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Research Article

Insecticidal Activity of Methanolic Seeds Extract of *Ricinus communis* on Adults of *Callosobruchus maculatus* (Coleoptera: Brauchidae) and Analysis of its Phytochemical Composition

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ABSTRACT
The objectives of this study were analysis of the secondary metabolite products of *Ricinus communis* and evaluation anti-insect activity against *Callosobruchus maculatus* (Coleoptera: Brauchidae). GC-MS analysis of *Ricinus communis* revealed the existence of the Pregn-5-ene-3,11-dione , 17,20,21-bis[methylene bis(oxy)]-, 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)- , 8-Octadecenal , Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) , Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl , 1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione , hexahydro- , Deoxyspergualin , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , Cyclohexanecarboxylic acid , 2-hydroxy-,ethyl ester , Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- , Pregna-3,5-dien-9-ol-20-one , 2-Methoxy-4-vinylphenol , Ascaridole epoxide , Trans-3,4,5-Trimethoxy-β-nitrostyrene , 3-(N,N-Dimethyllaurylammonio)propanesulfonate , Tetraacetyl-d-xylonic nitrile , Pentaerythritol , bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl , 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy) , 1H-Purin-2-amine , 6-methoxy-N-methyl- , 1,2-Cyclopentanedicarboxylic acid 4-(1,1-dimethylethyl)-dim , Glycyl-D-asparagine , 1-Tetradecanamine , N,N-dimethyl- , Gibberellic acid , 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrida , Propiolic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)- , l-(+)-Ascorbic acid2,6-dihexadecanoate and Phytol. Methanolic seeds extract of *Ricinus communis* was highly active on accumulative mortality of *Callosobruchus maculatus* (Coleoptera: Brauchidae) (adult).

Keywords: *Callosobruchus maculatus*, Insecticidal activity, Phytochemical analysis, *Ricinus communis*, GC/MS.

INTRODUCTION

The cowpea beetle, *Callosobruchus maculatus* (F.) is associated with cowpea storage, where it can attack the whole cowpea grains[1,2]. The use of plant materials in pest control could become important supplements or alternatives to imported synthetic pesticides. *C. maculatus* (F.) attacking vigna species was also tested against several oils. It is, therefore, important that appropriate technology is developed to promote a direct preparation of traditional pesticides at the farm level for resourcepoor farmers who have no access to commercial pesticides or cannot afford them[3]. Chemical insecticides can cause pest resistance, environmental and food contamination and toxicity to nontarget organisms[4]. Vegetables oils and plants products have been used for a long time for the protection of stored grains. But a very little work on the storage of pigeon pea seeds using vegetables oils has been carried out[5]. Many literature indicate the importance of plant extract in protecting seeds by way of direct mixing of dried leaves plant powdered, solvent extracts, vegetable, essential oil on seeds during post-harvest storage[6-10]. The oils could also act as antifeedants or modify the storage micro-environment, thereby discouraging insect penetration in the grain and feeding[11]. Bekele et al. (1997)[12] showed the effectiveness of ground leaves and essential oil extract of *Ocimum kilimandscharicum, O. suave,* and *O. kenyense* in protecting maize and sorghum against attack by S. *zeamais* (Mots.) (Curculionidae), *Rhyzopertha dominlca* (Fab.) (BQstrichidae), and *Sitotraga cerealel/a(Oliver)* (Gellechidae). The use of some of vegetable oils (rubber seed oil, palm oil and palm kernel oil) was evaluated against cowpea weevil, *Callosobruchus maculatus* in three cowpea varieties (Ife white, Ife brown and Kano white). There was no adverse effect of the oils on grains quality. Of the three plant oils used, rubber seed oil was the most effective. Plants produce secondary metabolites many of which can have insecticidal properties, as an alternative to synthetic insecticides[13]. Plant extracts and essential oils have traditionally been used to kill or repel stored product insects[14,15]. The insecticidal constituents of many essential oils against stored product insects are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol and myrcene[16]. Essential oils of several medical plant displayed considerable fumigant and repellent effects on adults of *C. maculates*[17]. The

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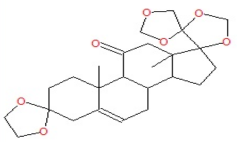
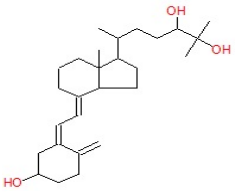
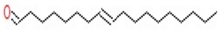
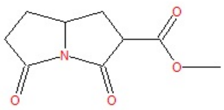
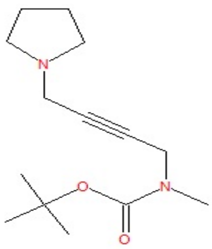
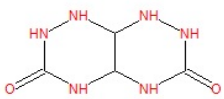
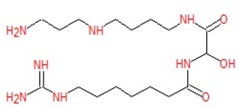
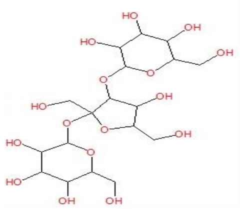
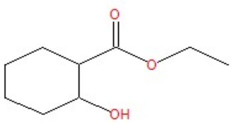
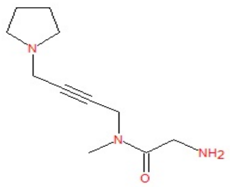
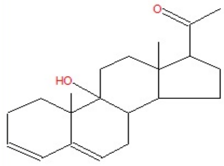
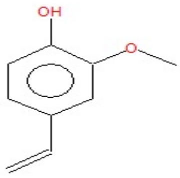
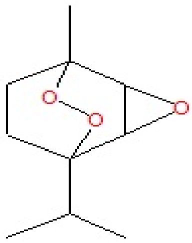
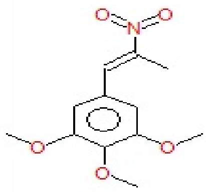
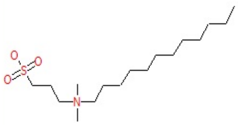
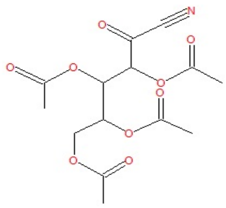
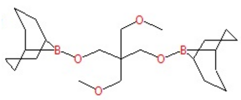
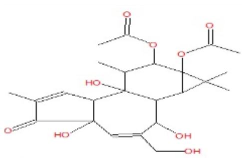
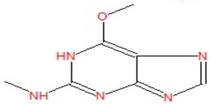
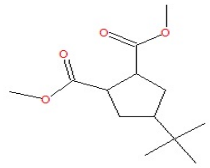
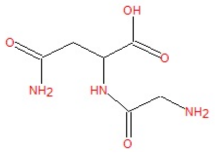
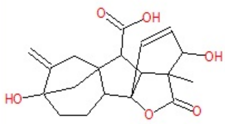
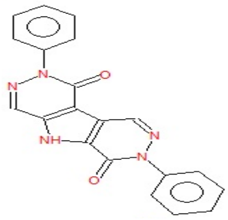
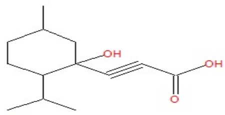
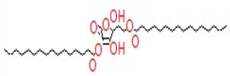
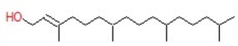
Table 1: Major phytochemical compounds identified in methanolic extract of *Ricinus communis*.

