Plant Omics Journal POJ

POJ 12(01):70-77 (2019) ISSN:1836-3644
doi: 10.21475/poj.12.01.19.pt1971

Assessment of camel thorn (*Alhagi maurorum*) as new sources of bioactive compounds using GC-MS technique

Reham M. Mostafa, Heba S. Essawy*

Botany Department, Facukty of Science, Benha University, Egypt

Corresponding author: hebasamyessawy@live.com

Abstract

Alhagi maurorum (A. maurorum) is one of the medicinally important plants belonging to the family leguminasae, commonly known as camel thorn. This research was amid to identify the chemical compounds in the aerial part of *A. maurorum* using GC-mass analysis. Three solvents with different polarities were used for the extraction of chemical constituents (water, methanol and petroleum ether). The results of GC-MS analysis led to identification of various compounds. In total, thirty-nine compounds from petroleum ether extract, thirty-two compounds in methanolic extract and seventeen compounds in aqueous extract were identified. Majority of the identified compounds have been reported to possess many biological activities. Among them, we reported 10 new anticancer compounds (Vitamin E; Hexadecanoic acid; Stigmast-5-en-3-ol; Phytol,2-hexadecen-1-ol,3,7,11,15-tetramethyl; Squalene; Hexadecanoic acid; 2-hydroxy-1-(hydroxymethyl) ethyl ester; Oxime,methoxy-phenyl,methyl N-hydroxyben-zenecarboximidoate; Ergost-5-en-3-ol; 9,12- Octadecad-ienoic acid and Farnesol) from *A. maurorum* using three solvent, while the best effective solvent was petroluem ether. Therefore, we report that *A. maurorum* has great potential to be developed into anticancer drugs.

Keywords: Secondary metabolites; *Alhagi maurarum*; GC-mass spectroscopy; anticancer compounds.

Introduction

Natural products have useful and interesting biological activities and they also do several functions. Researchers are progressively turning their attention toward natural products in order to develop better drugs against cancer as well as viral and microbial infections (Revathi and Parimelazhghan, 2010). In Egypt the medicinal plants represent a new promising resource, as there is a relatively high representation of medicinal species in the native flora. It is already seen the need to shed light on some medicinal plants because of their significance (Asmeda, 2013). Phytochemical studies on *A. maurorum* show carbohydrates, tannins, unsaturated sterols, triterpenes, flavonoids, and flavanone glycosides (Hamed et al., 2012).

Alhagi maurorum belongs to the Fabacea family and Fabales order. Fabacea comprises approximately 730 genera and 19,400 species. Plants belonging to this family are known to produce a large number of biologically important secondary metabolites. *A. maurorum* is one of the species of legumes. It is also known as camel thorn, Caspian manna, or Persian manna-plant (Duke, 2007). *A. maurorum* plant is extensively distributed and has wide ecological amplitude. It recorded from Nile region, oasis, Mediterranean region, Eastern and Western Desert, Red sea Coast and Sinai, also in Saudi Arabia deserts (Hassanein and Mazen, 2010). *Alhagi maurorum* is a very common woody perennial shrub, rich in flavonoid and phenolic compounds with more than twelve different isolated flavonoids (Al-Jaber et al., 2011). It is being used in diuretic, diaphoretic, and anti-ulcer treatments, and also has tissue-repairing properties (Al-Snafi, 2015). The plant is used in the treatment of diseases of the liver and urinary tract, and also as a laxative (Marashdah and Al-Hazimi 2010). The oil extracted from the leaves is applicable for the treatment of rheumatism, and the flowers are used for warts and migraines (Awaad et al., 2011). A study by Sulaiman showed that this plant has anti-bacterial properties (Sulaiman, 2013). *In vitro* anticancer activity of *Ocimum Basilicum*, *Alhagi maurorum*, *Calendula Officinalis* and their Parasite *Cuscuta Campestris* were studied by (Behbahani, 2014).

Results and Discussion

Results of GC-MS analysis in our study led to identification of various compounds, in the aerial part of *A. maurorum* using three different solvents and these compounds shown anticancer and cytotoxic properties. Thirty-nine compounds from petroleum ether extract (Table 1; Fig 1), thirty-two compounds from methanolic extract (Table 2; Fig 2) and seventeen compounds from aqueous extract (Table 3; Fig 3) were identified. The present study revealed that *A. maurarum* have fatty acids, phenols, steroids, alkanes, vitamine E and terpenoids as stated by (Ahmad et al., 2015). The chemical investigation of the *Alhagi* species by Karuppasamy et al. (2012), Kalhoro et al. (1997) and Verma et al. (2013) revealed the presence of many contents such as sterols, fatty acids and flavonoids (Behari and Gupt , 1980) and (Awaad et al., 2011) coumarins and alkaloids. Phenolic

compounds are considered as the biggest and widely dispersed groups of secondary metabolites in plants (Scalbert and Williamson, 2000).

