

Study of Bioactive Methanolic Extract of *Camponotus fellah* Using Gas Chromatography – Mass Spectrum

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Available Online: 25th December, 2016

ABSTRACT

GC-MS analysis of *Camponotus fellah* revealed the existence of the Cyclopentaneacetaldehyde, 2-formyl-3-methyl- α -methylene-, Cyclohexene, 1-methyl-5-(1-methylethenyl)-(R)-, Bicyclo[5.1.0]octane, 8-methylene-, 5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione, Decane, 1-Heptanol, 2-propyl, Octane, -4-methyl, Cyclopentanol, 2-(2-propynyloxy)-, trans-, Hydroxylamine, O-decyl, Trans-2-Ethyl-2-hexen-1-ol, Cyclopropene, 1-methyl-3-(2-methylcyclopropyl)-, 10-methyl-endo-tricyclo[5.2.1.0(2.6)]decane, Undecen-1-yne, (Z, Exo-Norbornyl propionate, 5,8,10-Undecatrien-3-ol, 3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol, Cyclohexane, 1,3-butadienyliidene-, Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)-, Pyrroline, 1,2-dimethyl, Cyclooctene-1-carboxaldehyde, Cyclopentanol, 1-(1-methylene-2-propenyl)-, Eicosanoic acid, phenylmethyl ester, Octahydrochromen-2-one, Methyl 12-oxo-9-dodecenoate, 14-Methylpentadec-9-enoic acid methyl ester, 10-Octadecenoic acid, methyl ester, 9-Octadecenoic acid (Z)-, phenylmethyl ester, 14-Octadecenal, Butyl 9-tetradecenoate. *Camponotus fellah* produce many important secondary metabolites with high biological activities.

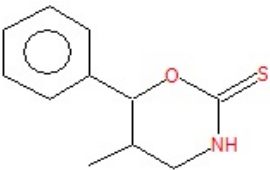
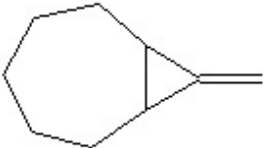
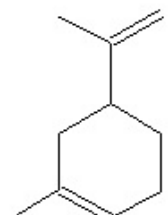
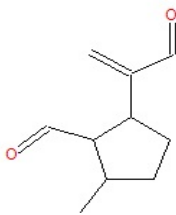
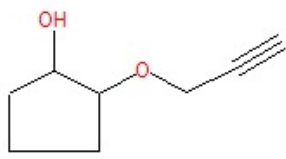
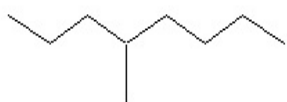
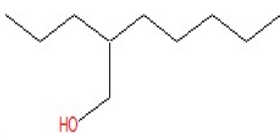

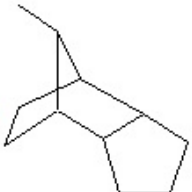
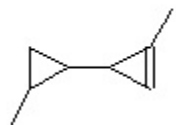
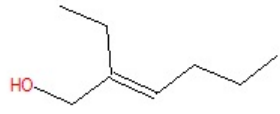

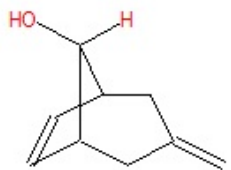
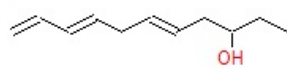
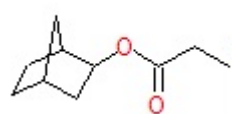

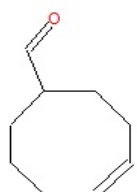
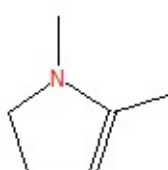
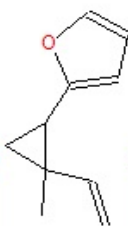
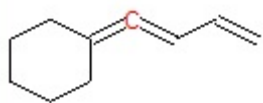
Keywords: Bioactive compounds, *Camponotus fellah*, GC-MS, Insect.

