Biochemical screening and evaluation of *Eucalptuscamaldulensis*Dehnh leaf n-hexane extracton *Callosobruchusmaculatus*Fabricius (Coleoptera: Bruchidae).

``

Abstracts

The phytochemical screening and GC-MS analysis of the leaf of *camaldulensis*was carried out in the laboratory, using standard methods, while the extract was also extracted with n-hexane with Soxhlet extractor and concentrated with vacuum evaporator. The extractl was tested on *Callosobruchusmaculatus*to evaluate the effects on mortality, oviposition and adult emergence. The of *E. camaldulensis* leaves was effective in controlling *C. maculatus*as it caused a significantly high mortality of the weevils. Weevil mortality increased with increased in number of days in which they were exposed to the treatments and exractdosage level. Weevil mortality of 100 % was achieved by 72 h when treated with 3 % and 4 % dosage level of the exract. The extracts significantly (P < 0.05) caused reduction in oviposition and adult emergence by the weevils. Oviposition and adult emergence decreased with increased in the dosage level of the extract. Oviposition was totally suppressed when insects were exposed to 4 % extract dosage level, while there was no adult emergence on exposure to 3 and 4 % extract dosage levels.The resultsobtainedfrom the phytochemical analysis of *E. camaldulensis*indicated the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and phenol indicating the quantitative phytochemical composition of phenol to be highest (76.955mg/100g), followed by alkaloids (33.025mg/100g), flavonoids (11.74mg/100g), while saponins has the least value of 9.88mg/100g. The GC-MS analysis revealed arrays of bioactive substances that are responsible for the insecticidal and medicinal properties of *E. camaldulensis.* The leaf of *E. camaldulensis*ischeap, biodegradable, ecological friendly and very effective bioinsecticides and therapeutic medicine. Hence, can be used as alternative to the poisonous conventional insecticides and drugs.

Keywords: Phytochemicals, *Eucalyptus camaldulensis, Callosobruchusmaculatus,* ovoposition, adult emergence, bioinsecticides.

.**Introdution**

"Cowpea (*Vignaunguiculata* L. walp) is an essential leguminous crop cultivated throughout all ecological zones of sub-Saharan Africa" (Nkhoma*et al.*, 2020). "As a major staple food, cowpea accounts for about 23-32% of the plant protein consumed by indigenous people in the tropics" (Kpoviessi*et al.,* 2019). Hiama*et al.* (2019) reported that, "aside from the nutritional benefits of cowpea, it is an important means of soil fertility improvement in tropical soil through nitrogen fixation". "In the tropics, infestation of cowpea seed by weevils is a major constraining issue in the longetivity of cowpea seeds in storage" (Adebayo and Anjorin, 2018; Mishra *et al.,* 2018;

Boukar*et al.,* 2020) "due to failure of small holder farmers to store seed using appropriate methods of storage" (Mobolade*et al.*, 2019; Omoigui *et al.*, 2020). During storage the cowpea weevils causes heavy qualitative and quantitative losses. The damage seeds are unsuitable for human and animal consumption and they cannot be used for planting. Preservation of the quality of the seeds for the following planting season is one of the worrying problems of farmers.

"The heavy post-harvest losses and the qualitative deteriorations caused by storage pests is a major problem facing agriculture in developing countries such as Nigeria" (Ashamo, 2007). "The main field pests during the growing season are the aphids while the main storage pests are the bruchids. The primary insects causing losses to stored cowpeas in West Africa are the cowpea weevils, *Callosobruchusmaculatus*. Infestation begins in the field at low level. After the crop is placed in storage, the insect population continues to grow until the cowpea is completely damaged" (Profit, 1997). "Another bruchids pest of cowpea is *Bruchidiusatrolineatus*. This insect causes losses primarily around harvest times and does not reproduce in storage" (Ntoukam, *et al.*, 2000). "A single female weevil can reproduce herself 20-fold every 3-4 weeks. Harvested cowpea grains with a very light infestation will have a heavy infestation within 2-3 months" (Carlos, 2000).