Total phenolic and flavonoids contents of *A. maurorum* showed high concentrations, especially in the methanol extract.

Most of the identified compounds have been reported to possess interesting biological activities as antimicrobial, antiviral, antioxidant, anticancer, antitumor and anti-inflammatory e.g. phenol, 4- (methoxymethyl); stigmasterol; 2H-1-benzopyran-7-ol,3-(2,4-dimethoxyphenyl) -3,4-dihydro; 2-furan- carboxaldehyde,5-(hydroxymethyl); tetradecanoic acid, methyl ester; dodecanoic acid;; cosane derivatives; naphalene derivatives; Erythritol; Bis (2-ethylhexyl) phthalatehalic acid; Loliolide; Ledol (Shubhangi, 2016; Himaja and Moonjit, 2014; De Oliveira et al., 2014; Kuppuswamy et al., 2013). In this study, we identified ten new anticancer bioactive compounds in *A. maurorum* in Egypt, including Hexadecanoic acid; Stigmast-5-en-3-ol; Phytol,2-hexadecen-1-ol, 3,7,11,15-tetramethyl; vitamin E; Ergost-5-en-3-ol; Farnesol; Squalene; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 9,12-Octadecadienoic acid and Oxime-,methoxy-phenyl,methyl N-hydroxybenzene-carboximidoate (Table 4).

Using GC MS method, Sianipar et al. (2016) recorded only four anticancer compounds such as Hexadecanoic acid, Stigmast-5-en-3-ol, Ergost-5-en-3-ol and Farnesol) in the leaves and tubers of the fourth generation of rodent tuber's *(Typhonium flagelliformeLodd.)* vegetative mutant clones (MV4). In this study, Hexadecanoic acid was a solitary compound that appeared with the three solvent. Hexadecanoic acid (palmitic acid) was selectively cytotoxic against leukemia cancer cells MOLT-4 due to its interaction with DNA topoisomerase I and its ability to induce apoptosis. Hexadecanoic acid had *in vivo* antitumor activity (Harada et al., 2002). Biological activities of hexadecanoic acid ethyl ester were antioxidant, antimicrobial (Bodoprost and Rosemeyer, 2007) which could reduce the risk of coronary heart disease. Hexadecanoic acid methyl ester was able to inhibit the growth and induce apoptosis of human gastric cancer cells (Yu et al., 2005). Hema et al. (2011); Pietro et al. (2010) and Hsouna et al. (2011) showed another activities as hypocholesterolemic, nematicide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial agent, antimalarial and antifungal. The n-hexadecanoic acid has been recognised as the major compound in the leaves of *Cleistanthus collinus* (Parasuraman et al., 2009). Siddiq Ibraham et al. (2009) recorded the presence of n-Hexadecanoic acid in ethyl acetate extract of *Goniothalamus umbrosus* using GC-MS analysis. Stigmast-5-en-3-ol is a phytosterol with various biological activities. It could reduce the cell's cholesterol level and modify membrane lipid profile (Awad et al., 1996). This is an antidiabetic compound (Sujatha et al., 2010), which is able to inhibit the growth of cancer cells such as lung (Mendilaharsu et al., 1998), gastric (De Stefani et al., 2000), and ovary (McCann et al., 2003) cancers. It also has anti-tumor activity (Ekade and Manik, 2014), antioxidant (Zawistowaski, 2010), anti-osteoarthitic (Gabay et al., 2010), cyto-toxicity activity (Huang et al., 2009) and anti-HIV reverse transcriptase (De Oliveira et al., 2014). Ergost-5-en-3-ol (campesterol) is a phytosterol which has been proven to be able to inhibit various cancer cells, such as lung (Mendilaharsu et al., 1998), gastric (De Stefani et al., 2000), and ovary (McCann et al., 2003) cancers. Farnesol is a non-sterol isoprenoid which is commonly found in various fruits and aromatic plants, such as citrus, sage, spearmint, nutmeg, basil, lemon grass, and chamomile. Farnesol could selectively inhibit the proliferation and induce apoptosis of leukemia and cervical cancer cells (Rioja et al., 2000; Yazlovitskaya and Melnykovych, 1995). Farnesol has *in vivo* antitumor and anticarcinogenic activities (Belanger, 1998); (Crowell, 1999). Farnesol is shown to have detrimental effects on many microbes including bacteria and other fungi, such as *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Aspergillus* species, *Paracoccidioides brasiliensis* and *Mycobacterium smegmatis* (Semighini et al., 2006; Jabra-Rizk et al., 2006).