S. No.	Pharmacological actions	MS Fragment-ions	Chemical structure	Exact Mass	Molecular Weight	RT (min)	Phytochemical compound
1.	anti-inflammatory	55,69,8199,161,256,314,372,446		446.230453	446	3.161	Pregn-5-ene-3,11-dione , 17,20,21-bis[methylene bis(oxy)]-,
2.	New chemical compounds	55,69,118,136,158,176,207,253,383,416		416.329044	416	3.230	9,10-Secocholesta - 5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)-
3.	Anti-inflammatory activity and antimicrobial activity	57,68,82,97,177,252		266.260965	266	4.054	8-Octadecenal
4.	Antioxidant, analgesic potential and anti-inflammatory activities	55,69,84,98,142,197		197.068808	197	4.031	Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester)
5.	anti-histaminc properties	57,70,84,108,151,195,221		252.183778	252	4.529	Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl
6.	anti-inflammatory activity	57,100,172		172.070873	172	4.861	1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione , hexahydro-
7.	anti-tumour activity	59,72,86,128,187,219,252		387.295788	387	4.941	Deoxyspergualin
8.	anti-inflammatory	60,73,85,97,126,145,199		504.169035	504	5.330	α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β-
9.	Unknown	57,73,101,127,144,172		172.109944	172	5.542	Cyclohexanecarboxylic acid , 2-hydroxy-,ethyl ester

10.	an anti-diabetic agent	55,70,84,121,139,192,208		209.152812	209	6.554	Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-
11.	*anti-inflammatory*	55,67,79,120,138,194,281,314		314.22458	314	6.646	Pregna-3,5-dien-9-ol-20-one
12.	Anti-inflammatory effect	51,77,89,107,135		150.06808	150	7.046	2-Methoxy-4-vinylphenol
13.	antimicrobial properties	55,69,91,107,135,150,168		184.109944	184	7.212	Ascaridole epoxide
14.	Unknown	63,77,91,106,133,161,177,191,206,222,253		253.095022	253	7.888	Trans-3,4,5-Trimethoxy-β-nitrostyrene
15.	Unknown	58,69,84,97,122,152,181,213		335.249414	335	8.877	3-(N,N-Dimethyllaurylammonio)propanesulfonate
16.	antiasthamatic, anti-inflammatory and antipyretic properties	60,73,112,133,238,281		343.090332	343	9.209	Tetraacetyl-d-xylonic nitrile
17.	anti-inflammatory	55,67,85,109,127,143,181,223,258,293,347,404		404.326921	404	9.490	Pentaerythritol , bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl
18.	Anti-tumor	53,69,109,149,269,326,345,405,464		464.204632	464	10.348	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy)
19.	antimycobacterial activity	55,83,95,150,179		179.080709	179	10.623	1H-Purin-2-amine , 6-methoxy-N-methyl-

20.	antimycobacterial activity	57,107,126,154,211		242.151809	242	10.852	1,2-Cyclopentanedicarboxylic acid 4-(1,1-dimethylethyl)-dim
21.	Unknown	55,113,154		189.074956	189	10.920	Glycyl-D-asparagine
22.	Anti-cancer activity	58,69,114,142,170,241		241.27695	241	11.000	1-Tetradecanamine , N,N-dimethyl-
23.	anti-inflammatory effect	55,77,91,136,203,239,300,346		346.141638	346	11.126	Gibberellic acid
24.	Anti-inflammatory	51,7793,149,187,224,267,327,355		355.106924	355	11.521	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrida
25.	Unknown	55,81,95,135,163,191		224.141245	224	11.910	Propiolic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-
26.	Antibacterial, antiviral, anti-thrombosis, anti-tumor, anti-inflammatory	57,73,85,98,129,157,213,256,278,353396,414		652.49142	652	13.684	l-(+)-Ascorbic acid2,6-dihexadecanoate
27.	Anti-inflammatory	57,71,81,95,111,123,137,196,221,249,278		296.307917	296	15.034	Phytol

objective of this study was to assess the efficacy of methanolic seeds extract of *Ricinus communis* L. against *Callosobruchus maculatus* (Coleoptera: Brauchidae) under laboratory conditions.

