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Exploration of Bioactive Compounds from *Camponotus compressus*using Gas Chromatography - Mass Spectroscopy for Therapeutic Applications

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ABSTRACT

Gas chromatography-mass spectrometry (GC-MS) analysis of the aqueous extract GC-MS analysis was performed using the Agilent technologies 6890 N JEOL GC-MS Mate II model instrument(IFGTC Coimbatore). The test showed the presence of 32 compounds. The identified compounds were 5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione, Bicyclo[5.1.0]octane,8-methylene, Pentadecane, 7-Chloro-2,3-dihydro-3-(4 nitrobenzilidene)-5-phenyl-1H-4-ben, Diphenyl r-2-methoxycarbonyl-2,c-5-diphenylpyrrolidin-c-3,c-4-d, Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R)-, 1-Heptanol, 2-propyl- , Pyrroline, 1,2-dimethyl- , Decane, Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)-, Butyl 9-tetradecenoate, Benzenemethanol, .alpha.-[1-(ethylmethylamino)ethyl]-, [R-(R*,S,Hexadecanoic acid (CAS), 3-Hydroxymyristic acid, 1-Dotriacontanol (CAS), Tritetracontane, Ethyl linoleate, 2,3-Dihydroxypropyl elaidate, Octadecanoic acid, Octane, -4-methyl-, 5,8,10-Undecatrien-3-ol , 3-Undecen-1-yne, (Z)- , 2-Pentacosanone, Oleic anhydride, N'-(2-Methyl-4-chlorophenyl)-N-cyclohexyformamidine, Acetic acid, (triphenylphosphoranylidene)-, methyl ester (CAS), Cholesta-3,5-diene (CAS), 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9a-tetrakis(a, Cholest-5-en-3-ol (3.beta.)-, 9-octadecenoate, (Z)-, Cholesta-4,6-dien-3-ol, (3.beta.)-, Lycoxanthin, Cholest-5-en-3-ol (3.beta.)-, acetate (CAS).From the results, it is evident that *Camponotus compressus* contains various bio components and it can be analysed for pharmaceutical applications.

KEYWORDS: Bioactive compounds, *Camponotus compressus,* Entomotheraphy.

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INTRODUCTION

Insects are the most prevailing species on earth. Insects play many significant roles for ecosystem services in nature, certain insects provide sources of commercially important products which can be of direct advantage to humans. Insects and the substances extracted from them have been used as therapeutic resources in the medical systems of many cultures. Many insect species all over the world have been used live, cooked, ground, in infusions, in plasters, in salves, and as ointments, both in curative and preventive medicines.[1] The therapeutic use of insects and their products is termed as entomotherapy.

Ants serve very useful purpose in nature. Despite their tiny size, they have made themselves extremely dominant and effective group by their huge population size, studiousness, survival even in very odd situations, division of labour and excellent intra colony cooperation.. Recent interest in the ant's medicinal qualities by British researchers has led to investigations into the extract's ability to serve as a cancer-fighting agent. Dunn and Sanchez[2] have argued for the use of social insects as medicine, particularly ants and termites, by comparing their social behaviour and nature to those of human beings, so they have evolved the use of different antibiotics and fungicides that can be directly used by humans. Soil-dwelling ants have been shown to make and employ compounds that kill both fungi and bacteria in their underground nests, and the predatory water beetle is known to use phenolic compounds to repel microbial attacks.[3]

Camponotus compressus is a common ant found in South and Southeast Asia, and is known to interact with plant sap-sucking hemiptera like aphids and treehoppers.[4] Bioactive aqueous extract of *Camponotus compressus* using gas chromatography – mass spectrum was the aim of this study. Metabolomics, as a part of functional genomics, plays an important role in medically oriented research on insects. Fingerprint analysis, performed by complementary gas and liquid chromatography, coupled with the mass spectrometry technique, is a fundamental approach in metabolomic studies. One of mass spectrometry's beneficial features is its usefulness in identification of metabolites in complex biological material. Such identified metabolites might next be recognized as specific biomarkers or so called "lead" structures for rational drug design. Substances with biological activity have been sought since ages but still searching for new bioactive agents is necessary. Nowadays, much attention is dedicated to insect's metabolites, because of their unique properties.