Squalene was able to inhibit carcinogenesis of various cancer cells, such as colon cancer (Rao et al*.,* 1998). Samejo et al. (2012) extracted squalene from the leaves of *A. maurorum* in Iraq. Squalene is being used in cosmetic products as a natural moisturizer (Sermakkani and Thangapandian, 2012). Vitamin E (α dan γ-tocopherol) has been proven to be able to reduce the risk of carcinogenesis (Jiang et al., 2001). Squalene has been reported in *Aloe vera* (Arunkumar and Muthuselvam, 2009) and (Praveen Kumar et al., 2010). The 9,12-Octadeca-dienoic acid (Z, Z) was found to have potential cancer preventive, anti-inflammatory and antiarthritic activities. Similar result was reported in *Croton tiglium* seed and found to have potential antioxidant and anticancer activity (Mangunwidjaja et al., 2006). Devi et al. (2009) reported that *Euphorbia longan* leaves mainly contains 9, 12-Octadecadienoic acid.

Phytol in *P. alatum* leaf is also found to be effective in different stages of arthritis (Parthipan et al., 2015). Similar results were also observed in the leaves of *Lantana camera* (Maria et al., 2011), *Mimosa pudica* (Sridharan et al., 2011) and aerial parts of *Flueggea leucopyrus* (Sudha et al., 2013). The results demonstrate the reactive oxygen species that promotes substances such as Phytol promising a novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi et al., 2009).

Vitamin E has antioxidant and many other medicinal activities (Stampfer et al., 1993; Rimm et al*.,* 1993). Vitamin E is a vital fat-soluble nutrient in the human body. It is essential due to the fact that body cannot manufacture its own vitamin E. So, foods and supplements must provide it (Sen et al., 2006). Epidemiological studies have reported that high vitamin E intakes are correlated with a reduced risk of cardiovascular diseases, whereas intakes of other dietary antioxidants (such as vitamin C and b-carotene) are not, suggesting that vitamin E plays specific roles beyond that of its antioxidant function (Stampfer et al., 1993; Rimm et al., 1993). Lipid-soluble antioxidants, such as vitamin E may protect breast tissue from oxidant damage that may lead to the development of cancer (Baum et al., 1991). It has been proposed that dietary supplementation of vitamin E in excess of dietary requirements may reduce risk of developing breast cancer in women (London et al., 1985). Dietary supplementation of vitamin E is potentially feasible and practical as preventive major measure for breast cancer.

Within a decade, there were several analytical techniques including NMR, TLC, UV, and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995). The GC-MS is a method used for screening, identification and quantification

Table 1. Compounds detected in petroleum ether extract.

