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Research Article

Determination of Bioactive Chemical Composition of *Callosobruchus maculutus* and Investigation of its Anti-Fungal Activity

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ABSTRACT

Methanolic extract of bioactive compounds of *Callosobruchus maculutus* was assayed for in *vitro* anti-fungal activity against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*, *Penicillium expansum*, and *Trichoderma viride*. GC-MS analysis of *Callosobruchus maculutus* revealed the existence of the 4-Cyclooctene-1-carboxaldehyde, Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, 2-Undecanone, 6,10-dimethyl-, Tricyclo[10.2.2.2(5,8)octadeca-5,7,12,14,15,17-hexaene, 1-Dimethyl(pentafluorophenyl)silyloxycyclopentane, 3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol, Benzenesulfonamide , N-(bicyclo[2.2.1]hept-5-en-2-ylmethyl), (-)-Norephedrine, 4-Bromo-7-methylenebicyclo[4.2.0]oct-2-ene, Benzene, 1-propenyl-, 1-Hexanol, 2-ethyl-, 1,2-Benzisothiazol-3-amine tbdms, 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione, Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl, 3,6-Octadecadiynoic acid, methyl ester, 1-Chloroundecane, 10-(Tetrahydro-puran-2-yloxy)-tricyclo[4.2.1.1(2,5)]dec-7-en, Pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino, Methyl salicylate, 2-Amino-nicotinic acid methyl ester, 2,5-Dimethylhexane-2,5-dihydroperoxide, 1,4-Oxathiane, 4-oxide, 3-Cyclohexene-1-propanal, Cyclobutane(1,6)spiro(2,3-diazabicyclo[3.1.0]hex-2-ene)-4-, Trans-8-Hydroxy-bicyclo(4,3,0)non-3-ene, Tricyclo[3.3.1.1(3,7)]decane, 1-[(hydrazinocarbonyl)amino]-, (7R)-cis-bicyclo[4.3.0]-3-nonen-7-ol, Ethaneperoxoic acid ,1-cyano-1,4-diphenylpentyl ester, 3-Amino-3-(4-isopropoxy-3-methoxy-phenyl)-propionic acid, Aziridinone, 1,3-bis(tricycle[3.3.1.1(3,7)]dec-1-yl), Hydrazinecarboxamide, 2-(2,6-cyclooctadien-1-ylidene, Cis-pinen-3-ol, Salicylaldehyde, thiocarbazone, Adamantane,1-isocyano, 2,7-Methanonaphthalen-3-amine , 1,2,3,4,4a,7,8,8a-octahyd, Pteridine-8-oxide, 6-aldoximino-2-amino-4(3H)-oxo-, 2-Dodecenal, 5H-Cyclohepta-1,4-dioxin ,2,3,4a,6,7,9a-hexahydro-,cis-, Hexadecanoic acid, methyl ester, Cyclohexanebutanoic acid, 2-methyl-3-oxo-, methyl ester, Octadecanoic acid, Butyl 9-tetradecenoate, 7-[3-Chloro-2-hydroxypropyl]guanine, 4-Heneicosanone, 1-cyclopentyl-, 4-Methoxycarbonylmethylundec-3-enedioic acid, dimethyl ester, 5-(4,5-Dihydro-3H-pyrrol-2-ylmethylene)-4,4-dimethylpyrrolidin, Phthalic acid ,octyl oct-3-yl ester. The results of anti-fungal activity produced by *Callosobruchus maculutus* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus niger*. *Callosobruchus maculutus* produce many important secondary metabolites with high biological activities.

Keywords: GC/MS, Bioactive compounds, *Callosobruchus maculutus*, Anti-fungal.

