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Research Article

Analysis of the Secondary Metabolite Products of *Ammi majus* and Evaluation Anti-Insect Activity

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ABSTRACT

Aims of our study were analysis of the secondary metabolite products of *Ammi majus* and evaluation insecticidal activity against *Tribolium castaneum*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Ammi majus* revealed the existence of the Pregn-5-ene-3,11-dione , 17,20:20,21-bis [methylenebis(oxy)]- , Penta-2,4-dien-1-one , 5-dimethylamino-1-[5-(4-dimethylamino)] , 8-Octadecenal , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)- , 3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole , Piperidine , 2,3-dimethyl- , d-Mannose , 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol , Paromomycin , d-Glycero-d-ido-heptose , Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- , Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) , 5-Allyl-6-methyl-2-phenyl-5,6-dihydro-4H-oxazolo[4,5-c]pyridin- , 3-O-Methyl-d-glucose , Dasycarpidan-1-methanol , acetate (ester) and Corynan-17-ol,18,19-didehydro-10-methoxy-,acetate (ester). Methanolic extract of *Ammi majus* was active on accumulative mortality of *Tribolium castaneum* (adult).

Keywords: *Ammi majus*, Anti-insect, Bioactive chemical analysis, GC/MS, *Tribolium castaneum*.

INTRODUCTION

Several plant derived medicine are rich in phenolic and other compounds such as those used in protection against the coronary heart diseases and carcinogenesis[1]. Fructus Ammi Majoris consists of the dried ripe fruits of *Ammi majus* L. (Apiaceae)[1,2], and widely distributed in Europe, the Mediterranean region, and western Asia, now cultivated in India[2]. *Ammi majus* has different names, the Arabic name khillah, khillah shytani, English name bishops weed, Latin and German name ammi, French name ammi commun, belongs to family Apiaceae. Is an upright annual herb which grows up to 1 meter or more, whitish tap-roots; stem erect, slender, leaves alternate with long petiole, flower whitish actinomorphic or zygomorphic, fruit a cremocarp with slender prominent ribs, *A. majus* is one of the best medicinal plants that was first found in Mediterranean and Egypt[3]. It is used as an emmenagogue to regulate menstruation, as a diuretic, and for treatment of leprosy, kidney stones, and urinary tract infections[4]. This is widely used for the treatment of skin disorders such as psoriasis and vitiligo (acquired leukoderma), and of vitiligo[5]. In Europe due to climate conditions the growth of *Ammi majus* is poor, attempts to acclimatize *Ammi majus* in cool climate were not successful, the fruits failed to ripen and plants were highly susceptible to infection[6]. The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants[7-9]. The objectives of this study were analysis of the secondary metabolite products and evaluation anti-insect activity against adults of *Tribolium castaneum*.

MATERIALS AND METHODS

Plant material, extraction and isolation of Ammi majus

One kg of *Ammi majus* have been collected from Hilla city, middle of Iraq. *Ammi majus* was stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. Methanolic extract of *Ammi majus* powdered were soaked in 500 mL methanol for 14 hours in a rotatory shaker. The filtrates were used for further phytochemical analysis[10-16].

Evaluation of anti-insect activity

Tribolium castaneum was obtained from laboratory cultures maintained in the dark in incubators at 26 ± 1°C. This insect was reared on wheat flour mixed with yeast (10:1, w:w). A control was prepared in the same way but extract application was omitted. Three replicates were set up for the treated. Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests[17-22].

Gas chromatography – mass spectrum analysis

The Gas chromatography – mass spectrum analysis of methanolic extract of *Ammi majus* was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the

