantly against many bacterial strains. Balaj et al. (2014)<sup>30</sup> also reported that the GC-MS analysis of various extracts of *clerodendruphlomidis* leaf. Similarly, our present result showed that the GC-MS analyses of methanolic extract of *Adiantum capillusveneris*. Generally its application is oriented towards the specific detection and potential identification of compounds based on the molecular mass in a complex mixture.

#### Evaluation of antimicrobial activity

In the current study, the anti-microbial activity of the methanolic extract was evaluated by determining the zone of inhibition against five bacteria and fourteen fungi and yeast. Clinical pathogens were selected for antibacterial activity namely, (*Bacillus subtilis*, *Pseudomonas eurogenosa*, *Streptococcus faecalis*, *Salmonella typhi* and *Staphylococcus aureus*. Maximum zone formation was against *Streptococcus faecalis*. Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*, *Fusarium sp.*, *Microsporium canis*, *Streptococcus faecalis*, *Mucor sp.*, *Penicillium expansum*, *Trichoderma viride*, *Trichoderma horzianum* and *Trichophyton mentagrophytes*. *Adiantum capillus-veneris* was very highly active against *Aspergillus terreus* (7.09 $\pm$ 0.32). Results of antimicrobial activity are presented in Table 2 and Table 3. In comparison to the antibiotics used in this study, the plants extracts were far more active

Figure 33: FT-IR profile of *Adiantum capillus-veneris*

against the test bacterial strains. Similarly our extract of *Adiantum capillus-veneris* was active against microbes which is almost in agreement with the studies by Jain et al., (2012)<sup>31</sup> and Rondon et al., (2006)<sup>32</sup>. A previously published study by Dildar et al., (2005)<sup>33</sup> revealed that the *Pseudomonas aeruginosa* was the most susceptible, and the aqueous and methanolic extracts exhibited a slightly higher efficiency against this pathogen than the drug amoxicillin.

## CONCLUSION

Medicinal property of plant extract is due to presence of secondary metabolites identified by GC-MS analysis. In the present study determined that thirty one phytoconstituents were identified from methanol extract of the whole plant of *A. capillusveneris* by Gas chromatogram and mass spectrometry (GC-MS) analysis. This plant derived bioactive compounds used as source of antibiotic, antioxidant, anti-inflammatory, anticancer properties and pharmaceutical industries used for drug formulation. This plant crude extract showed the phytochemical constituent has great potential for food resource and malnutrition of human health. Hence these compounds may act as potent drugs to treat obesity.

## ACKNOWLEDGMENTS

Special thanks to Prof. Abdul-Kareem, Babylon University, Faculty of science for women, for his special care.

## REFERENCES

- 1.Chandra S. The ferns of India (Enumeration, Synonyms and Distribution). International book distributors Dehradun, India. 2000; 459.
- 2.Dixit RD. Ferns- A much neglected group of medicinal plants. International Journal of Res Indian Med. 1974; 9:74-90.
- 3.Jain N, Magan A, Sondhi SM. Determination of mineral elements present in medicinal plants used for development of health, for treatment of cough and vomiting, pyorrhoea and rheumatic and allied disorder. Indian Drugs. 1992; 30:190- 194.
- 4.Kumar M, Ramesh M, Sequiera S. Medicinal pteridophytes of Kerala, South India. Indian Fern J. 2003; 20:1-28.
5. Singh KK, Saha S, Maheshwari JK. Ethnomedicinal uses of some ferns among the tribals of Uttar Pradesh. Indian Fern J. 1989; 6:63-67.
- 6.Santhoshkumar S, Nagarajan N, Screening of Preliminary phytochemical constitution and antimicrobial activity of *Adiantum capillus-veneris*. Journal of Research in antimicrobial. 2012; 1(1):056-061.
7. Sheela D, Uthayakumari F. GC-MS analysis of bioactive constituents from coastal sand Dune taxon – *Sesuvium portulacastrum*. Bioscience Discovery 2013; 4(1):47-53.
8. Lewis K, Ausubel FM. Prospects for plant-derived antibacterials. Nat Biotech. 2006; 24 (12):1504-1507.
9. Olagunju JA, Fagbohunka BS, Oyedapo OO, Abdul AIA. Effects of an ethanolic root extract of *Plumbago*