"Awareness of the environmental health hazards posed by synthetic pesticides, development of resistance to these chemicals leading to recurrent pest outbreaks, danger of misuse and presence of toxic residues in food, has led to a search for safe and environmentally-friendly alternatives" (Akinkurolere*et al., 2006;* Oni and Ileke, 2008; Adedire et al., 2011). "Several groups of insecticidal chemicals have been identified in plants. These compounds have different behavioural and physiological effects on insects" (Varma&Dubey, 1999)."Eugenol, isoeugenol and methyleugenol (benzene derivatives) exhibited contact toxicity towards weevils" (Huang *et al*. 2002). "Efficacy of the meliacarpin derivatives, 1,3-dicinnamoyl-11-hydroxymeliacarpin, 1-cinnamoyl-3-methacrylyl-11- hydroxymeliacarpin and 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin extracted from China-berry leaves, compared very well with that of the wellknownazadirachtin. Many plant species synthesize their own chemicals in defense against attack by herbivores, pests and pathogens. Presently, efforts have shifted to the use of edible plants materials as protectants and the tropics are well endowed with these plants, thus limiting the reliance on synthetic chemicals for use in storage" (Akinkurolere*et al.*, 2006; Adedire*et al.*, 2011). These botanicals have become more relevant in the control of stored product insect pests because of their advantages over the synthetic pesticides.

"Eucalyptuscamaldulensis belong to the family Myrtaceae. They are highly exploited because of their wood and essential oil. These essential oil are reported to have antimicrobial, antifungal, antiviral and insecticidal activities, especially in contact and fumigant insecticidal action against stored product pests and other insects". (Mans and Kaufman, 2012).In this present study, *Eucalyptus camaldulensis*leafextract was screened for it phytochemical and the insecticidal potential of the extract was evaluated on cowpea weevils.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Eucalytuscamaldulensis* were collected from Ekiti State University campus, Ado Ekiti,Ekiti State, Nigeria. They were washed in distilled water and spread on the laboratory tables to air-dry for 4 weeks. Thereafter, the leaves were pulverized into fine powder with a Binatoneblender(Model BL 400). The fine powder was kept inside an air tight sample containers and put inside the refrigerator before application.

Preparation of plant extracts

Two hundred grams (200g) of the pulverized leaves of *E. camaldulensis* were measured and packed in thimble using muslin cloth. Then, 500 mL of the *n*-hexane was measured with measuring cylinder and poured into the Soxhlet apparatus. The apparatus was then connected with water supply to the condenser. The temperature of the heating mantle was maintained at $68^{o}C$ to $70^{o}C$ for 3 h after which the thimble was removed from the unit and the solvent was recovered by redistilling in the rotary evaporator. The resulting extract was poured into a brown bottleto prevent photo-oxidation and stored as stock solution until required.

Collection and preparation of insect culture

The parent stock of cowpea bruchids, *Callosobruchusmaculatus*used for this work was obtained from naturally infested *Vignaunguiculata* seeds obtained from MojereMarket in Ado Ekiti, Ekiti state, Nigeria. The infested seeds along with the weevils were put inside plastic container and taken to the laboratory. The insects were allowed to acclimatize to the laboratory condition for five days before they were used for experiment. On the fifth day, 500g of clean un-infested Ife Brown Variety of cowpea seeds were weighed into transparent plastic containers. Afterward, twenty (20) copulating pairs (20 males:20 females) of *C. maculatus* were introduced into the plastic containers containing the disinfested cowpea seeds. The plastic was covered with muslin cloth held tightly in place with rubber band to enhance ventilation and to prevent the entry and escape of theinsects; the insect culture was kept on the laboratory benches for 30 days for the insect to lay eggs and for the adults to emerge. The newly hatched beetles (0-24 h old) produced were used for subsequently experiments.

Phytochemical Analysis

Phytochemical screening of the crude extract of *E camaldulensis*leaves were carried out using the procedure as described by Harbone (1973, 1978)The presence of alkaloids, flavonoids, tannins, terpenoids, phenol, and saponins were tested. GC-MS Analysis of Bioactive Compounds of leaves of *E. camaldulensis*was also carried out to allow for the separation ofcomponents in a gas mixture**.**

Effect of *E. camaldulensis*extracts on the mortality of adult *C.maculatus*

An aliquot of 1.0ml of 1, 2,3 and4 % of *n*-hexane extracts of *E. camaldulensis*leaveswas measured using graduated syringes and mixed with 20g cowpea seeds inside Petri dishes. They were thoroughly mixed together manually by shaking.The Petri dishes were left opened for 1 h to allow the solvent to dry off. afterward, 20 newly emerged (0-24 h old) adult *C. maculatus* were introduced into each of the Petri dishes and covered with Petri plates, Untreated seedswere set up to serve as the control experiment. Each treatment was replicated four times and arranged in Completely Randomized Design in a wooden cage. The number of dead weevils were sorted, counted and recorded at 24 h interval for a period of 96 h. The weevils were confirmed dead when there was no response to probing with sharp pin at the abdomen.