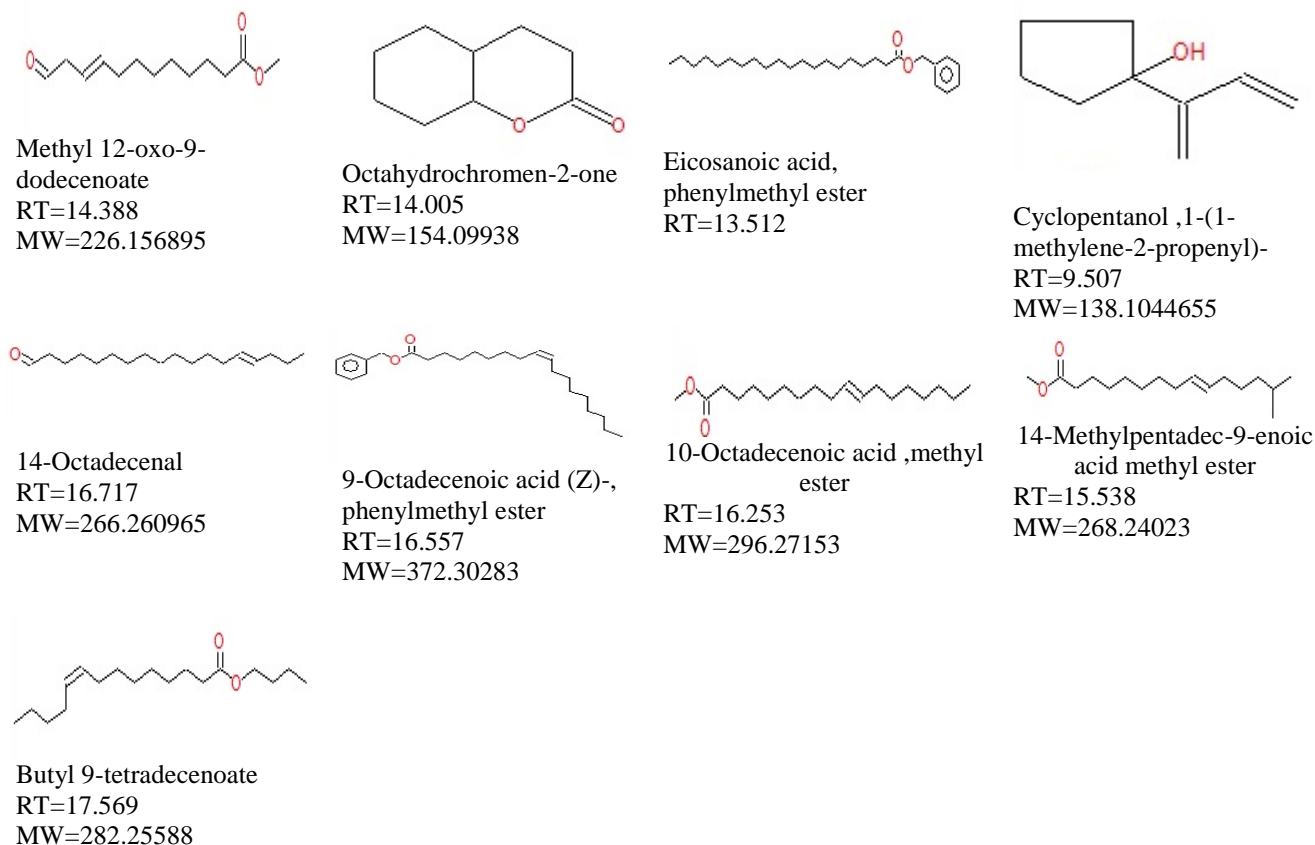
INTRODUCTION

C. fellah is a large species of *Tanaemyrmex*, with accentuated ground sculpture (major worker is mostly matte) and prismatic hindtibia that is provided ventrally with a row of bristles^{1,2}. *C. fellah* is similar to *Camponotus xerxes*, *Camponotus thoracicus sensu lato*, and *Camponotus oasium* from which it differs by the presence of erect setae on the ventral head surface, as opposed to a lack of such setae. *C. fellah* is distributed in Syria and Lebanon, Jordan, Iraq, Iran, Egypt, and the Arabian Peninsula. It also showed that the cuticular hydrocarbon profile is polymorphic, and that its homogeneity within a colony is maintained by frequent exchanges of hydrocarbons between workers. Behavioural observations of resident workers, in their nest, towards nestmates reintroduced after isolation indicated that a short isolation period (3-5 days), which induced a minor change in hydrocarbon profile, provoked frequent trophallactic solicitations³⁻⁶. These were likely to permit the isolated ants to readjust their hydrocarbon profile to that of the ants in the mother colony. Longer isolation periods (20-40 days) induced a greater change in hydrocarbon profile and made the residents intolerant towards their introduced nestmates. Therefore, our results clearly support the existence of a Gestalt colony odour in *C. fellah*. They also show that since individual hydrocarbon production is dynamic, workers are obliged to exchange hydrocarbons continually (mainly by