MATERIALS AND METHODS:

The insects were collected from various wild habitats, corn fields, vegetable gardens, grasslands and from the local markets. An amount of 10 g raw insects, were weighed and homogenised using a pestle and motor in 60 ml of distilled water, and this extraction product was centrifuged using bench centrifuge at 3 000 rpm for 10 min.[5]

Gas chromatography – mass spectrum analysis:

GC-MS analysis was performed using the Agilent technologies 6890 N JEOL GC-MS Mate II model instrument(IFGTC Coimbatore). The aqueous extract of the *Camponotus compressus* was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Mass spectra were obtained at an ionization voltage of 70 eV with ion source temperature of 250°C and the interface temperature was maintained at 250°C. The scanning range was 1- 2540 mass units.

Identification of compounds

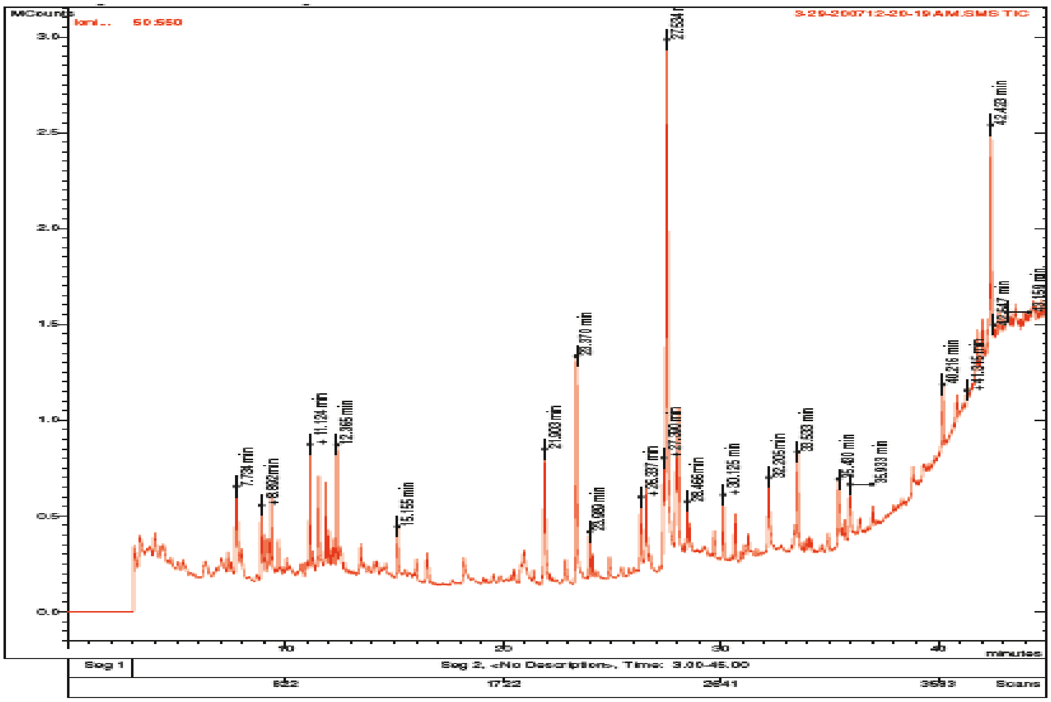
Interpretation of Gas Chromatogram mass spectrum (GC-MS) was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the NIST Library. The chemical name, peak are a and molecular weight of the compounds of the test material were ascertained.

RESULTS AND DISCUSSION

GCMS qualitative analysis:

Figure 1 shows a typical chromatogram after GCMS analysis of the aqueous extract from *Camponotus compressus*.