Effect of *E. camaldulensis*extracts on oviposition and adult emergence of *C. maculatus*

An aliquot of 1.0 mL of 1,2,3 and 4 % of *n*-hexane extracts of *E. camaldulensis*leaveswas measured using graduated syringes and mixed with 20g clean un-infested cowpea seeds of Ife brown variety inside Petri dishes. They were thoroughly mixed togethermanually by shaking for 2 minutes to enhance uniform coating of the extracts on the seeds. The Petri dishes were left opened for 1h to allow the solventto dry off. Thereafter, 2 copulating pairs (2 male : 2 females) adult *C. maculatus* (0-24 h old) were introduced into each of the Petri dishes and covered with Petri plates. Untreated seeds were set up to serve as the control experiment. The Petri experiment was left on the shelf for 7 days for oviposition to take place. The number of eggs laid for every extract dosage was counted and recorded. The experiment was kept inside wooden cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adults that emerged from each replicate was counted and recorded. Percentage adult emergence was calculated using the method of Odeyemi and Daramola (2000).

Data Analysis

Data obtained were subjected to Analysis of Variance (Anova), while Turkey test was used in separating the means.

RESULTS and DISCUSSION

Result of the qualitative phytochemical composition of *E. camaldulensis*is shown in Table 1. It indicated the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and phenol. Result of quantitative phytochemical composition of *E. camaldulensis*is shown in Table 2. The results showed that the total phenol has the highest composition of 76.955mg/100g, followed by alkaloids (33.025mg/100g), flavonoids(11.74mg/100g), while saponins has the least value of 9.88mg/100g.

The list of bioactive compounds identified in the leaf extracts of *E.camaldulensis*is presented in Table 3. Results revealed the presence of some volatile compounds in the leaf extracts. These include,hexanal, octanoic acid, 6-octen-1-ol,3,7-dimethyl-, 4-Hexen-1-ol,methyl-2-(1-methylethenyl)-,(R)-, caryophyllene oxide, hexadecanoic acid methyl ester, 2-pentadecanone,6,10,14-trimethy, n-Hexadecanoic acid, phytol, 12-Octadecadienoic acid (z,z)-, 11-octadecenoic acid, methyl ester, Isopropyl stearate, and squalene.

The GC-MS spectra of the identified compounds are shown in Fig 1 to 11. The figures revealed the structure of active volatile compounds in the leaf extracts of the plant samples.

Table 1: Qualitative phytochemical constituent of *E. camaldulensis*.

PARAMETER	OCCURRENCE
Alkaloids	++
Flavonoids	+
Tannins	++
Terpenoids	+
Phenol	++
Saponins	+

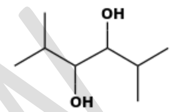
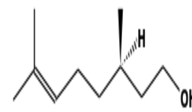
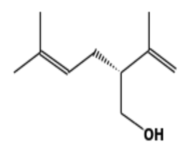
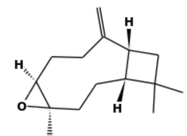
Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test.

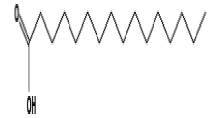
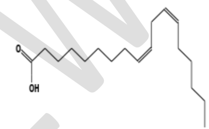
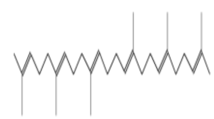
Table 2: Quantitative phytochemicals estimation of *E. camaldulensis*

PARAMETER (mg/100g)	**OCCURRENCE**
Alkaloids	33.025 ± 0.12^{b}
Flavanoids	11.74 ± 2.83c
Total phenol	76.96 ± 7.07^{a}
Saponin	9.88 ± 2.83^{d}

Means in the column followed by the different alphabet(s) are significantly different at $p < 0.05$ using Tukey's test