INTRODUCTION

Callosobruchus maculatus is a species of beetle known commonly as the cowpea weevil or cowpea seed beetle[1]. The beetle most likely originated in West Africa and moved around the globe with the trade of legumes and other crops[1,2]. As only a small number of individuals were likely present in legumes carried by people to distant places, the populations that have invaded various parts of the globe have likely gone through multiple bottlenecks. A female adult can lay over a hundred eggs, and most of them will hatch. She lays an egg on the surface of a bean, and when the larva emerges about 4 to 8 days later, it burrows into the bean[3]. The bean beetle, *Callosobruchus maculatus*, oviposit their eggs on the cowpea bean. The varieties of cowpea that are most common for the beetle to lay their eggs on are black eyed peas, mung beans, and adzuki beans[4]. As only a small number of individuals were likely present in legumes carried by people to distant places, the populations that have invaded various parts of the globe have likely gone through multiple bottlenecks. If more than one host is available, the beetle will choose its host depending on the variety and size of the bean as well as the texture of the seed coat[5]. The time it takes the larvae to develop varies across hosts, with longer development times on less suitable hosts[4]. It has been found that beetles that choose to oviposit their eggs on the black eyed pea have a shorter development time, suggesting that the black eyed pea is a more suitable host[6]. Larval crowding can occur when up to 8 or 10 larvae feed and grow within one bean. The emerged adult beetles mate assortatively, meaning they mate with others that developed on the sam

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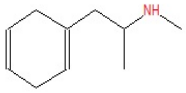
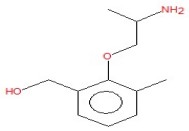
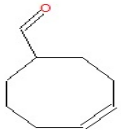
Table 1: Bioactive chemical compounds identified in methanolic extract of Callosobruchus maculutus



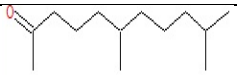
1-(1,4-cyclohexadienyl)-2-methylaminopropane
Rt=3.293
Mw=151.1361



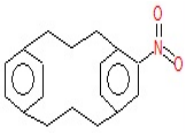
Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-
Rt=3.259
Mw=195.125929



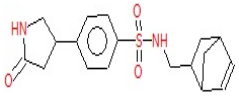
4-Cyclooctene-1-carboxaldehyde
Rt=3.133
Mw=138.1044655



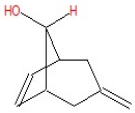
2-Undecanone ,6,10-dimethyl-
Rt=3.362
Mw=198.198365



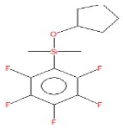
Tricyclo[10.2.2.2(5,8)octadeca-5,7,12,14,15,17-hexaene
Rt=3.396
MW=281.141579



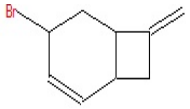
Benzenesulfonamide , N-(bicyclo[2.2.1]hept-5-en-2-ylmethyl)-
RT=3.545
MW=346.135113



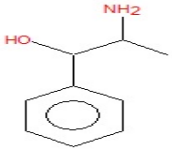
3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol
RT=3.499
MW=136.088815



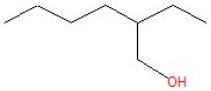
1 Dimethyl (pentafluorophenyl) silyloxycyclopentane
RT=3.430
MW=310.081232



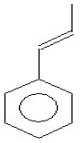
4-Bromo-7-methylenebicyclo [4.2.0]oct-2-ene
RT=3.722
MW=198.004412



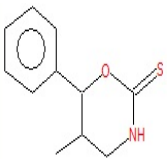
(-)-Norephedrine
RT=3.653
MW=151.099714



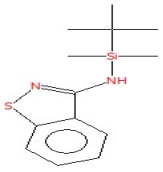
1-Hexanol ,2-ethyl-
RT=3.997
MW=130.135765



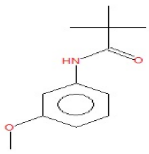
Benzene , 1-propenyl-
RT=3.785
MW=118.0782504



5-Methyl-6 phenyltetrahydro-1,3-oxazine-2-thione
RT=4.260
MW=207.071785

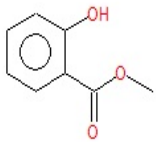


1,2-Benzisothiazol-3-amine tbdms
RT=4.146
MW=264.111647

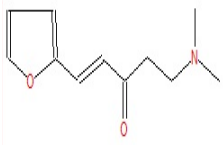


Propanamide ,N-(3-methoxyphenyl)-2,2-dimethyl-
RT=4.552
MW=207.125929

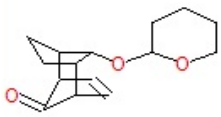
3,6-Octadecadiynoic acid , methyl ester
RT=4.626
MW=290.22458



Methyl salicylate
RT=6.245
MW=152.047344

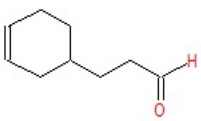


Pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino-
RT=4.872
MW=193.110279