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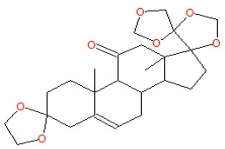
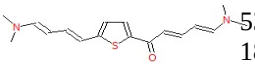
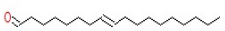
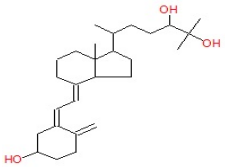
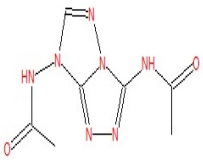
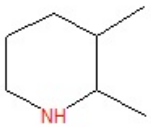
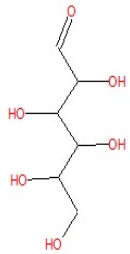
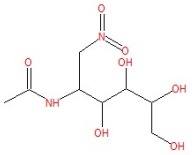
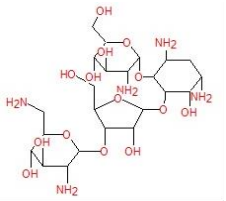
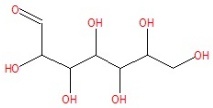
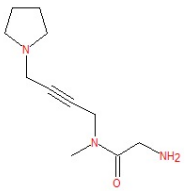
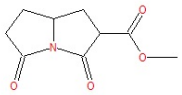
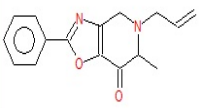
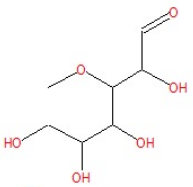
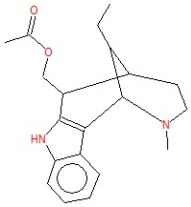
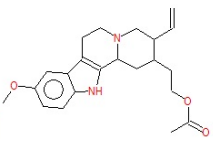
Table 1: Major phytochemical compounds identified in methanolic extract of *Ammi majus*.

S. No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-	3.178	446	446.230453		55,69,81,99, 161,256, 314,372,446	Unknown
2.	Penta-2,4-dien-1-one, 5-dimethylamino-1-[5-(4-dimethylamino)]	3.402	302	302.145285		53,81,94,124, 189, 221,258,302	*anti*-inflammatory properties
3.	8-Octadecenal	3.556	266	266.260965		57,68,82,97, 177,252	*anti* bacterial and *anti* fungal *activities*
4.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	3.653	416	416.329044		55,69,118,136, 158,176, 207,253,383,416	antitumor *activity*
5.	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	4.237	223	223.081773		59,69,84,97, 153,207	*anti*-mycobacterium tuberculosis *activity*
6.	Piperidine, 2,3-dimethyl-	4.546	113	113.1204495		56,70,84,98	antibacterial and antifungal *activity*
7.	d-Mannose	4.666	180	180.063388		60,73,103,149	*Anti*-Bacterial Agents
8.	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	4.832	252	252.095751		60,86,98,114, 161, 188,219,252	antifungal *activity*

9.	Paromomycin	5.055	615	615.296303		57,67,80,94, 109,191, 227,,259,292,324	*anti*-HIV-1 agents
10.	d-Glycero-d-ido-heptose	5.141	210	210.073953		60,73,85,133	*anti*-inflammatory and *anti*-septic *activities*
11.	Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	6.240	209	209.152812		55,70,84,121, 139,192	Unknown
12.	Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester)	8.122	197	197.068808		55,69,84,98, 142,197	Unknown
13.	5-Allyl-6-methyl-2-phenyl-5,6-dihydro-4H-oxazolo[4,5-c]pyridin-	7.739	268	268.121178		56,67,83, 105,129, 165,197,240	*anti*-inflammatory, antitumor and *anti*-parasitic *activity*
14.	3-O-Methyl-d-glucose	13.283	194	194.079039		73,87,103, 116, 145,163,177	*anti*-cancer, *anti* inflammatory
15.	Dasycarpidan-1-methanol , acetate (ester)	16.877	326	326.199429		69,97,180, 222, 256,326	antimicrobial, antioxidant and *anti*-inflammatory
16.	Corynan-17-ol,18,19-didehydro-10-methoxy-,acetate (ester)	17.975	368	368.209993		69,97,143, 186, 281,308,368	*anti*-diarrhoeal *activity*

GC-MS using a micro syringe and the scanning was done for 45 minutes. The time from when the injection was made (Initial time) to when elution occurred referred to as the Retention time (RT)[23-30]. While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called