MATERIALS AND METHODS

Extraction and isolation of Ricinus communis

Seeds (2 kg) of *Ricinus communis* have been collected from gardens in Hilla city, middle of Iraq). *Ricinus communis* was stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. Methanolic extract of *Ricinus communis* powdered were soaked in 1000 mL methanol for ten hours in a rotatory shaker[18-27]. The filtrates were used for further phytochemical analysis.

Evaluation of anti-insect activity

Laboratory culture of *Callosobruchus maculatus* (Coleoptera: Brauchidae) was obtained from college of science for woman university of Babylon. Two hundred insects were released in plastic containers having 700 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[28]. A control was prepared in the same way but extract application was omitted. Five replicates were set up for the treated[29-30].

Statistical analysis

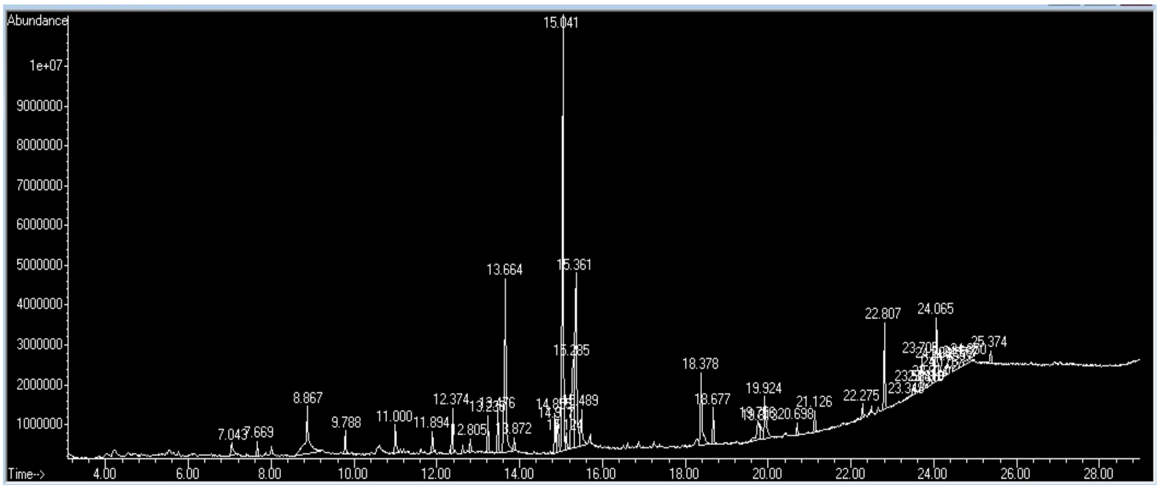


Figure 1: GC-MS chromatogram of methanolic extract of *Ricinus communis*.

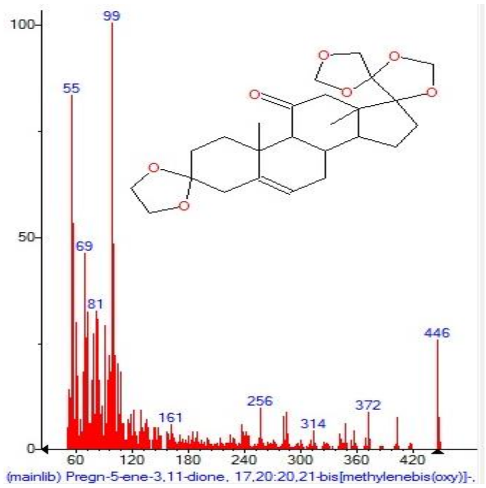


Figure 2: Mass spectrum of Pregn-5-ene-3,11-dione , 17,20,21-bis[methylene bis(oxy)]-, with Retention Time (RT)= 3.161.

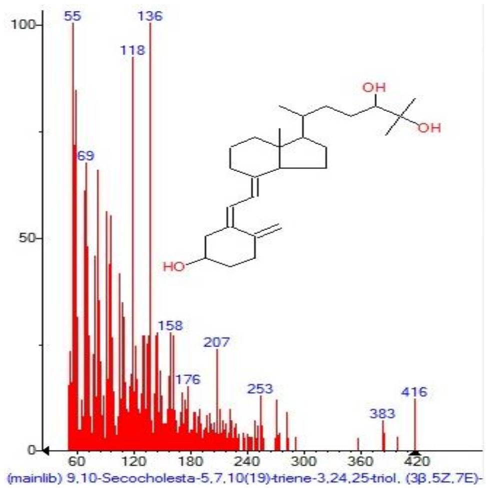


Figure 3: Mass spectrum of 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)- with Retention Time (RT)= 3.230.

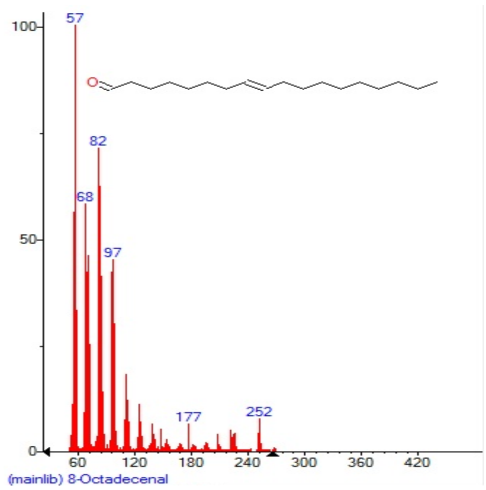


Figure 4: Mass spectrum of 8-Octadecenal with Retention Time (RT)= 4.054.