RT	Chemical name	Peak area	Nature of compound
7.44	Decane	(+) 0.39	alkanes
8.98	Naphthalene,decahydro-[4.4.0] decane	(+) 0.04	Aromatic hydrocarbon
10.20	Undecane	(+) 0.93	alkanes
10.60	Benzeneethanol	(+) 0.67	Phenol
10.77	Maltol 4H-pyran-4-one,3-hydroxy-2-methyl	(+) 2.74	Phenol
13.03	Dodecane	(+) 1.23	alkanes
15.03	Octane,2,6-di methyl	(+) 0.16	alkanes
21.83	Benzene, (1-butylhexyl)decane, 5-phenyl-(5-decyl) benzene	(+) 2.32	Phenol
22.40	Dodecanoic acid	-	Fatty acid
22.90	4-Fluoroveratrole ,fluorobenzene,3,4-methoxy	-	Phenol
24.14	Benzene , (1-butylhebtyl)- undecane, 5-phenyl	(+) 8.75	Phenol
24.36	Benzen, (1-propyloctyl) – undecane, 4-phenyl- (1- Propyloctyl) benzene	(+) 4.36	Phenol
24.49	2-Furanethanol,-.beta.-ethoxy	-	Phenol
24.81	Benzene, (1-ethylnonyl Undecane, 3-phenyl-3-phenylundecane	(+) 3.87	Phenol
25.62	Benzen, (1-methyldecyl) -2-phenylundecane , undecane,2-phenyl	(+) 4.45	Phenol
26.22	Benzene, (1-pentylheptyl)-dodecane, 6-phenyl-6-phenyldodecan	(+) 2.82	Phenol
26.33	Benzene, (1-butyloctyl)-dodecane,5-phenyl-5-phenyldodecane	(+) 3.68	Phenol
26.56	Benzene, (1-propylnonyl)-4-phenyldodecane	(+) 3.81	Phenol
27.01	Benzene, (1-ethyldecyl)-3-phenyldodecane	(+) 3.26	Phenol
27.82	Benzene, (1-methylundecyl)-dodecane, 2-phenyl-	(+) 3.50	Phenol
28.27	Benzene, (1-pentyloctyl)- tridecane, 6-phenyl-	(+) 4.28	Phenol
29.25	1-Hexadecene	(+) 0.30	Alkanes
29.5	9,12,15-Octadecatrienoic acid,	(+) 0.12	Unsaturated fatty acids
30.09	Farnesol	(+) 0.08	Phenol
30.83	Hexadecanoic acid	(+) 0.82	Fatty acids
33.17	1-Octadecene	(+) 0.48	alkanes
33.72	Phytol,2-hexadecen-1-ol, 3,7,11,15-tetramethyl	(+) 7.69	Alkanes
34.27	9,12,15-Octadecatrienoic acid,linolenic acid	(+) 2.45	Unsaturated fatty acids
34.73	Ethyl 9, 12, 15-octadecatrienoate	(+) 0.60	Unsaturated fatty acids
36.94	Tricosane	(+) 0.18	alkanes
40.15	Pentacosane	(+) 0.33	alkanes
41.67	Hexacosane	(+) 0.34	alkanes
42.67	2H-1-Benzopyran-7-ol,3-(2,4-dimethoxyphenyl) -3,4-dihydro	(+) 0.23	Phenol
44.57	Octacosane	(+) 0.65	alkanes
45.03	Squalene	(+) 3.08	triterpene
45.96	Tetracosane	(+) 2.61	alkanes
46.61	Oxirane,2,2-dimethyl-3-(3,7,12,16,20)pentamethyl-3,7,11,15,19 –heneicosapentaeny	(+) 0.43	alkanes
48.56	Docosane	(+) 1.00	alkanes
48.87	Cholest-5-en-3-ol	(+) 0.36	Phenol
49.08	Vitamin E	(+) 5.15	vitamin
50.27	Ergost-5-en-3-ol	(+) 0.10	Phenol
50.69	Stigmasterol	(+) 0.85	Steroid
51.49	Stigmast-5-en-3-ol	(+) 1.79	Steroid

of several compounds in plant extracts. Gas chromatography (GC) is used to separate drugs that exist in the sample. The retention time (RT) is an identifying distinctive of a drug. The mass spectrometry (MS) is the detector for the GC.

Materials and methods

Plant material

Collection and Identification of the tested plant *A. maurorum* plants were collected from arid land at railway stations in Banha, Qulyubia government, Egypt. The collected plant was identified and authenticated by Botany Dept, Faculty of Science, Benha University, according to (Tackholm, 1974). The aerial part (leaves and stems) were washed with tap water and air-dried. Shoots of *A. maurorum* were oven dried at 50 º C. After 10 days they reached a constant weight. The plant shoots were grounded to very fine powder in mixer and stored in glass jars until use.

Extraction

The extraction of dried materials was done by petroleum ether (non-polar) and methanol (have a moderate polarity) using Soxhlet method (Nikhal et al., 2010), and by soaking in water (high polar) at room temp. The extraction was done after 24 hours and the extracts were filtered and concentrated to 5 ml using rotatory evaporator at room temperature.

Analysis method

The secondary metabolite compounds in three extracts were analyzed by Gas chromatography-mass spectrometry (GC-MS) analysis as described by (Wagay and Rothe, 2016). Aglient 6890 gas chromatograph supplied with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5ms (30m x 0.32 mm x 0.25µl film thickness). Samples were injected under the following conditions. Helium was used as carrier gas at

Table 2. Compounds detected in methanolic extract.