Table 3: The bioactive compounds of *E. camaldulensis*revealed by GC-MS analysis

Peak #	RT	Compound Detected	Molecular Formula	MW	Peak Area (%)	Structures
1	10.00	Hexanal	$C_6H_{12}O$	100	2.84	
2	11.61	Octanoic acid	$C_8H_{16}O_2$	144	3.66	
3	17.46	6-Octen-1-ol,3,7-dimethyl-	$C_{10}H_{20}O$	156	10.09	
4	19.50	4-Hexen-1-ol,5-methyl-2-(1-methylethenyl)-, (R)-	$C_{10}H_{18}O$	154	1.83	
5	20.50	Caryophyllene oxide	$C_{15}H_{24}O$	220	10.08	
6	22.00	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.32	
7	23.50	2-Pentadecanone, 6,10,14-trimethy	$C_{18}H_{36}O$	268	2.75	

8	24.83	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.69	
9	27.00	Phytol	$C_{20}H_{40}O$	296	3.85	
10	27.48	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	270	10.01	
11	32.25	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	4.96	
12	35.92	Isopropyl stearate	$C_{21}H_{42}O_2$	326	20.94	
13	43.50	Squalene	$C_{30}H_{50}$	410	2.93	

RT-: Rotation Time

Mw-: Molecular weight

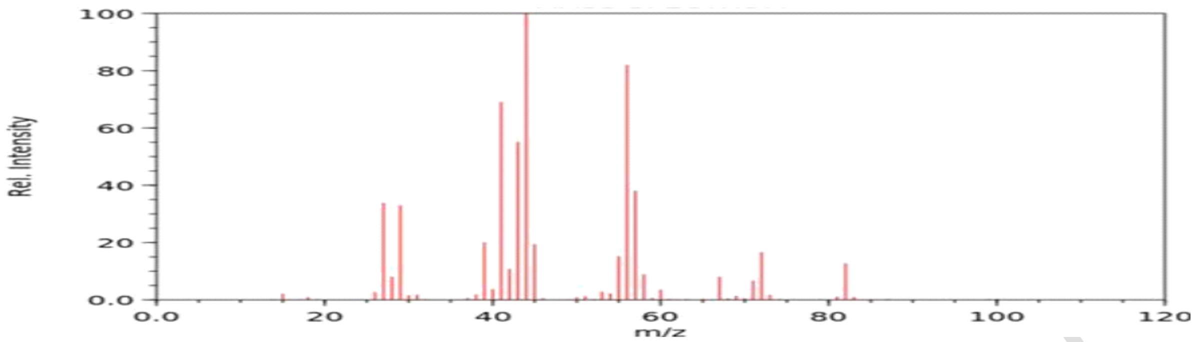


Fig 1: Structure of Hexanal, identified in methanoic extract of *E. camaldulensis*

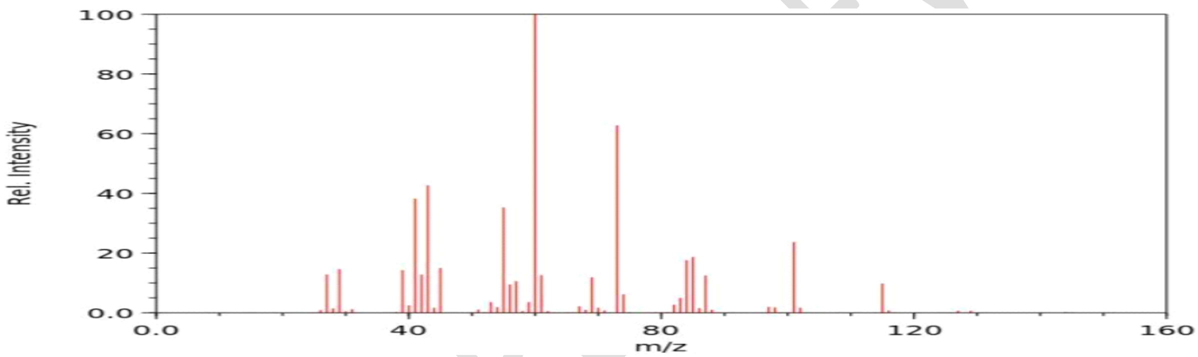


Fig 2: Structure of Octanoic acid identified in methanoic extract of *E. camaldulensis*