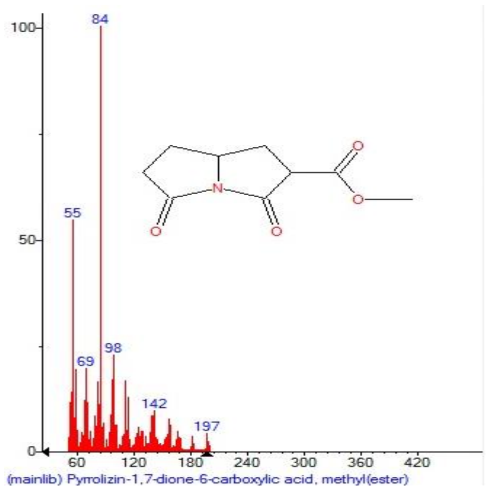


Figure 5: Mass spectrum of Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) with Retention Time (RT)= 4.031.

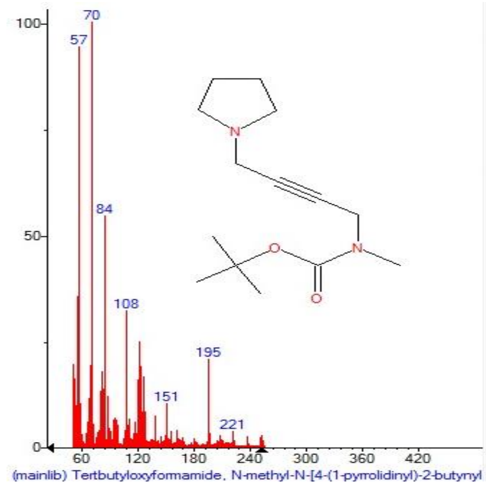


Figure 6: Mass spectrum of Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl with Retention Time (RT)= 4.529.

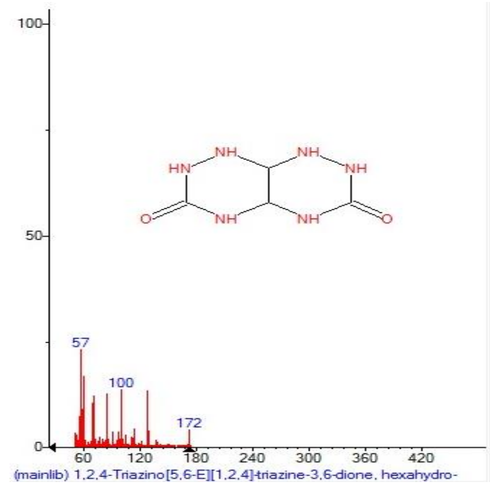


Figure 7: Mass spectrum of 1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione , hexahydro- with Retention Time (RT)= 4.861.

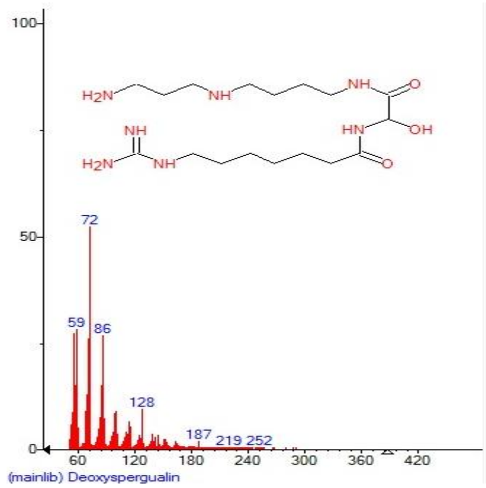


Figure 8: Mass spectrum of Deoxyspergualin with Retention Time (RT)= 4.941.

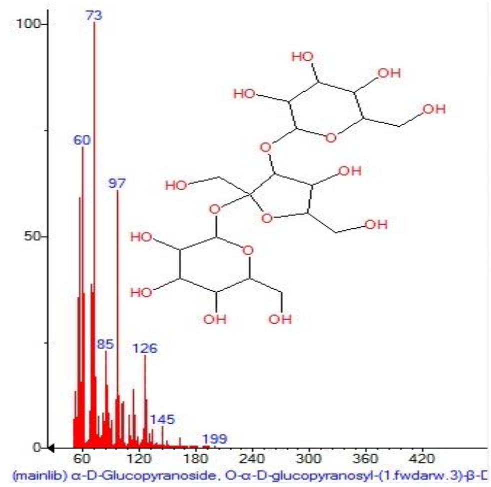


Figure 9: Mass spectrum of α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- with Retention Time (RT)= 5.330.

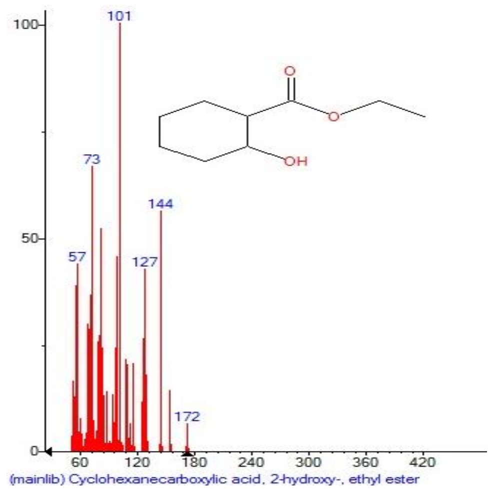


Figure 10: Mass spectrum of Cyclohexanecarboxylic acid , 2-hydroxy-,ethyl ester with Retention Time (RT)= 5.542.