RT	Chemical name	Peak area	Nature of compound
5.95	Propanedioic acid, dimethyl ester	(+) 0.78	Fatty acid methyl ester
7.16	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	(+) 1.06	Phenol
11.78	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	(+) 3.66	Phenol
14.28	2-Furan- carboxaldehyde,5-(hydroxymethyl)	(+) 3.33	Aldehyde
15.30	2-Ethoxyethyl-.beta.-phenylpropionate ,2-ethoxyethyl,3-phenylpropanoate	(+) 1.19	Phenol
18.30	(E) -1-(2,3,6-trimethylphenyl)buta-1,3-diene	(+) 3.59	Phenol
19.08	Acetic acid , (1,2,3,4,5,6,7,8-octahydro -3,8,8-trimethylnaphth-2-yl)methyl ester	(+) 0.96	Unsaturated fatty acids
19.19	Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1 h –inden-4-yl)	(+) 0.60	Phenol
20.218	Benzene , 1-ethyl-3,5-diisopropyl-benzene	(+) 4.54	Aromatic compound
20.56	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	(+) 2.13	alkanes
22.40	Dodecanoic acid	(+) 0.19	Fatty acid
26.83	Tetradecanoic acid	(+) 0.93	Fatty acid
27.16	(-)-Loliolide	(+) 1.06	Terpene
30.18	9,11-Octedecediynoic acid, 8-hydroxy-methyl ester	(+) 1.24	Fatty acids
30.83	Hexadecanoic acid	(+) 4.46	Fatty acids
30.84	Mome inositol	(+) 2.36	Phenol
33.50	9,12,15-Octadecatrienoic acid , methylester	(+) 1.30	Fatty acids,methyl ester
33.72	Phytol,2-hexadecen-1-ol, 3,7,11,15-tetramethyl	(+) 3.65	Alkanes
34.13	9,12-Octadecadienoic acid	(+) 0.88	Unsaturated fatty acids
34.27	9,12,15-Octadecatrienoic acid,linolenic acid	(+) 3.43	Unsaturated fatty acids
34.61	Octadecanoic acid	(+) 1.53	Unsaturated fatty acids
40.32	Hexadecanoic acid , 2-hydroxy-1-(hydroxymethyl)ethyl ester	(+) 1.20	Fatty acids ethyl ester
40.96	Bis(2-ethylhexyl) phthalatehalic acid , bis(2-ethylhexyl)ester	(+) 5.58	Fatty acids ethyl ester
42.67	2H-1-Benzopyran-7-ol,3-(2,4-dimethoxyphenyl) -3,4-dihydro	(+) 1.31	Phenol
43.14	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	(+) 2.23	Fatty acids methyl ester
44.42	1a,12b-Dihydrobenzo[b] oxireno-[9,10] –phenanthro(3,2-d)thiophene	(+) 1.57	Aromatic compound
49.08	Vitamin E	(+) 8.62	vitamin
50.26	(E) -5,10-secocholest-1(10) – en-3,5-dione	(+) 1.17	Phenol
50.69	Stigmasterol	(+) 2.62	Steroid
51.49	Stigmast-5-en-3-ol	(+) 4.21	Steroid

Table 3. Compounds detected in water extract.

RT	Chemical name	Peak area	Nature of compound
4.00	2,3-Butanediol,2,3-butanediol	(+) 3.14	Phenol
4.13	1,3-Butanadiol,1,3-butylene glycol	(+) 17.40	Phenol
5.65	Oxime-,methoxy-phenyl,methyl -hydroxybenzenecarboximidoate	(+) 1.05	Phenol
14.85	1,2-Benzenediol, 3-methoxy-pyrocatechol, 3-methoxy	(+) 1.36	Phenol
16.05	1,2-Ethanediol,1-phenyl- styrene glycol	(+) 1.25	Phenol
17.48	Erythritol	(+) 0.40	Terpene
18.30	(E) -1-(2,3,6-trimethylphenyl)buta-1,3-diene	(+) 0.70	Phenol
19.25	Phenol, 4-(methoxymethyl)	(+) 1.23	Phenol
20.18	Ledol	(+) 4.01	TERPEN
20.82	2,3-Dimethylpenzene-1,4-dicarbonitrile	(+) 20.26	Phenol
22.90	4-Fluoroveratrole ,fluorobenzene,3,4-methoxy	(+) 19.52	Phenol
24.49	2-Furanethanol,-.beta.-ethoxy	(+) 2.08	Phenol
25.61	Tetradecanoic acid, methyl ester	(+) 0.51	Fatty acid
27.29	(3,4-Dihydroxyphenyl) hexylamine	(+) 1.39	Phenol
28.82	Oxacyclododeca-6,9-dien-2-one,7-methyl	(+) 1.32	Alkanes
30.83	Hexadecanoic acid	(+) 0.87	Fatty acids
50.99	2-p-Nitrophenyl-1,3,4-oxadiazol-5-one	(+) 2.29	Phenol

Table 4. Anticancer compounds in the three extracts.