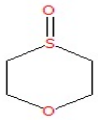


10-(Tetrahydro-puran-2-yloxy)-tricyclo[4.2.1.1(2,5)]dec-7-en
RT=4.775
MW=248.141245

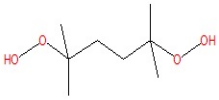
1-Chloroundecane
RT=4.706
MW=190.1488285



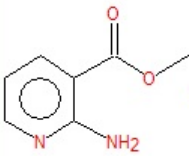
3-Cyclohexene-1-propanal
RT=8.900
MW=138.1044655



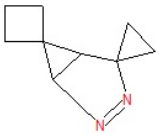
1,4-Oxathiane, 4-oxide
RT=8.569
MW=120.0245004



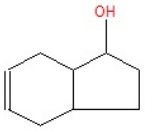
2,5-Dimethylhexane-2,5-dihydroperoxide
RT=8.145
MW=178.120509



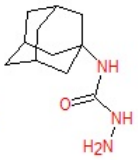
2-Amino-nicotinic acid methyl ester
RT=7.785
MW=152.058578



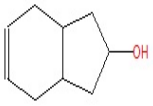
Cyclobutane (1,6) spiro (2,3 diazabicyclo [3.1.0] hex-2-ene)-4- RT=9.152 MW=148.100048



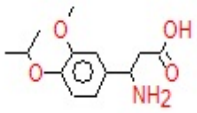
(7R)-cis-bicyclo[4.3.0]-3-nonen-7-ol RT=9.982 MW= 138.1044655



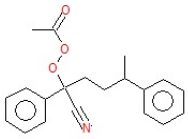
Tricyclo [3.3.1.1(3,7)] decane, 1-[(hydrazinocarbonyl)amino- RT=9.713 MW=209.152812



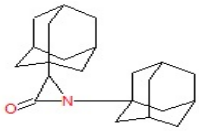
Trans-8-Hydroxy-bicyclo (4,3,0) non-3-ene Rt=9.261 MW=138.1044655



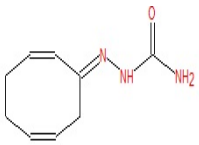
3-Amino-3-(4-isopropoxy-3-methoxy-phenyl)-propionic Acid RT=10.297 MW=253.131408



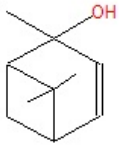
Ethaneperoxoic acid ,1-cyano-1,4-diphenylpentyl ester RT=10.136 MW=323.152143



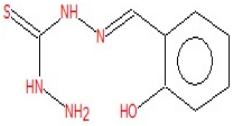
Aziridinone, 1,3 bis (tricycle [3.3.1.1(3,7)] dec-1-yl)- RT=10.428 MW=325.240564



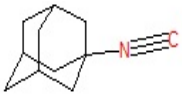
Hydrazinecarboxamide ,2-(2,6-cyclooctadien-1-ylidene RT=11.504 MW=179.105862



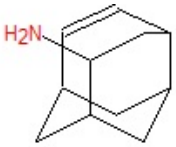
Cis-pinen-3-ol RT=11.916 MW=152.120115



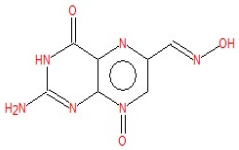
Salicylaldehyde, thiocarbazone RT=12.179 MW=210.057532



Adamantane,1-isocyano- RT=12.122 MW=161.120449



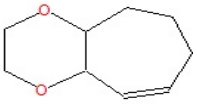
2,7-Methanonaphthalen-3-amine , 1,2,3,4,4a,7,8,8a-octahyd RT=13.758 MW=163.1361



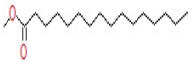
Pteridine-8-oxide , 6-aldoximino-2-amino-4 (3H)-oxo- RT=13.896 MW=222.050138

Dodecenal RT=13.198 MW=182.167066

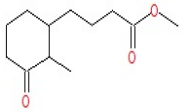
2-



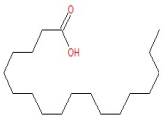
5H-Cyclohepta-1,4-dioxin ,2,3,4a,6,7,9a-hexahydro-, cis- RT=14.382 MW=14.382



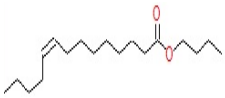
Hexadecanoic acid, methyl ester RT=14.577 MW=270.25588