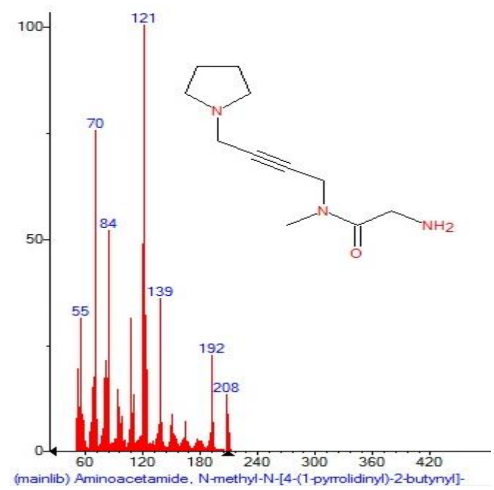


Figure 11: Mass spectrum of Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- with Retention Time (RT)= 6.554.

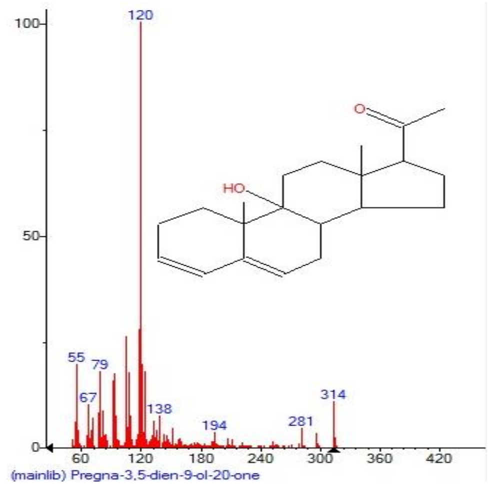


Figure 12: Mass spectrum of Pregna-3,5-dien-9-ol-20-one with Retention Time (RT)= 6.646.

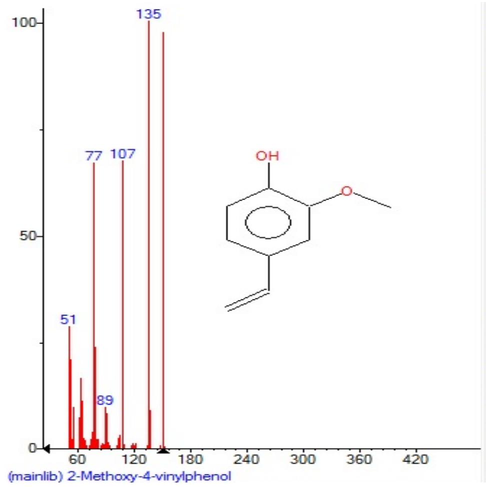


Figure 13: Mass spectrum of 2-Methoxy-4-vinylphenol with Retention Time (RT)= 7.046.

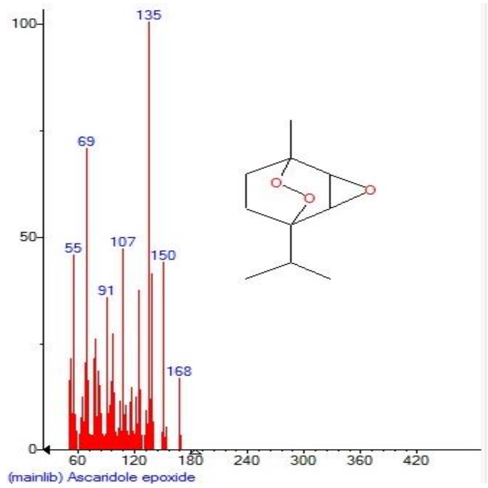


Figure 14: Mass spectrum of Ascaridole epoxide with Retention Time (RT)= 7.212.

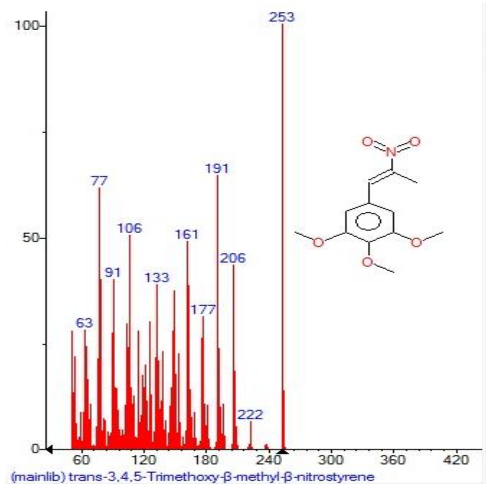


Figure 15: Mass spectrum of Trans-3,4,5-Trimethoxy-β-nitrostyrene with Retention Time (RT)= 7.888.

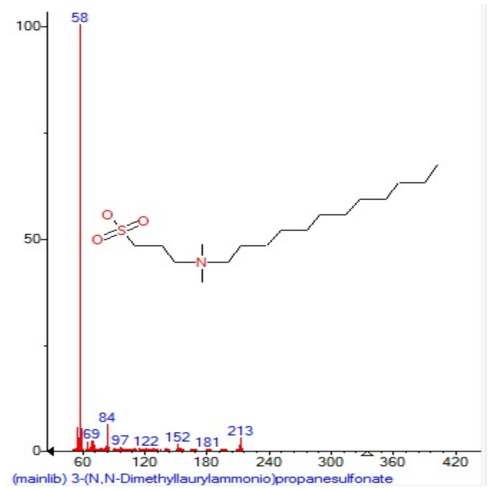


Figure 16: Mass spectrum of 3-(N,N-Dimethyllaurylammonio)propanesulfonate with Retention Time (RT)= 8.877.

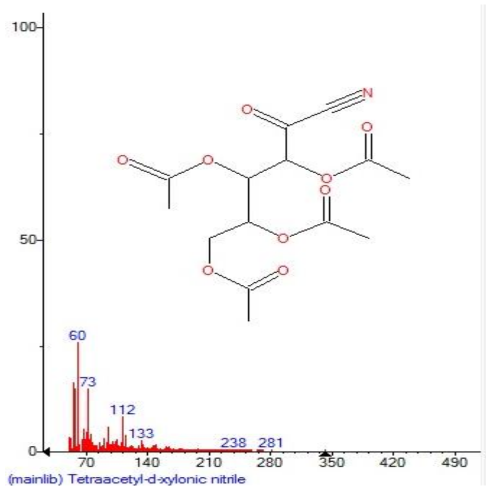


Figure 17: Mass spectrum of Tetraacetyl-d-xylonic nitrile with Retention Time (RT)= 9.209.