Name of compound	Peak area			Nature of compound
	Petroleum ether	Methanol	Water	
Vitamin E	+(5.15)	+(8.62)	-	Vitamin
Hexadecanoic acid	+(0.82)	+(4.46)	+(0.87)	Fatty acid
Stigmast-5-en-3-ol	+(1.79)	+(4.21)	-	Steroid
Phytol,2-hexadecen-1-ol, 3,7,11,15-tetramethyl	+(7.69)	+(3.65)	-	Terpinoid
Squalene	+(3.08)	-	-	Triterpene
Hexadecanoic acid , 2-hydroxy-1-(hydroxymethyl) ethyl ester	-	+(1.20)	-	Fatty acid ethyl ester
Oxime-,methoxy-phenyl,methyl N-hydroxybenzenecarboximidoate	-	-	+(1.05)	Phenol
Ergost-5-en-3-ol	+(0.10)	-	-	Phenol
9,12-Octadecadienoic acid	-	+(0.88)	-	Unsaturated fatty acid
Farnesol	+(0.08)	-	-	Phenol

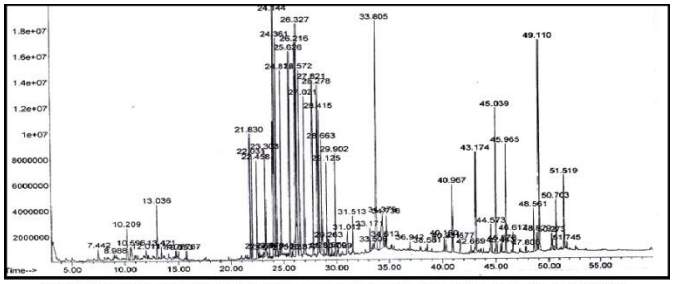

Fig (1) :Chromatogram showing bioactive compounds by petroleum ether solvent using GC-MS.

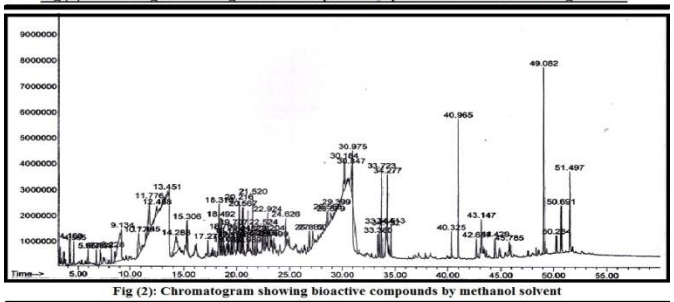

Fig (2): Chromatogram showing bioactive compounds by methanol solvent

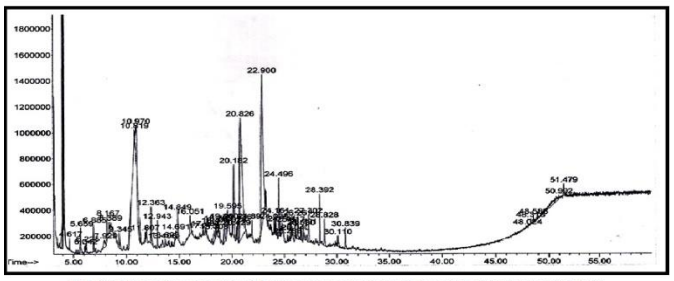

Fig (3): Chromatogram showing bioactive compounds by aqueous solvent using GC-MS.

The electron multiplier voltage (EM voltage) was maintained at 1650 V above auto tune. The instrument was manually tuned using perfluorotriputyl amine (PFTBA). The GC temperature program was started at 60°C (2 min) then elevated to 300°C at rate of 5°C /min. The injector temperature was set to 280 °C. Wiley and Wiley Nist mass spectral data base was used in the identification of the separated peaks. The previous analyses were done at the central pesticides laboratory.

Conclusion

This study provides information on *A. maurorum* and its natural products which have many biological activities. It is documented that *A. maurorum* is rich in anticancer compounds and has great potential to be developed into anticancer drug.

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