- zeylanica* L on some serum parameters of the rats. RPMP-Drug Dev Mol. 2006;11:268-276.
- 10.10.Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 2010; 4 (7):191-195.
  - 11.11.O'Bryan CA, Crandall PG, Chalova VI, Ricke SC. Orange essential oils antimicrobial activities against *Salmonella* spp. J. Food. Sci 2008;73:264-267.
  - 12.Hamza LF, Kamal SA, Hameed IH (2015) Determination of metabolites products by *Penicillium expansum* and evaluating antimicrobial activity. Journal of Pharmacognosy and Phytotherapy 7(9): 194-220.
  - 13.13.Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Journal of Pharmacognosy and Phytotherapy. 2015;7(8):132-163.
  - 14.14.Altameme HJ, Hameed IH, Idan SA and Hadi MY. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 2015;7(9):221-237.
  - 15.15.Rukayadi Y, Yong D, Hwang JK. In vitro anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. J Antimicrob Chemother 2006; 57:1231-1234.
  - 16.16.Altameme H J, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy 2015; 7(10):238-252.
  - 17.17.Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy 2015; 7 (7):107-125.
  - 18.18.Hameed IH, Abdulzahra AI, Jebor MA, Kqueen CY, Ommer AJ. Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions. Mitochondrial DNA. 2015;26(4):544-9.
  - 19.19.Al-Marzoqi AH, Hameed IH, Idan SA. Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). African Journal of Biotechnology. 2015;14(40):2812-2830.
  - 20.20.Hameed IH, Ibraheem IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus officinalis* leaves. Journal of Pharmacognosy and Phytotherapy. 2015;7 (6):90-106.
  - 21.21.Hameed IH, Jebor MA, Ommer AJ, Abdulzahra AI, Yoke C (2014). Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions. Mitochondrial DNA. 11,719-12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. 4:1-4.
  - 22.22.Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicrobial activity. Journal of Pharmacognosy and Phytotherapy. 2015;7(9):194-220.
  - 23.23.Karthishwaran K, Muthukumarasamy S, Sankaran M. GCMS analysis of methanolic extract of aerial parts of *Pergularia daemia*. Journal of Life Science 2012; 1(1):50-55.
  - 24.24.Swenson JM, Killgore GE, Tenover FC. Antimicrobial susceptibility testing of *Acinetobacter* spp. by NCCLS broth microdilution and disk diffusion methods. J Clin Microbiol 2004; 42:5102-5108.
  - 25.25.Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicrobial activity. Journal of Pharmacognosy and Phytotherapy 2015;7(9):194-220.
  - 26.26.Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy 2015;7(4):56-72.
  - 27.27.Idan SA, Al-Marzoqi AH, Hameed IH. Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry. African Journal of Biotechnology 2015; 14 (46):3131-3158.
  - 28.28.Adekunle AS, Adekunle OC. Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. Biol Med 2009; 1(3): 20-24.
  - 29.29.Bharathy V, Maria Sumathy B, Uthayakumari F. Determination of phytocomponents by GC-MS in leaves of *Jatropha gossypifolia*. Science Research Reporter 2012; 2(3):286-290.
  - 30.30.Balaj K, Kilimozhi D, Parthasarathy V. GC-MS analysis of various extracts of *clerodendrum phlomidis* leaf. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6 (1):226-232.
  - 31.31.Jain, R, Katore N, Kumar V. In vitro anti bacterial potential of different extracts of *Tagetes Erecta* and *Tagetes Patula*. J. Nat. Sci. Res 2012; 2:84-90.
  - 32.32.Rondon, M, J. Velasco, J. Hernandez. Chemical composition and antibacterial activity of the essential oil of *Tagetes patula* L. (Asteraceae) collected from the Venezuela Andes. Rev. Latinoam. Quim 2006; 34:1-3.
  - 33.33.Dildar A, Muhammad MK, Ramsha S. Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantum caudatum* Leaves. Antioxidants 2015; 4:394-409