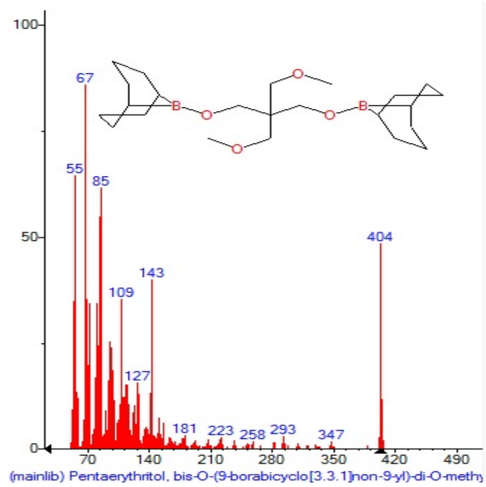


Figure 18: Mass spectrum of Pentaerythritol , bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl with Retention Time (RT)= 9.490.

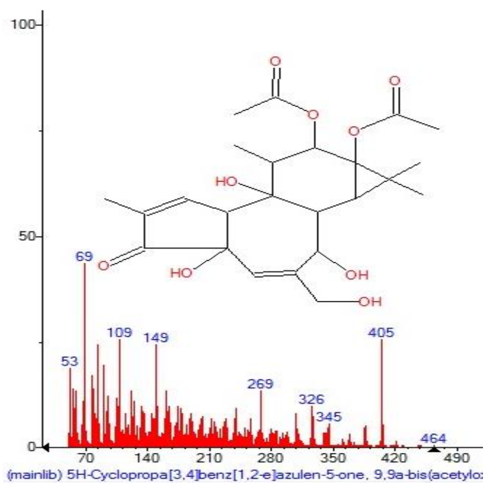


Figure 19: Mass spectrum of 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy) with Retention Time (RT)= 10.348.

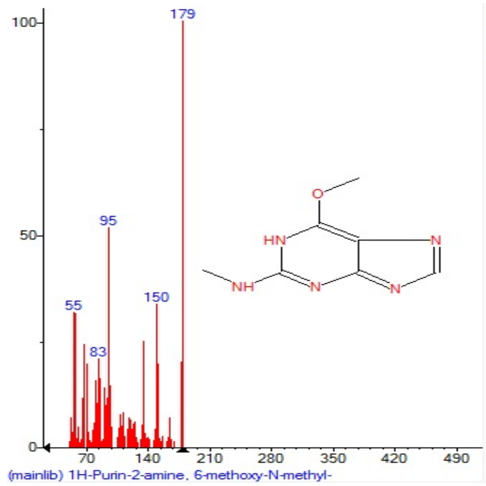


Figure 20: Mass spectrum of 1H-Purin-2-amine , 6-methoxy-N-methyl- with Retention Time (RT)= 10.623.

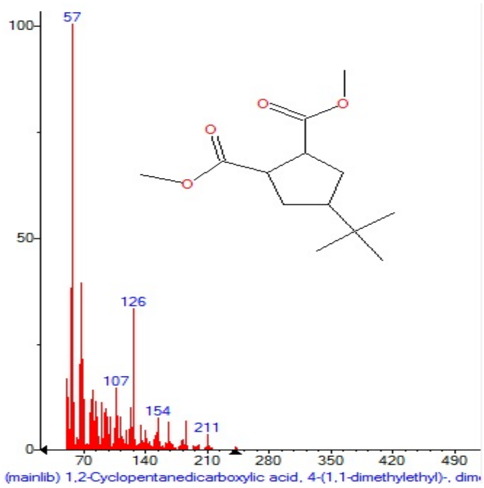


Figure 21: Mass spectrum of 1,2-Cyclopentanedicarboxylic acid 4-(1,1-dimethylethyl)-dim with Retention Time (RT)= 10.852.

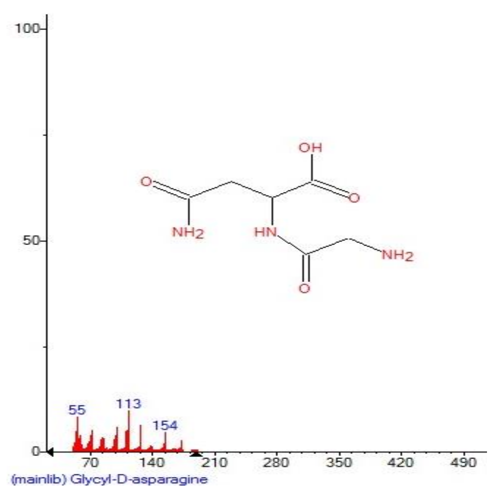


Figure 22: Mass spectrum of Glycyl-D-asparagine with Retention Time (RT)= 10.920.

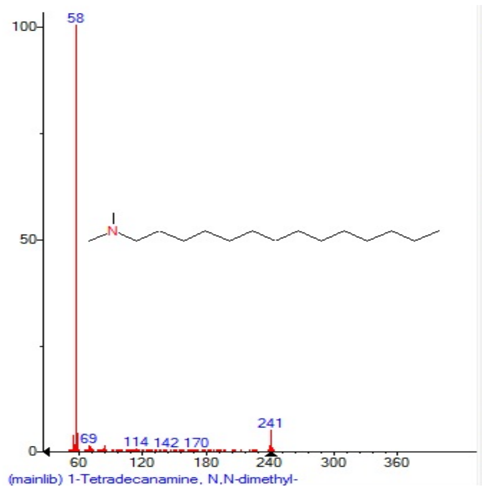


Figure 23: Mass spectrum of 1-Tetradecanamine , N,N-dimethyl- with Retention Time (RT)= 11.000.