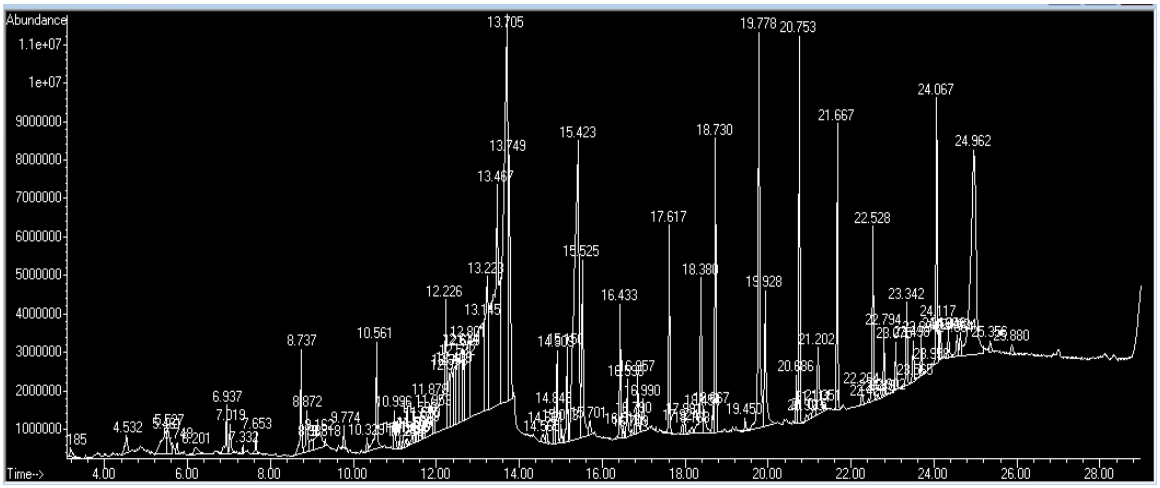


Figure 1: GC-MS chromatogram of methanolic extract of *Ammi majus*.

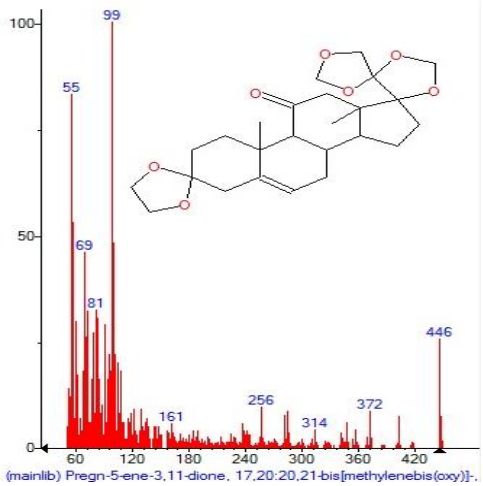


Figure 2: Mass spectrum of Pregn-5-ene-3,11-dione , 17,20:20,21-bis[methylenebis(oxy)]- with Retention Time (RT)= 3.178.

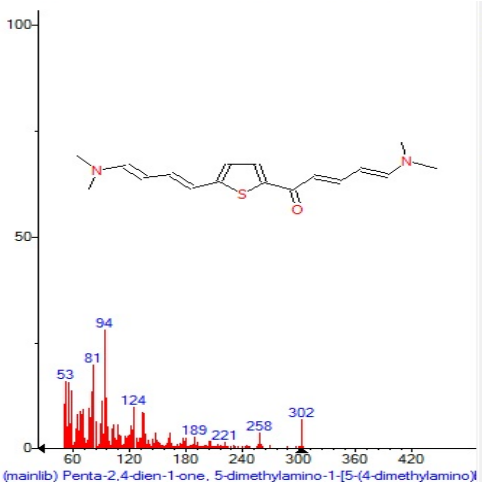


Figure 3: Mass spectrum of Penta-2,4-dien-1-one , 5-dimethylamino-1-[5-(4-dimethylamino)] with Retention Time (RT)= 3.402.

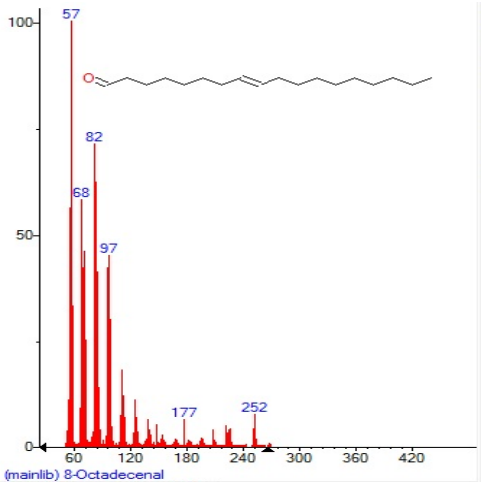


Figure 4: Mass spectrum of 8-Octadecenal with Retention Time (RT)= 3.556.