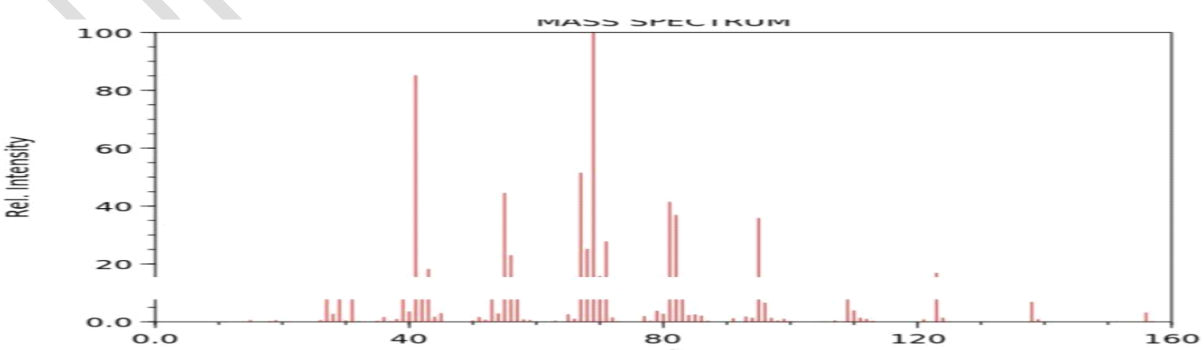


Fig 3: Structure of 6-Octen-1-ol, 3,7-dimethyl-, identified in methanoic extract of *E. camaldulensis*

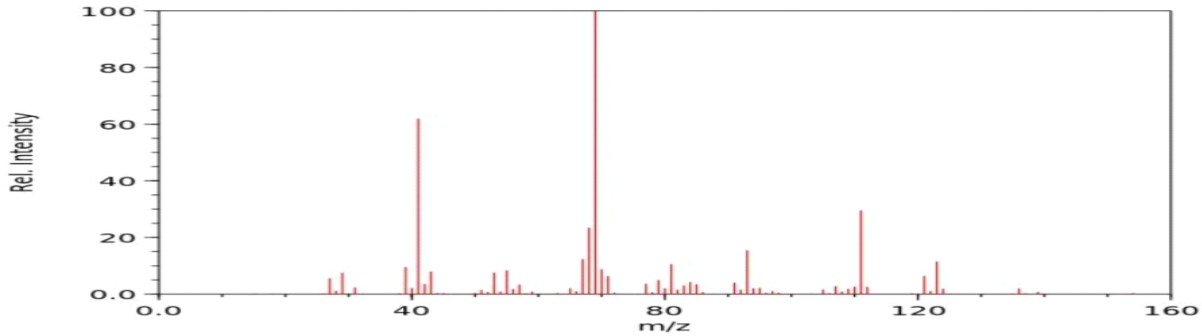


Fig 4:Structure of 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-,Identified in methanoic extract of *E. camaldulensis*

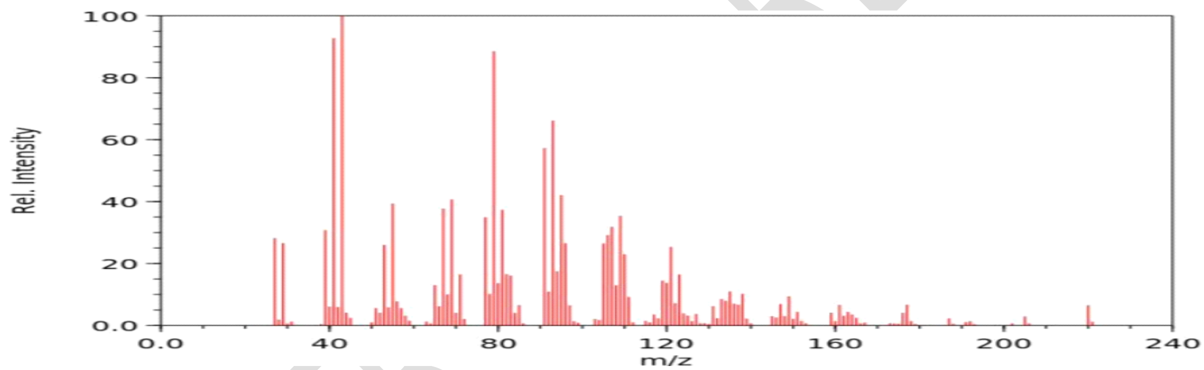


Fig 5: Structure of Caryophyllene oxide identified in methanoic extract of *E. camaldulensis*