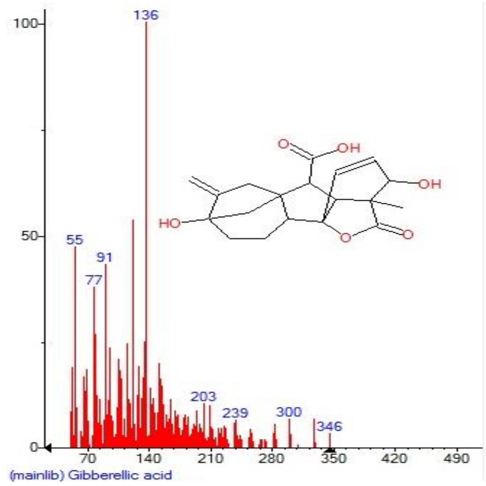


Figure 24: Mass spectrum of Gibberellic acid with Retention Time (RT)= 11.126.

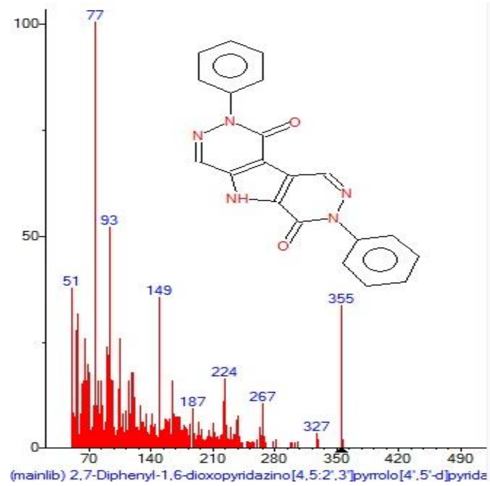


Figure 25: Mass spectrum of 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrida with Retention Time (RT)= 11.521.

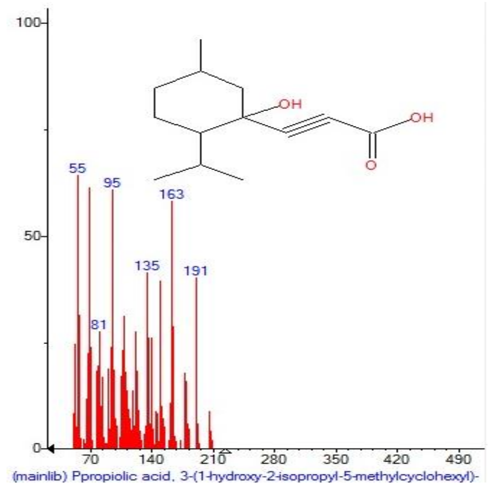


Figure 26: Mass spectrum of Propiolic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)- with Retention Time (RT)= 11.910.

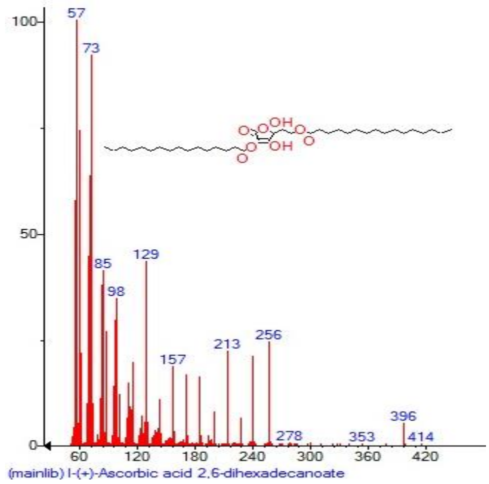


Figure 27: Mass spectrum of l-(+)-Ascorbic acid2,6-dihexadecanoate with Retention Time (RT)= 13.684.

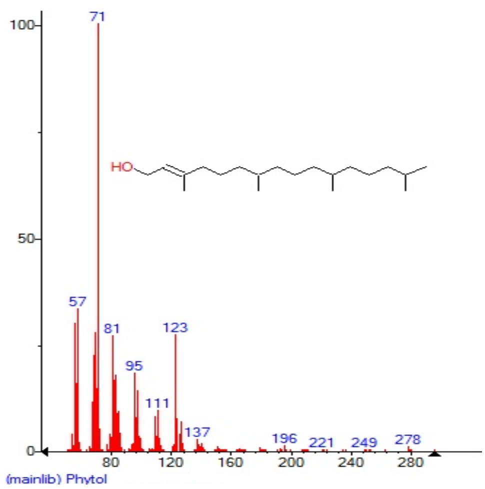


Figure 28: Mass spectrum of Phytol with Retention Time (RT)= 15.034.

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[34-36].