Fig 1: GC-MS chromatogram of *Camponotus compressus*



The identification of the components of the extracts with their respective retention times, chemical formulae, mass and percentage content as obtained from the elution profile are given in Table 1. Chromatogram GC-MS analysis of the methanol extract of *C. compressus* showed the presence of thirty two major peaks and the components corresponding to the peaks were determined as5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione, Bicyclo[5.1.0]octane,8-methylene, Pentadecane, 7-Chloro-2,3-dihydro-3-(4 nitrobenzilidene)-5-phenyl-1H-4-ben, Diphenyl r-2-methoxycarbonyl-2,c-5-diphenylpyrrolidin-c-3,c-4-d, Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R)-, 1-Heptanol, 2-propyl- , Pyrroline, 1,2-dimethyl- , Decane, Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)-, Butyl 9-tetradecenoate, Benzenemethanol, .alpha.-[1-(ethylmethylamino)ethyl]-, [R-(R*,S,Hexadecanoic acid (CAS), 3-Hydroxymyristic acid, 1-Dotriacontanol (CAS), Tritetracontane, Ethyl linoleate, 2,3-Dihydroxypropyl elaidate, Octadecanoic acid, Octane, -4-methyl-, 5,8,10-Undecatrien-3-ol , 3-Undecen-1-yne, (Z)- , 2-Pentacosanone, Oleic anhydride, N'-(2-Methyl-4-chlorophenyl)-N-cyclohexyformamidine, Acetic acid, (triphenylphosphoranylidene)-, methyl ester (CAS), Cholesta-3,5-diene (CAS), 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9a-tetrakis(a, Cholest-5-en-3-ol (3.beta.)-, 9-octadecenoate, (Z)-, Cholesta-4,6-dien-3-ol, (3.beta.)-, Lycoxanthin,Cholest-5-en-3-ol (3.beta.)-, acetate (CAS).

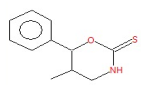
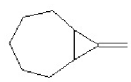
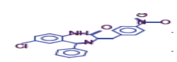
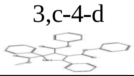
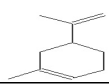
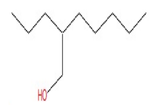
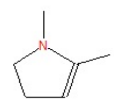
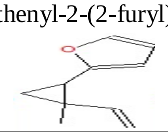
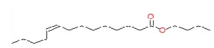
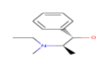
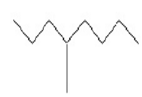
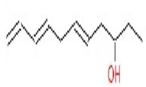
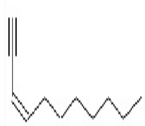
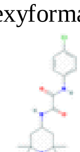
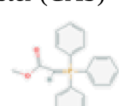
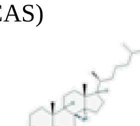
Antimicrobial peptides have been developed as alternatives for the treatment of infectious diseases. They generally act in destabilizing the cell membrane permeability or interacting with the specific targets in cells which cause signalling pathway disruption. Interestingly, insect whole body is also an alternative source for the discovery of a new class of small AMPs which may have a role in processes other than immunity.[6-11.] Several insect proteins also show cytotoxic effects against diverse cancer cell lines, such as mouse myeloma, melanoma, lymphoma, leukemia, breast cancer, lung cancer, and also in wound healing process.[12-15] These anticancer peptides (ACPs) are characterized by high therapeutic efficacy, a low probability of resistance emerging in target cells, and limited or no toxicity against mammalian erythrocytes, macrophages, and fibroblasts.[16-18] Hence to discover therapeutics for various diseases this preliminary analysis through GCMS has been done.

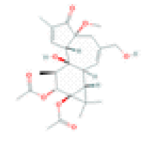
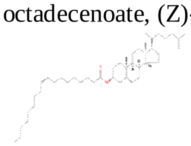
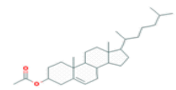
Table 1: Bioactive compounds identified from *Camponotus compressus*

Compound	Structure	Retention time	Molecular weight	Area	Percentage of total
5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione	C11H13NOS	7.734	207	2.357 e+6	3.419
Bicyclo[5.1.0]octane , 8-methylene	C24H20O5	8.892	122	1.388 e+6	2.013
Pentadecane	C15H32	9.001	212	1.05e 4+6	1.529
7-Chloro-2,3-dihydro-3-(4-nitrobenzilidene)-5-phenyl-1H-1,4-ben	C22H14ClN3O3	9.172	403	633188	0.918
Diphenyl r-2-methoxycarbonyl-2,c-5-diphenylpyrrolidin-c-3,c-4-d	C32H27NO6,	9.339	521	2.169e+6	3.146
Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R)-	C25H28NO2P	9.668	136	424552	0.616
1-Heptanol, 2-propyl-	$C_{10}H_{22}O$	11.124	158	1.729e+6	2.507
Pyrroline, 1,2-dimethyl-	C6H11N	11.524	097	1.395	2.023
Decane	C10H22	11.832	142	966958	1.402
Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)-	C10H12O	12.365	148	1.744e+6	2.529
Butyl 9-tetradecenoate	$C_{18}H_{34}$	15.155	282	858820	1.246
Benzenemethanol, .alpha.-[1-(ethylmethylamino)ethyl]-, [R-(R*,S	C12H19NO	21.903	193	3.499e+6	2.529
Hexadecanoic acid (CAS)	C16H32O2	23.370	193	5.536e+6	8.030
3-Hydroxymyristic acid	C14H28O3	23.989	256	921599	1.337
1-Dotriacontanol (CAS)	C32H66O	26.337	244	1.524e+6	2.210
Tritetracontane	C43H88	26.580	604	1.833e+6	2.658
Ethyl linoleate	C20H36O2	27.390	308	2.532e+6	3.672
2,3-Dihydroxypropyl elaidate	C21H40O4,	27.534	356	1.478e+7	21.439
Octadecanoic acid	C18H36O2,	27.974	284	2.205e+6	3.198
Octane, -4-methyl-	C_8H_{18}	28.062	128	3.087e+6	4.477
5,8,10-Undecatrien-3-ol	C11H18O	28.466	166	809670	1.174
3-Undecen-1-yne, (Z)-	C11H18	30.125	05	1.231e+6	1.785
2-Pentacosanone	C25H50O	30.677	466	959982	1.392
Oleic anhydride	C36H66O3	32.205	366	2.046e+6	2.968