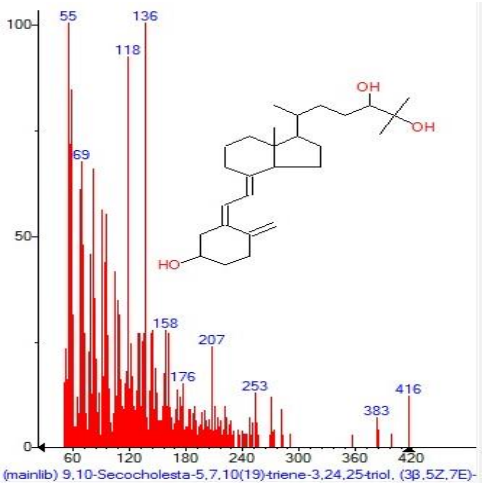


Figure 5: Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)- with Retention Time (RT)= 3.653.

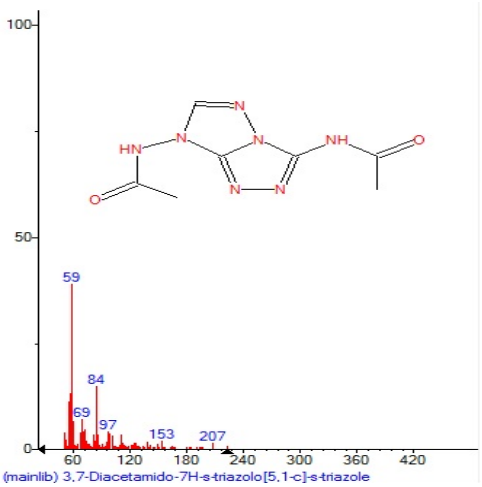


Figure 6: Mass spectrum of 3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole with Retention Time (RT)= 4.237.

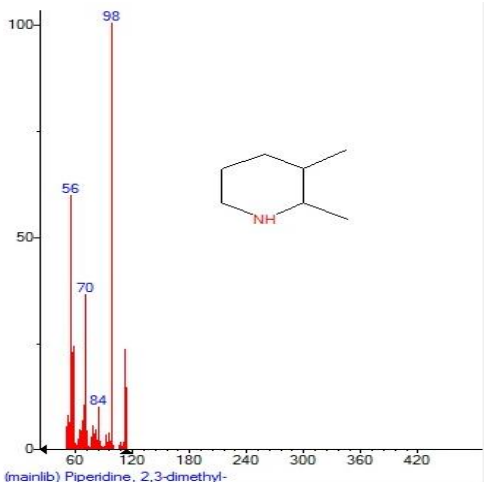


Figure 7: Mass spectrum of Piperidine , 2,3-dimethyl- with Retention Time (RT)= 4.546.

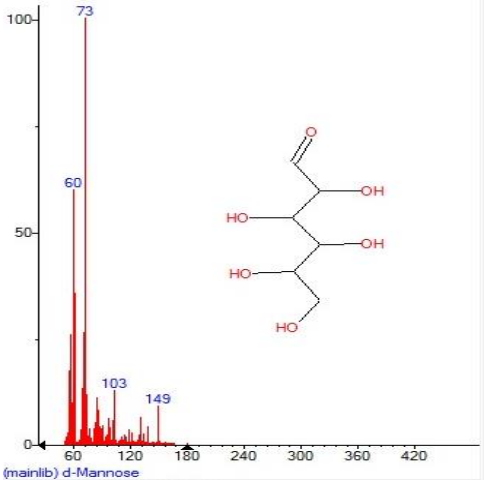


Figure 8: Mass spectrum of d-Mannose with Retention Time (RT)= 4.666.

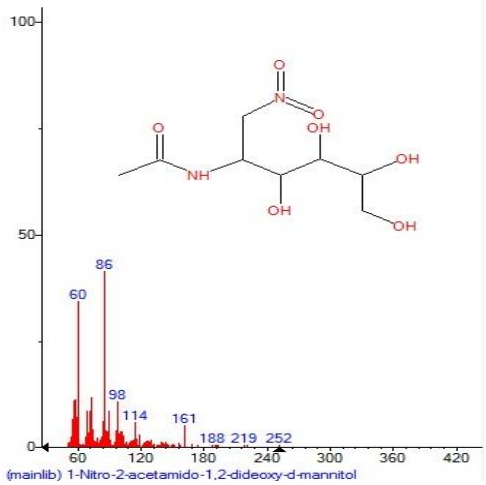


Figure 9: Mass spectrum of 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol with Retention Time (RT)= 4.832.