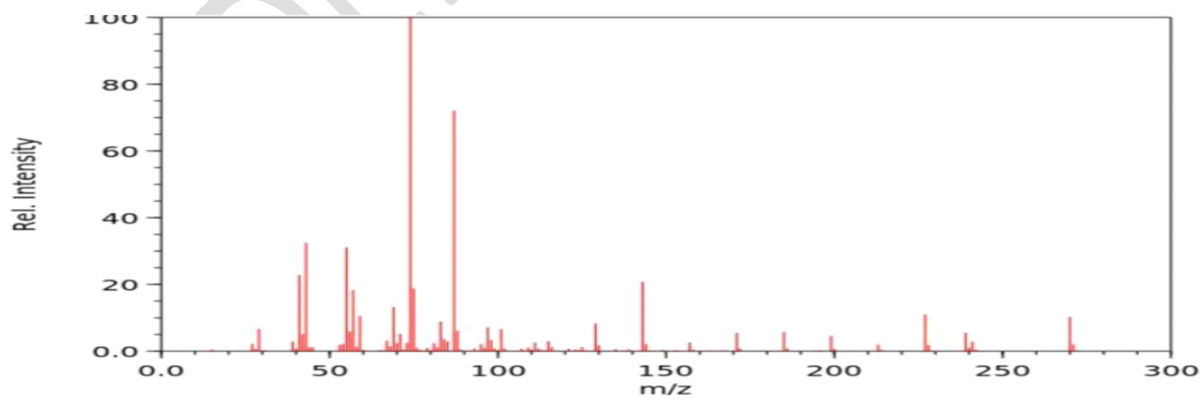


Fig 6: Structure of Hexadecanoic acid, methyl ester, identified in methanoic extract of *E. camaldulensis*

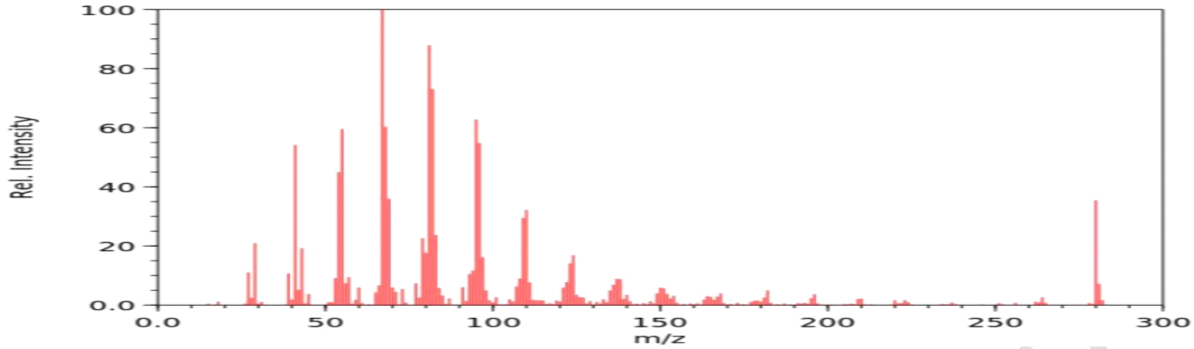


Fig 7: Structure of 9,12-Octadecadienoic acid (Z,Z)- , identified in methanoic extract of *E. camaldulensis*