N'-(2-Methyl-4-chlorophenyl)-N-cyclohexyformamidine	C11H16N2	33.533	546	1.773e+6	2.571
Acetic acid, (triphenylphosphoranylidene)-, methyl ester (CAS)	C21H19O2P	35.430	176	1.296e+6	1.879
Cholesta-3,5-diene (CAS)	C27H44	35.933	334	1.472e+6	2.135
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9a-tetrakis(a	C30H40O13	40.216	368	1.135e+6	1.646
Cholest-5-en-3-ol (3.beta.)-, 9-octadecenoate, (Z)-	C45H78O2	41.315	608	267233	0.388
Cholesta-4,6-dien-3-ol, (3.beta.)-	C27H44O	41.839	650	712039	1.033
Lycoxanthin	C40H56O,	42.423	552	5.438e+6	7.887
Cholest-5-en-3-ol (3.beta.)-, acetate (CAS)	C29H48O2	42.547	502	141821	0.206

Table 2: Bioactive chemical compounds and their structure

5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione 	Bicyclo[5.1.0]octane , 8-methylene- 	Pentadecane	7-Chloro-2,3-dihydro-3-(4-nitrobenzilidene)-5-phenyl-1H-1,4-ben 
Diphenyl r-2-methoxycarbonyl-2,c-5-diphenylpyrrolidin-c-3,c-4-d 	Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R)- 	1-Heptanol, 2-propyl- 	Pyrroline, 1,2-dimethyl- 
Decane 	Cyclopropane. -1-methyl-1-ethenyl-2-(2-furyl)- 	Butyl 9-tetradecenoate 	Benzenemethanol, .alpha.-[1-(ethylmethylamino)ethyl]-, [R-(R*,S 
Hexadecanoic acid (CAS) 	3-Hydroxymyristic acid	1-Dotriacontanol (CAS)	Tritetracontane
Ethyl linoleate 	2,3-Dihydroxypropyl elaidate 	Octadecanoic acid	Octane, -4-methyl- 
5,8,10-Undecatrien-3-ol 	3-Undecen-1-yne, (Z)- 	2-Pentacosanone	Oleic anhydride 
N'-(2-Methyl-4-chlorophenyl)-N-cyclohexyformamidin 	Acetic acid, (triphenylphosphoranylidene)-, methyl ester (CAS) 	Cholesta-3,5-diene (CAS) 	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9a-tetrakis(a

			
Cholest-5-en-3-ol (3.beta.)-, 9-octadecenoate, (Z)- 	Cholesta-4,6-dien-3-ol, (3.beta.)- 	Lycoxanthin	Cholest-5-en-3-ol (3.beta.)-, acetate (CAS 

CONCLUSION

Thirty two bioactive chemical constituents have been identified from aqueous extract of the *Camponotus compressus* by GC-MS technique. The purpose of the present study is to focus on the use of insect natural products as potential source for alternative medicine that is beneficial for curing as well as giving protection from the diseases that modern human civilization is combating for. This primary analysis is significant in developing insect natural products as potential new alternative medicinal drugs. This field of investigation provides a promising research topic due to the importance to man in various fields including ethnobiology, medicine and pharmaceutical development.

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