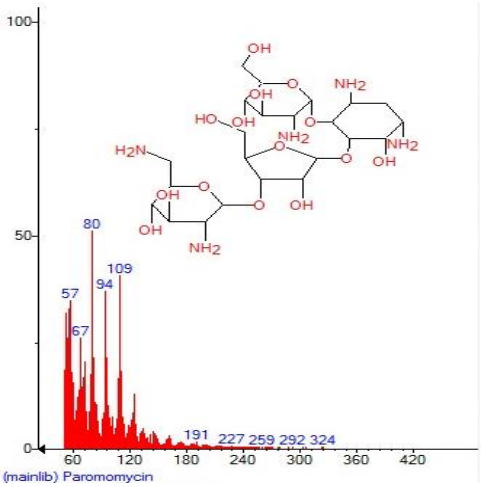


Figure 10: Mass spectrum of Paromomycin with Retention Time (RT)= 5.055.

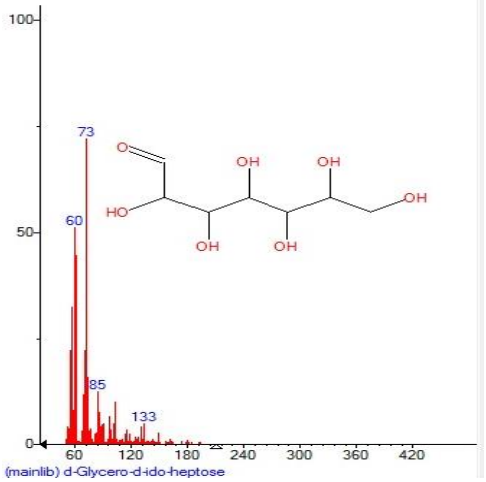


Figure 11: Mass spectrum of d-Glycero-d-ido-heptose with Retention Time (RT)= 5.141.

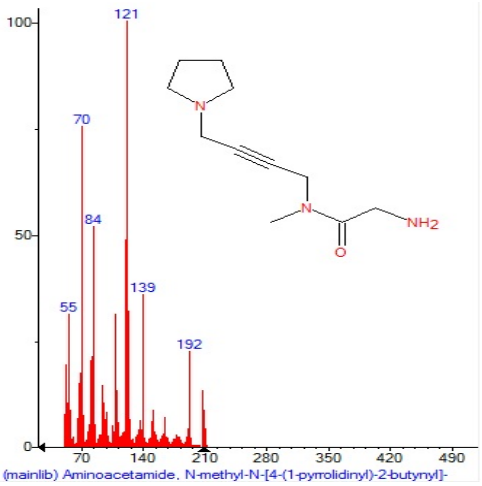


Figure 12: Mass spectrum of Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- with Retention Time (RT)= 6.240.

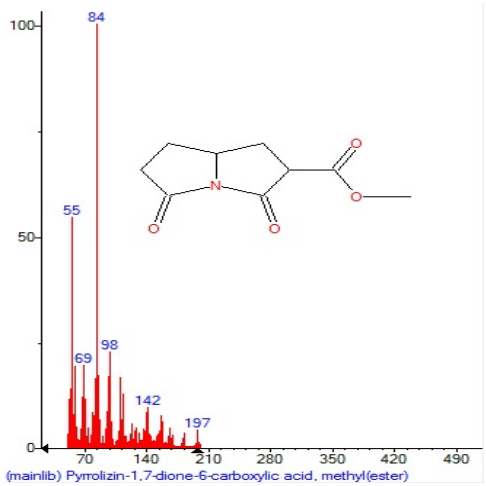


Figure 13: Mass spectrum of Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) with Retention Time (RT)= 8.122.

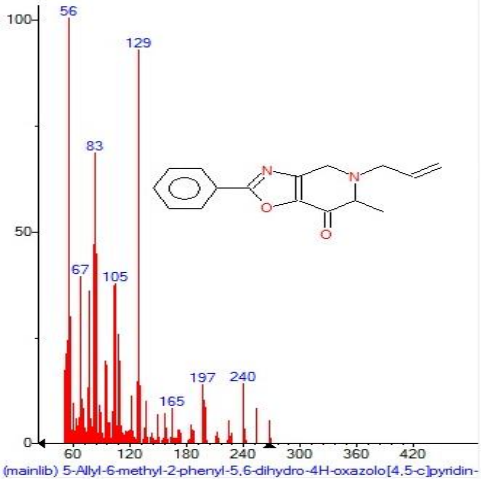


Figure 14: Mass spectrum of 5-Allyl-6-methyl-2-phenyl-5,6-dihydro-4H-oxazolo[4,5-c]pyridin- with Retention Time (RT)= 7.739.