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. The combination of a principle separation technique (GC) with the best identification technique (MS) made GC–MS an ideal for qualitative and quantitative analysis for volatile and semi-volatile compounds[37]. The GC-MS analysis of the plant 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 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made

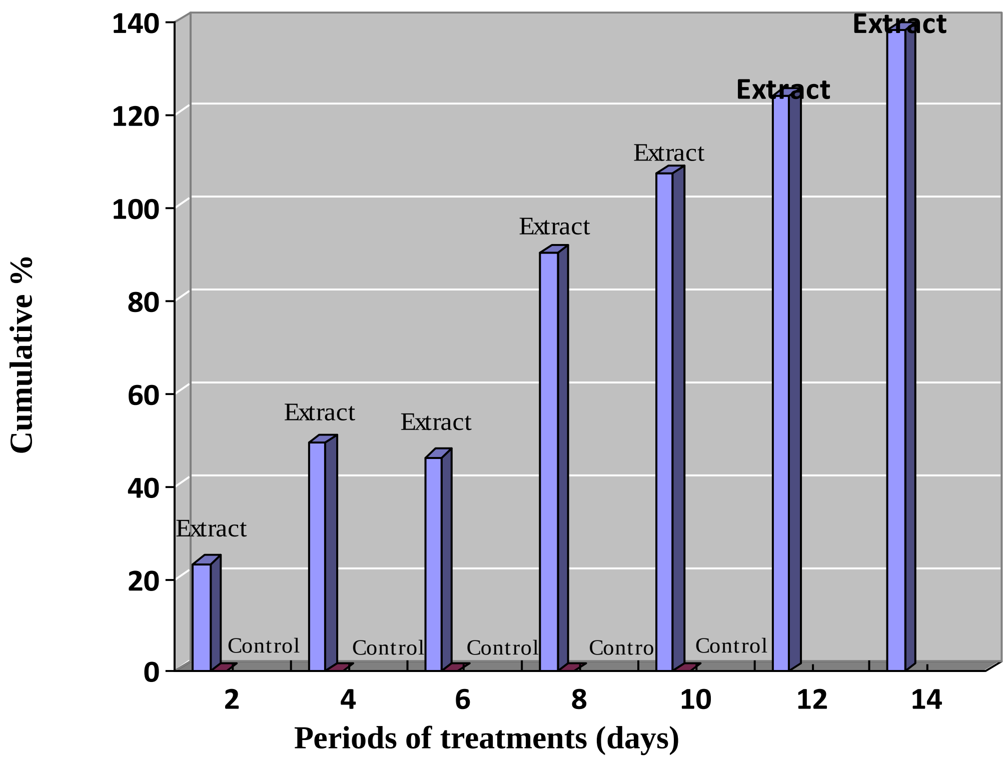


Figure 29: Effect of methanolic leaves extract of *Ricinus communis* on accumulative mortality of *Callosobruchus maculatus* (Coleoptera: Brauchidae) (adults).

(Initial time) to when elution occurred referred to as the Retention time (RT)[38-40]. 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 X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called 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 temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). 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[41-46].

RESULTS AND DISCUSSION

Identification of phytochemical compounds

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic seeds extract of *Ricinus communis*, shown in Table 1. The GC-MS chromatogram of the 27 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 , 17,20,21-bis[methylene bis(oxy)]-, 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol , (3β,5Z,7E)- , 8-Octadecenal , Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) , Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl , 1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione , hexahydro- , Deoxyspergualin , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , Cyclohexanecarboxylic acid , 2-hydroxy-,ethyl ester , Aminoacetamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- , Pregna-3,5-dien-9-ol-20-one , 2-Methoxy-4-vinylphenol , Ascaridole epoxide , Trans-3,4,5-Trimethoxy-β-nitrostyrene , 3-(N,N-Dimethyllaurylammonio)propanesulfonate , Tetraacetyl-d-xylonic nitrile , Pentaerythritol , bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl , 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis(acetyloxy) , 1H-Purin-2-amine , 6-methoxy-N-methyl- , 1,2-Cyclopentanedicarboxylic acid 4-(1,1-dimethylethyl)-dim , Glycyl-D-asparagine , 1-Tetradecanamine , N,N-dimethyl- , Gibberellic acid , 2,7-

Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrida , Propiolic acid , 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)- , l-(+)-Ascorbic acid2,6-

dihexadecanoate and Phytol Figure 2-28.

Determination of anti-insect (Callosobruchus maculatus) activity

In the current study, the anti-insect activity of the methanolic seeds extract was evaluated. The methanol extracts of *Ricinus communis* significantly affected survival of adult with 100%, during 14 days after treatment Figure 29. The relation between exposure period and treatment was very significant $p < 0.05$. Significant insecticidal activity against *Callosobruchus maculatus* adults was observed with crude methanol extract from *Ricinus communis*. Adults were more susceptible to extract of *Ricinus communis*. Pacheco et al. (1995)[47] used refined soybean and crude castor oils to control infestations of beetles *Callosobruchus maculatus* and *Callosobruchus phaseoli* (Gyllenhal) in stored chickpea (*Cicer arietinum* L.). Niber (1994)[48] showed the effectiveness ofleaf and seed powders and slurries of several plant species, including *Azadirac$a indica* Juss (Meliaceae), *R. communis* and *S. nigrum*, in protecting stored wheat and maize grains against damage caused by *S. oryzae* and *P. truncatus* (Horn). Shaaya et al., (1991)[49] suggest good potential for the use of materials derived from *R. communis* and *S. nigrum* as toxicant agents in storage pest management systems, particularly for resource poor farmers in developing countries. Some essential oils extracted from various plants had been shown to possess insecticidal and protectant properties. Previous studies have demonstrated the effectiveness of different vegetable oils in protecting grains against major stored-product insect pests[50-54]. The insecticidal activity of plant materials derived from *R. cummunis* is attributed to its major components of protein ricins and alkaloid ricinine which are lethal at very low concentrations[51]. Our results agree with De Sousa et al. (2005)[55] studying the effect of seven plant powders against *C. maculatus* in *V. unguiculata* seeds found that the *E. caryophyllata* and *P. nigrum* reduced ovipostion in 100% and adult emergence in 100% too.

CONCLUSION

Medicinal property of plant extract is due to presence of secondary metabolites identified by GC-MS analysis. In the present study determined that thirtyone phytoconstituents were identified from methanol extract of *R. cummunis*. This plant was highly active on accumulative mortality of *Callosobruchus maculatus* (Coleoptera: Brauchidae) (adult).

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