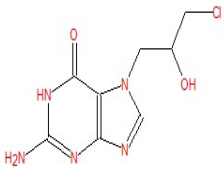
Cyclohexanebutanoic acid, 2-methyl-3-oxo-, methyl ester RT=14.806 MW=212.141245



Octadecanoic acid RT=16.831 MW=284.27153

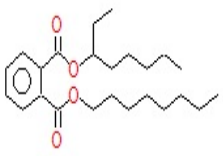


Butyl 9-tetradecenoate RT=17.501 MW=282.25588

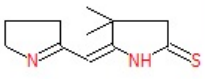


7-[3-Chloro-2-hydroxypropyl] guanine RT=17.649 MW=243.052302

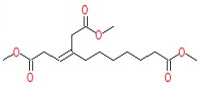
4-Heneicosanone, 1-cyclopentyl- RT=17.958 MW=378.386166



Phthalic acid, octyl oct-3-yl ester RT=20.018 MW=390.27701



5-(4,5-Dihydro-3H-pyrrol-2-ylmethylene)-4,4-dimethylpyrrolidin RT=18.376 MW=208.103419



4 Methoxycarbony lmethylundec-3-enedioic acid, dimethyl ester RT=18.187 MW=314.17294

Table 2: Antifungal activity of Callosobruchus maculutus metabolite products.

Fungi	Metabolite products Callosobruchus maculutus /Antibiotics			
	Metabolite products of Callosobruchus maculutus	Amphotericin B	Fluconazol	Miconazole nitrate
Aspergillus niger	4.00±0.20 [a]	1.00±0.07	1.08±0.04	0.15±0.03
Aspergillus flavus	4.02±0.20	1.04±0.09	1.79±0.20	1.04±0.04
Aspergillus terreus	3.98±0.19	1.99±0.08	1.00±0.07	1.95±0.08
Penicillium expansum	1.67±0.08	2.00±0.20	3.77±0.28	0.86±0.03
Aspergillus fumigatus	4.00±0.20	0.86±0.05	2.07±0.25	1.11±0.06
Trichoderma horzianum	2.00±0.10	1.08±0.04	1.99±0.20	1.08±0.04
Saccharomyces cerevisiae	2.02±0.10	1.33±0.09	1.93±0.20	2.13±0.16
Candida albicans	4.01±0.21	1.00±0.07	0.79.±0.05	1.98±0.10
Trichoderma viride	2.51±0.07	1.99±0.20	1.45±0.10	1.47±0.08

[a] The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract and 30 µg/mL of (Amphotericin B; Fluconazol and Miconazole nitrate).

host bean. The adult requires neither food nor water, but if offered water, sugared water, or yeast, it may consume it. A female given nutrients may lay more eggs[6]. The beetle tolerates a range of humidity and temperature, making it adaptable in climates worldwide. Its developmental time varies with factors such as humidity, temperature, legume type, crowding, and inbreeding levels in the population[7]. Temperature and humidity in legume storage areas are relatively constant and the food density is high[8]. Antibiotic resistance has been a major problem in impeding the efficiency of commonly used antibiotics exhibited by a plethora of microbes as a result of the excessive use of the drugs in medical treatments and animal feed[9-12]. Due to the unpredictable degree of resistance, antibiotic research has now turned its direction to the manipulation of antimicrobial peptides (AMPs). In insects, AMPs are constitutively expressed or highly inducible in response to bacterial infection by various epithelia of midgut and salivary glands, fat bodies and hemocytes in which they are secreted into the hemolymph. In holometabolous insects with complete metamorphosis, AMPs are produced by the fat bodies[5,13,14] whereas the hemocytes act as the vital synthesizer for AMP in heterometabolous insects with incomplete metamorphosis[15].