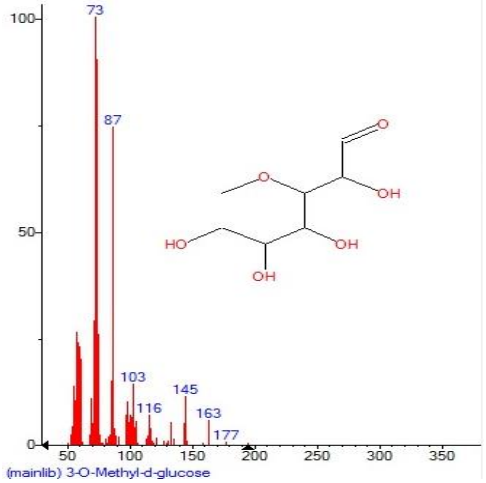


Figure 15: Mass spectrum of 3-O-Methyl-d-glucose with Retention Time (RT)= 13.283.

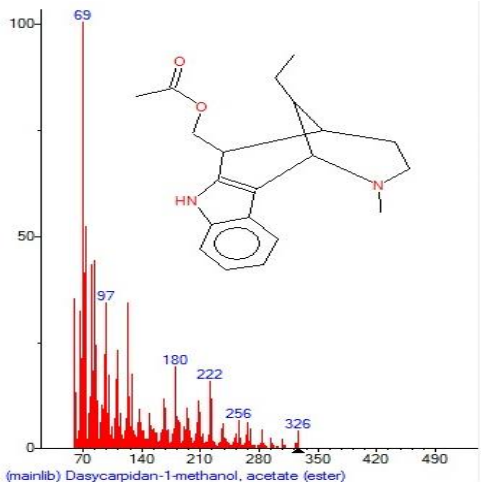


Figure 16: Mass spectrum of Dasycarpidan-1-methanol , acetate (ester) with Retention Time (RT)= 16.877.

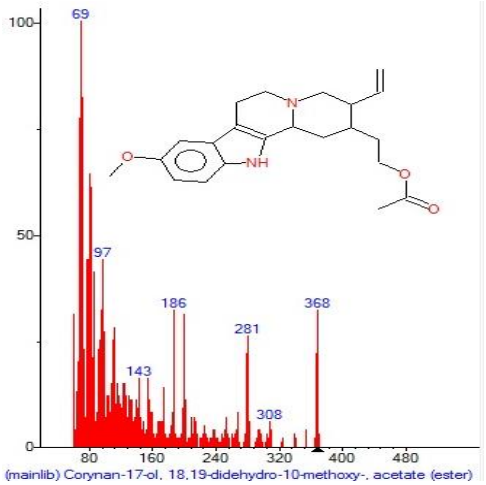


Figure 17: Mass spectrum of Corynan-17-ol,18,19-didehydro-10-methoxy-,acetate (ester) with Retention Time (RT)= 17.975.

