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 [methylenbis(oxy)]-. Penta-2,4-dien-1-one, 5-dimethylamino-1-[5-(4-dimethylamino)], 8-Octadecenal, 9,10-secosterol-5,7,10(19)-triene-3,24,25-triol, [3f,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-Glucos-d-id-o-heptose, Aminoacetamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butylnyl].. Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester), 5-Allyl-6-methyl-2-phenyl-5,6-dihydro-4H-oxazolo[4,5-c]pyridine, 3-O-Methyl-d-glucose, Dasyrcarpin-1-methanol, acetate (ester) and Corynanth-17-ol,18,19-didehydro-10-methoxy-acetate (ester). Methanic 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. Fructus Ammi Majoris consists of the dried ripe fruits of *Ammi majus* L. (*Apiaceae*) and, widely distributed in Europe, the Mediterranean region, and western Asia, now cultivated in India. *Ammi majus* has different names, the Arabic name khillah, khillah shytani, English name bishop 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. It is used as an emmenagogue to regulate menstruation, as a diuretic, and for treatment of leprosy, kidney stones, and urinary tract infections. This is widely used for the treatment of skin disorders such as psoriasis and vitiligo (acquired leukoderma), and of vitiligo. 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. The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. 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* were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. Methanic 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.

*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 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. Gas chromatography – mass spectrum analysis

The Gas chromatography – mass spectrum analysis of methanic 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
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Molar Weight</th>
<th>Exact Mass</th>
<th>Chemical structure</th>
<th>MS Fragment ions</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pregn-5-ene-3,11-dione , 17,20:20,2-(methyl enebis(oxy))</td>
<td>3.178</td>
<td>446</td>
<td>446.2304</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>55.69, 81.99, 161.256, 314.372, 446</td>
<td>Unknown</td>
</tr>
<tr>
<td>2.</td>
<td>Penta-2,4-dien-1-one , 5-dimethylamino-1-{5-(4-dimethylamino)}</td>
<td>3.402</td>
<td>302</td>
<td>302.1452</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>53, 81.94, 124, 189, 221.258, 302</td>
<td>anti-inflammatory properties</td>
</tr>
<tr>
<td>3.</td>
<td>8-Octadecenal</td>
<td>3.556</td>
<td>266</td>
<td>266.2609</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>57.68, 82.97, 177.252</td>
<td>anti bacterial and anti fungal activities</td>
</tr>
<tr>
<td>4.</td>
<td>9,10-Secocholest-5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)</td>
<td>3.653</td>
<td>416</td>
<td>416.3290</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>55.69, 118.136, 158.176, 207.253, 383.41</td>
<td>antitumor activity</td>
</tr>
<tr>
<td>5.</td>
<td>3,7-Diacetamido-7H-s-triazolo[5, 1-c]-s-triazole</td>
<td>4.237</td>
<td>223</td>
<td>223.0817</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>59.69, 84.97, 153.207</td>
<td>anti-mycobacterium tuberculosis activity</td>
</tr>
<tr>
<td>6.</td>
<td>Piperidine , 2,3-dimethyl-</td>
<td>4.546</td>
<td>113</td>
<td>113.1204</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>56.70, 84.98</td>
<td>antibacterial and antifungal activity</td>
</tr>
<tr>
<td>7.</td>
<td>d-Mannose</td>
<td>4.666</td>
<td>180</td>
<td>180.0633</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>60.73, 103.149</td>
<td>Anti-Bacterial Agents</td>
</tr>
<tr>
<td>8.</td>
<td>1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol</td>
<td>4.832</td>
<td>252</td>
<td>252.0957</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>60.86, 98.114, 161, 188.219, 252</td>
<td>antifungal activity</td>
</tr>
</tbody>
</table>
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)\textsuperscript{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 \text{ /} charge) ratio obtained was calibrated from the graph obtained, which was called anti-HIV-1 agents
109,191, 227,259,292,324
anti-inflammatory and anti-septic activities
Unknown
anti-inflammatory, antitumor and anti-parasitic activity
Unknown
anti-cancer, anti-inflammatory
antimicrobial, antioxidant and anti-inflammatory
anti-diarrhoeal activity
Figure 1: GC-MS chromatogram of methanolic extract of *Ammi majus*.

Figure 2: Mass spectrum of Pregn-5-one-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 Dasyocardian-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.
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. The GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. GC-MS analysis of compounds detected was 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-idoo-heptose, Aminosacetamide, N-methyl-N-[4-(1-pyrolidinyl)-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]pyrindin-, 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, insecticidal, and anti-leech properties, as well as in integrated disease management against phytopathogenic fungi, nonspecific skin infections. Ammi majus belong to the family Apiceae 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. 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.

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-idoo-heptose, Aminosacetamide, N-methyl-N-[4-(1-pyrolidinyl)-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]pyrindin-, 3-O-Methyl-d-glucose, Dasycarpidan-1-methanol, acetate (ester) and Corynan-17-ol,18,19-didehydro-10-methoxy- acetate (ester) Figure 2-17.

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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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