MATERIALS AND METHODS

Laboratory culture of *C. maculatus* was obtained from infested seeds from college of science for woman university of Babylon. The stock culture of the insects maintained on *Vigna sinensis*, seeds to obtain newly emerged beetles of same generation, 100 insects were released in plastic containers having 500 of cowpea seed covered by muslin cloth, containers kept in acclimatized chambers at 28 ±2 C° and 65 ±5% humidity, after ten days' adults were removed. The insects emerged after four weeks were used in entire investigation[16]. The extraction was performed by adding 100 ml methanol to the whole body insect powder. Methanol was used as solvent control. The studied yeast and fungi, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*, *Penicillium expansum*, and *Trichoderma viride* were isolated and maintained in potato dextrose agar slants. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for 16 days at 130 rpm. The tests were carried out in triplicate[17-20]. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity[21-24]. The GC-MS analysis of the insect extract was made in a (Agilent 789 A) instrument under computer control at 70 eV[25,26]. About 1µL of the methanol extract was injected into the 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). 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 libraries[27-29].

Statistical analysis

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.

RESULTS AND DISCUSSION

Identification of biochemical compounds

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Callosobruchus maculutus*, 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 *Callosobruchus maculutus* showed the presence of forty-eight major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 4-Cyclooctene-1-carboxaldehyde, Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, 2-Undecanone, 6,10-dimethyl-, Tricyclo [10.2.2.2 (5,8) octadeca-5,7,12,14,15,17-hexaene, 1-Dimethyl (pentafluoropenyl) silyloxycyclopentane, 3-Methylene-bicyclo [3.2.1] oct-6-en-8-ol, Benzenesulfonamide, N-(bicyclo [2.2.1] hept-5-en-2-ylmethyl), (-)-Norephedrine, 4-Bromo-7-

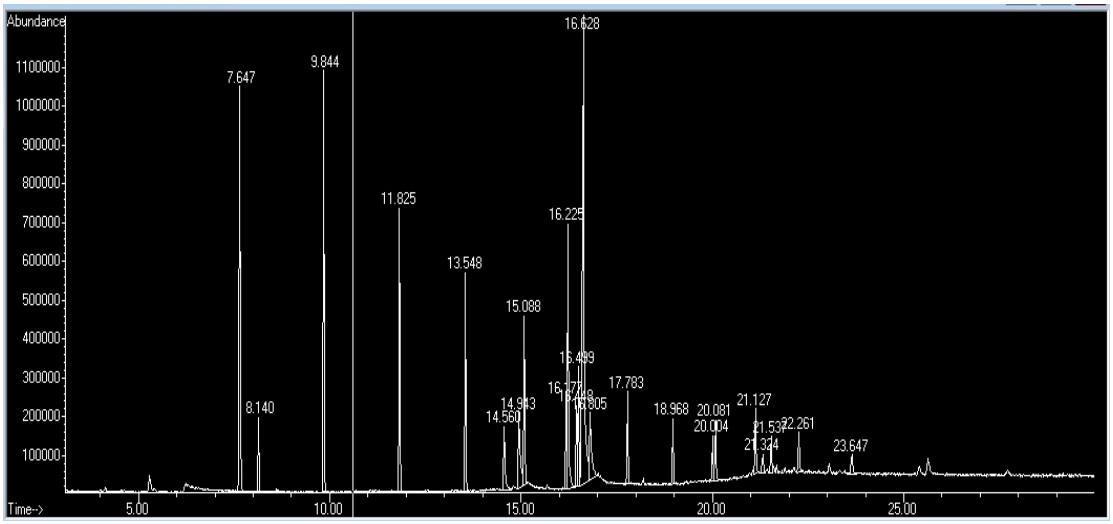


Figure 1: GC-MS chromatogram of methanolic extract of Callosobruchus maculutus.