trophallaxis) in order to be in the Gestalt, and properly integrate into the colony. Copyright 2000. The Association for the Study of Animal Behaviour. Ants are widespread and abundant in almost every terrestrial ecosystem, yet surprisingly little is known about their reproductive ecology^{7,8}. In many ant species, mating occurs in nuptial flights, in which males and females emerge from their maternal nest in order to mate and establish new colonies. Most ant species are exogamous and invest much energy and biomass in their reproductive castes, making the precise timing of nuptial flights also of paramount importance for their predators^{9,10}. Synchronization among colonies could serve as an antipredator strategy by swamping the predator population. Such a reproductive strategy is known for many ant and termite species, as well as for tropical trees with mass flowering. In temperate latitudes, nuptial flights are brief, usually synchronized with meteorological events such as first rain of the season or an extremely hot day or concentrated at the beginning of summer or early fall. There are two main types of ant mating syndromes: (1) male aggregation, in which large swarms of male alates gather in aggregations that function as leks, at which the females arrive to select males. These gatherings tend to be short in time and are assumed to be synchronous across a large area and (2) female "calling," in which females attract males^{11,12}. This

Table 1: Bioactive chemical compounds identified in methanolic extract of *Camponotus fellah*.

			
5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione RT=4.231 MW=207.071785	Bicyclo[5.1.0]octane, 8-methylene- RT=3.974 MW=122.1095505	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)- RT=3.756 MW=136.1252	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene- RT=3.156 MW=166.09938
			
Cyclopentanol, 2-(2-propynyloxy)-, trans- RT=4.701 MW=140.08373	Octane, -4-methyl- Rt=4.672 Mw=128.156501	1-Heptanol, 2-propyl- RT=4.638 MW=158.167066	Decane RT=4.586 MW=142.172151
			
Anti-10-methyl-endo-tricyclo[5.2.1.0(2.6)]decane RT=5.055 MW=150.140851	Cyclopropene, 1-methyl-3-(2-methylcyclopropyl)- RT=4.941 MW=108.0939	Trans-2-Ethyl-2-hexen-1-ol RT=4.781 MW=128.120115	Hydroxylamine, O-decyl- RT=4.729 MW=173.177964
			
3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol RT=5.593 MW=136.088815	5,8,10-Undecatrien-3-ol RT=5.496 MW=166.135765	Exo-Norbornyl propionate RT=5.158 MW=168.115029	3-Undecen-1-yne, (Z)- RT=5.084 MW=5.084
			
4-Cyclooctene-1-carboxaldehyde RT=8.494 MW=138.1044655	Pyrroline, 1,2-dimethyl- RT=8.111 MW=97.0891495	Cyclopropane, -1-methyl-1-ethenyl-2-(2-furyl)-, RT=5.856 MW=148.088815	Cyclohexane, 1,3-butadienylidene- RT=5.742 MW=134.1955



latter syndrome can last for very long periods or even year-round, with occasional males flying out in search of calling females. These species usually perform mass nuptial flights on spring afternoons, synchronized with a low-pressure system from North Africa that brings dry air and high temperatures (“sharav”), and is usually followed by a burst of rain¹². Bioactive methanolic extract of *Camponotus fellah* using gas chromatography – mass spectrum was the aim of this study.

MATERIALS AND METHODS

The colonies of *C. fellah* were obtained by rearing newly mated queens collected in hillah in April 2015. They were maintained in a temperature-controlled room conditions. Colonies were installed in artificial plaster nests allowing direct observations of intranidal activities. Each nest was connected with a foraging area. The ants were fed with diet consisting of dead insects (mealworms, flies and moths) and sugar solution supplied twice a week. We used 15 colonies containing each one queen, at least 250 workers and abundant brood. We used as subjects media workers present in the foraging area. Laboratory culture of *C. fellah* was obtained from infested seeds from college of science for woman university of Babylon. The insects emerged after four weeks were used in entire investigation¹³⁻¹⁸. The extraction was performed by adding 100 ml methanol to the whole body insect powder. Methanol was used as solvent control.

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. The GC-MS analysis of the insect (*C. fellah*) extract was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes¹⁹⁻³³. The time from when the injection was made (Initial time) to when elution occurred referred to as the Retention time (RT). Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries³⁴⁻⁴⁴.