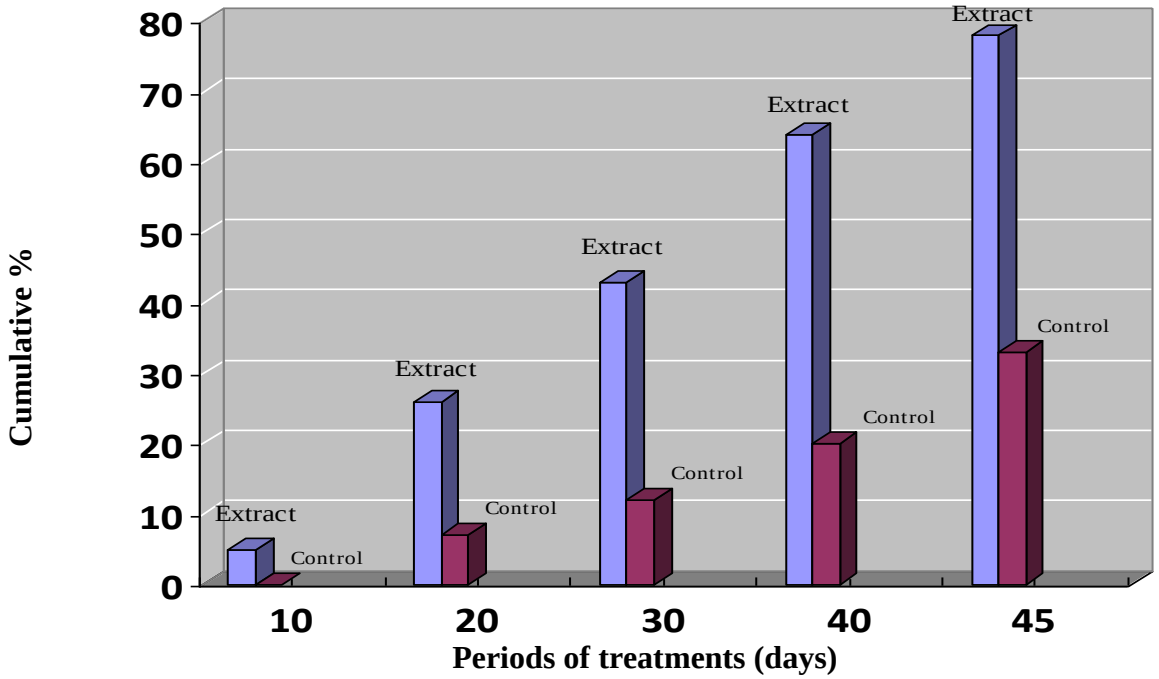


Figure 18: Effect of methanolic leaves extract of *Ammi majus* on accumulative mortality of *Tribolium castaneum* (adult).

as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries[31-33].

RESULTS AND DISCUSSION

Characterization of phytochemical compounds

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. GC-MS analysis of compounds was carried out in methanolic extract of *Ammi majus*, shown in Table 1. The GC-MS chromatogram of the 16 peaks of the compounds detected was shown in Figure 1. The First set up peak were determined to be Pregn-5-ene-3,11-dione , 17,20:20,21-bis [methylenebis(oxy)]- , Penta-2,4-dien-1-one, 5-dimethylamino-1-[5-(4-dimethylamino)], 8-Octadecenal, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)-, 3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole, Piperidine, 2,3-dimethyl-, d-Mannose, 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, Paromomycin, d-Glycero-d-ido-heptose, Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-, Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester), 5-Allyl-6-methyl-2-phenyl-5,6-dihydro-4H-oxazolo[4,5-c]pyridin- , 3-O-Methyl-d-glucose, Dasycarpidan-1-methanol, acetate (ester) and Corynan-17-ol,18,19-didehydro-10-methoxy-, acetate (ester) Figure 2-17.

Anti-insect activity

In the current study, the anti-insect activity of the methanolic extract was evaluated. Extract of *Ammi majus* caused 50% mortality during the 30 days after treatment. The methanol extracts of *Ammi majus* significantly affected survival of adult with 79%, during 45 days after treatment Figure 18. The relation between exposure period and treatment was very significant $p < 0.05$. Significant insecticidal activity against *T. castaneum* adults was observed with crude methanol extract from *Ammi majus*. Adults were more susceptible than larvae to extract of *Ammi majus*. Beside antimicrobial activity, the essential oil and its constituents have also been used for their herbicidal[26-35], insecticidal, and anti-leech properties, as well as in integrated disease management against phytopathogenic fungi, nonspecific skin infections. *Ammi majus* belong to the family Apiaceae that provide a huge number of compounds with important medicinal activities. Numerous clinical trials have assessed the efficacy of fructus Ammi Majoris and xanthotoxin for the treatment of vitiligo, psoriasis, and hypopigmentation tinea versicolor. The natural source of producing huge numbers of phytoconstituents in a most efficient way and with precise selectivity are plants and different bioactive phytoconstituents have been isolated and characterized in the middle of the 19th century, many of these are used as the active ingredients of the modern medicine or as the lead compounds for new drug discovery[36-43].

CONCLUSION

Sixteen bioactive chemical constituents have been identified from methanolic extract of the *Ammi majus* by

Gas Chromatography-Mass Spectrometry technique. Evaluation of anti-insect of secondary metabolite products of *Ammi majus* forms a primary platform for further pharmacological investigation for the development of new potential anti-insect compounds.

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