methylenebicyclo [4.2.0] oct-2-ene, Benzene, 1-propenyl, 1-Hexanol ,2-ethyl-, 1,2-Benzisothiazol-3-amine tbdms, 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione, Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl, 3,6-Octadecadiynoic acid, methyl ester, 1-Chloroundecane, 10-(Tetrahydro-puran-2-yloxy)-tricyclo [4.2.1.1(2,5)] dec-7-en, Pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino, Methyl salicylate, 2-Amino-nicotinic acid methyl ester, 2,5-Dimethylhexane-2,5-dihydroperoxide, 1,4-Oxathiane, 4-oxide, 3-Cyclohexene-1-propanal, Cyclobutane (1,6) spiro(2,3-diazabicyclo [3.1.0] hex-2-ene)-4-, Trans-8-Hydroxy-bicyclo (4,3,0) non-3-ene, Tricyclo [3.3.1.1(3,7)] decane, 1-[(hydrazinocarbonyl)amino]-, (7R)-cis-bicyclo[4.3.0]-3-nonen-7-ol, Ethaneperoxoic acid,1-cyano-1,4-diphenylpentyl ester, 3-Amino-3-(4-isopropoxy-3-methoxy-phenyl)-propionic acid, Aziridinone, 1,3-bis(tricycle[3.3.1.1(3,7)]dec-1-yl), Hydrazinecarboxamide, 2-(2,6-cyclooctadien-1-ylidene, Cis-pinen-3-ol, Salicylaldehyde, thiocarbazone, Adamantane, 1-isocyano, 2,7-Methanonaphthalen-3-amine, 1,2,3,4,4a,7,8,8a-octahyd, Pteridine-8-oxide, 6-aldoximino-2-amino-4(3H)-oxo-, 2-Dodecenal, 5H-Cyclohepta-1,4-dioxin,2,3,4a,6,7,9a-hexahydro-,cis-, Hexadecanoic acid, methyl ester, Cyclohexanebutanoic acid , 2-methyl-3-oxo-, methyl ester, Octadecanoic acid, Butyl 9-tetradecenoate, 7-[3-Chloro-2-hydroxypropyl]guanine, 4-Heneicosanone, 1-cyclopentyl-, 4-Methoxycarbonylmethylundec-3-enedioic acid , dimethyl ester, 5-(4,5-Dihydro-3H-pyrrol-2-ylmethylene)-4,4-dimethylpyrrolidin, Phthalic acid ,octyl oct-3-yl ester. Solvent extract was found to exhibit significant inhibitory effects on the fungi ($p < 0.05$). The results of anti-fungal activity produced by *Callosobruchus maculutus* showed that the volatile compounds were the largest zone inhibition in *Aspergillus niger* (4.00±0.20 mm), *Aspergillus terreus* (3.98±0.19 mm), *Aspergillus flavus* (4.02±0.20 mm), *Aspergillus fumigatus* (4.00±0.20 mm), *Candida albicans* (4.01±0.21 mm), *Saccharomyces cerevisiae* (2.02±0.10 mm), *Penicillium expansum* (1.67±0.08 mm), *Trichoderma viride* (2.51±0.07 mm) and *Trichoderma horzianum* (2.00±0.10mm). *Callosobruchus maculutus* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Callosobruchus maculutus* can be useful. Maximum zone formation against *Aspergillus niger* (4.00±0.20 mm) mm, Table 2. AMPs are short bioactive molecules either cationic or non-cationic in nature with broad-spectrum antimicrobial effects against many pathogens[2,30-36]. AMPs are short bioactive molecules either cationic or non-cationic in nature with broad-spectrum antimicrobial effects against many pathogens[2,37]. They generally act in destabilizing the cell membrane permeability or interacting with the specific targets in cells which cause signaling pathway disruption[38]. 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. 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[39]. 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[39].

CONCLUSION

Forty-eight bioactive chemical constituents have been identified from methanolic extract of the *Callosobruchus maculutus* by GC-MS technique. *In vitro* antifungal evaluation of secondary metabolite products of *Callosobruchus maculutus* forms a primary platform for further pharmacological investigation for the development of new potential antifungal compounds.