RESULTS AND DISCUSSION

Identification of biochemical compounds

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *C. fellah*, shown in Table 1. The GC-MS chromatogram of the peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *C. fellah* showed the presence of twenty nine major peaks and the components corresponding to the peaks were determined as follows. The set up peak were determined to be Cyclopentaneacetaldehyde, 2-formyl-3-methyl- α -methylene-, Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-, Bicyclo[5.1.0]octane, 8-methylene-, 5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione, Decane, 1-

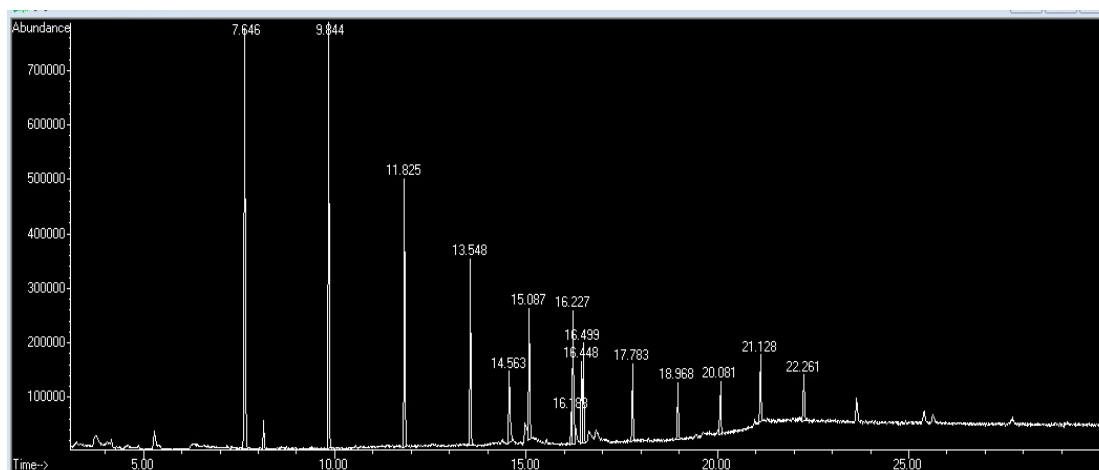


Figure 1: GC-MS chromatogram of methanolic leaves extract of *Camponotus fellah*.

Heptanol, 2-propyl, Octane, -4-methyl, Cyclopentanol, 2-(2-propynyloxy)-, trans-, Hydroxylamine, O-decyl, Trans-2-Ethyl-2-hexen-1-ol, Cyclopropene, 1-methyl-3-(2-methylcyclopropyl)-, 10-methyl-endo-tricyclo[5.2.1.0(2.6)]decane, Undecen-1-yne, (Z, Exo-Norbornyl propionate, 5,8,10-Undecatrien-3-ol, 3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol, Cyclohexane, 1,3-butadienylidene-, Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)-, Pyrroline, 1,2-dimethyl, Cyclooctene-1-carboxaldehyde, Cyclopentanol, 1-(1-methylene-2-propenyl)-, Eicosanoic acid, phenylmethyl ester, Octahydrochromen-2-one, Methyl 12-oxo-9-dodecenoate, 14-Methylpentadec-9-enoic acid methyl ester, 10-Octadecenoic acid, methyl ester, 9-Octadecenoic acid (Z)-, phenylmethyl ester, 14-Octadecenal, Butyl 9-tetradecenoate. AMPs are short bioactive molecules either cationic or non-cationic in nature with broad-spectrum antimicrobial effects against many pathogens. AMPs are short bioactive molecules either cationic or non-cationic in nature with broad-spectrum antimicrobial effects against many pathogens. They generally act in destabilizing the cell membrane permeability or interacting with the specific targets in cells which cause signaling pathway disruption. Interestingly, insect whole body is also an alternative source for the discovery of a new class of small AMPs which may have a role in processes other than immunity⁴⁵⁻⁵⁰. The AMP extraction from the whole body is governed by the following facts: (i) AMP production is not restricted to fat bodies and hemocytes, (ii) unknown physiological location of the molecules and (iii) small-sized body of insects. Initial peptide recovery from insect whole body requires the sample to be first subjected to homogenization. The beetle is known for attacking the cowpea (*Vigna unguiculata*), but it readily attacks other beans and peas such as the mung bean (*Vigna radiata*) and adzuki bean (*Vigna angularis*). The adult is more likely to seek the legume in which it developed as a larva, but if it is not available or less common, the beetle will utilize another type. Research devoted to the impact of immune responses on insect social behaviour is still at its beginning, especially in the case of ants. If we assume that both social relations and immune responses are

costly, we will have to assume as well that each individual has to choose where to invest its energy. To throw more light on that question, we studied the relations between immune responses and social behaviour in the ant *Camponotus fellah*, a species known to engage in intense social interactions⁵¹. PGNs are the major component of the cell wall of Gram-positive organisms that confer to the cell wall great mechanical strength, eliciting immune reactions in the infected organisms^{52,53}.

CONCLUSION

Twenty nine bioactive chemical constituents have been identified from methanolic extract of the *Camponotus fellah* by GC-MS technique.

ACKNOWLEDGMENT

The authors thank the department of Medical Science in college of nursing for providing all necessary facilities to conduct this study.

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