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REFERENCES

1. Cezard C, Pires V, C. Mullie, Sonnet P. Antimicrobial peptides: A review. Science against microbial pathogens: communicating current research and technological advances. Eds, Mendez-Vilas, A.: FORMATEX. 2011; 926-937.
2. Joerger RD. Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophage. Poultry Science. 2003; 82: 640-647.
3. Chiou SY, S Kotanen, A Cerstiaens, Daloze D. Purification of toxic compounds from larvae of the gray fleshfly: The identification of paralysins. Biochemical & Biophysical Research Communications. 1998; 246: 457.
4. Meylears K, A Cerstiaens, E. Vierstraete, G. Baggerman, C.W. Michiels, Loof A, Schoofs L. Antimicrobial compounds of low molecular mass are constitutively present in insects: Characterisation of β-alanyl-tyrosine. Current Pharmaceutical Design. 2002; 8: 99-110.
5. Bulet P, M Charlet, Hetru C. In Innate Immunity, Eds., Ezekowitz RA, Hoffman JA: Humana Press, Totowa, N.J, 2003; pp: 89-107.
6. Cirioni O, R Ghiseli, C Silvestri, W Kamsay, F Orlando, F Mocchegiani, FD Matteo, Riva A. Efficacy of tachyplesin III, colistin, and imienem against a multiresistant *Pseudomonas aeroginosa* strain. Antimicrobial Agents Chemotherapy. 2007; 51: 2005-2010.
7. Jakopic J, R Veberic, Stampar F. Extraction of phenolic compounds from green walnut fruits in different solvents. Acta Agriculturae Slovenica. 2009; 93: 11-15.
8. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 127-146.
9. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 889-905.
10. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016; 7(2): 1843- 1868.
11. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). Journal of Pharmacognosy and Phytotherapy. 2016; 8(3): 49-59.
12. Sosa AA, Bagi SH, Hameed IH. Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(5): 109-126.
13. Altameme H J, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2015a; 7(10): 238-252.
14. Lamberty M, D Zachary, R Lanot, Bordereau C, Robert A, Hoffmann JA, Bulet P. Insect immunity. Constitutive expression of a cysteine-rich antifungal and a linear antibacterial peptide in a termite insect. Journal of Biology Chemistry. 2001; 276: 4085-4092.
15. Shukla R, Siravatava B, Kumar R, Dubey NK. Potential of some powders in reducing infection of chickpea by *Callosobruchus maculatus* (Coleoptera: Brauchidae). J. Agricultur. Technol. 2007; 3(1): 11-19.
16. Mohammed GJ, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 977-996.
17. Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. Journal of Pharmacognosy and Phytotherapy. 2015; 7(9): 194-220.
18. Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 2015; 7(4): 56-72.
19. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2016; 8(2): 8-24.
20. Hameed IH, Ibraheam IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. Journal of Pharmacognosy and Phytotherapy. 2015; 7 (6): 90-106.
21. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. Oriental Journal of Chemistry. 2016; 32(2): 20-40.
22. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(5): 304-315.
23. Mohammed GJ, Al-Jassani MJ, Hameed IH. Anti-bacterial, Antifungal Activity and Chemical analysis of

Punica grantanum (Pomegranate peel) using GC-MS and FTIR spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(3): 480-494.
24. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. Journal of Pharmacognosy and Phytotherapy. 2016; 8(2): 25-48.
25. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemila valgaris* seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Malays. Appl. Biol. 2015b; 44(4): 47–58.
26. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Journal of Pharmacognosy and Phytotherapy. 2015b;7(8): 132-163.
27. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 2015; 7 (7): 107-125.
28. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of *Adiantum Capillus-Veneris* using GC-MS and FT-IR spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(3): 369-385.
29. Hussein HJ, Hadi MY, Hameed IH. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2016; 8(3): 60-89.
30. Kadhim MJ, Mohammed GJ, Hameed IH. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. Oriental Journal of Chemistry. 2016; 32(2): 10-30.
31. Hussein HM. Determination of phytochemical composition and ten elements content (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) of *CARDARIA DRABA* by GC-MS, FT-IR and AAS technique. Int. J Pharm Bio Sci. 2016;7(3): (B) 1009 – 1017.
32. Hussein HM. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-transform infrared spectroscopy. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(4): 2529 – 2555.
33. Jaddoa HH, Hameed IH, Mohammed GJ. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas Chromatography-Mass spectrometry and determination of its antifungal activity. Orient J Chem. 2016;32(4).
34. Hameed IH, Salman HD, Mohammed GJ. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. Orient J Chem. 2016;32(4).
35. Kadhim MJ, Mohammed GJ, Hussein HM. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(7): 655-670.
36. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(7): 655-670.
37. Altameme HJ, Hameed IH, Idan SA, Hadi MY. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 2015c; 7(9): 221-237.
38. Hull R, Katete R, Nywasa M. Therapeutic potential of antimicrobial peptides from insects. Biotechnology and Molecular Biology Review. 2012; 2: 31-47.
39. Gundappa, S, Jayappa J, Chandrashekara K. Bioprospecting for antimicrobial peptides from insects: In vitro antimicrobial activity of acidified methanol extract of dung beetles. Journal of Entomology Research. 2012; 36: 41- 44.
40. Chernysh, HF, Kim SL, Bekker G, Pleskach VA, Filatova NA, Bulet P. Antiviral and antitumor peptides from insects. Proceeding National Academy Science USA. 2002; 99: 12628-12623.