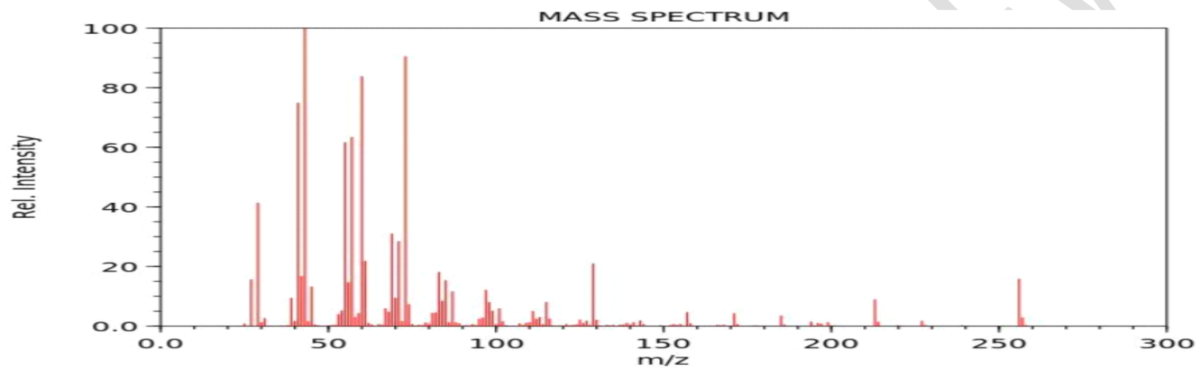


Fig 8: Structure of n-Hexadecanoic acid, identified in methanoic extract of *E. camaldulensis*

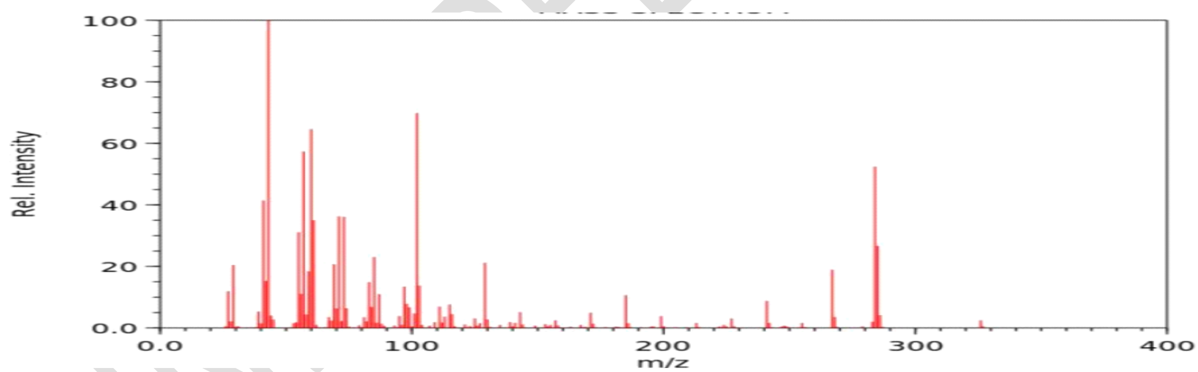


Fig 9: Structure of Isopropyl stearate, Identified in methanoic extract of *E. camaldulensis*

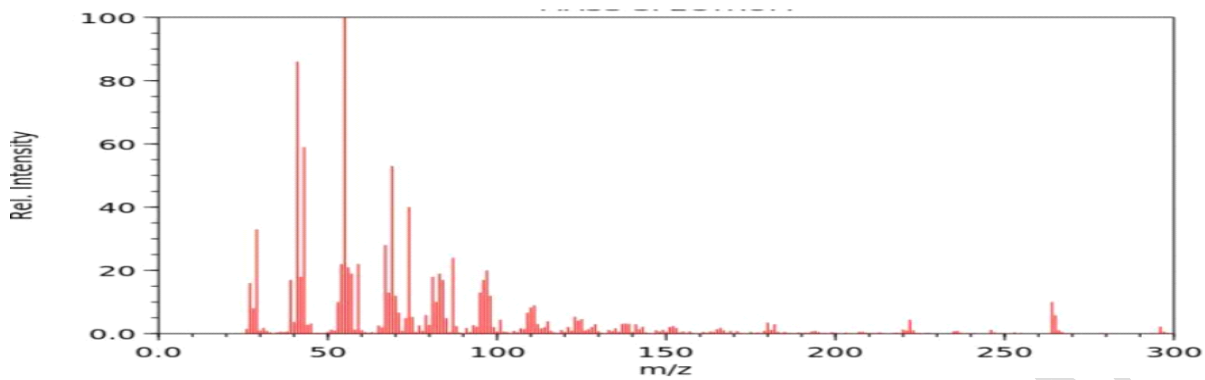


Fig 10: Structure of 11-Octadecenoic acid, methyl ester, identified in methanoic extract of *E. camaldulensis*

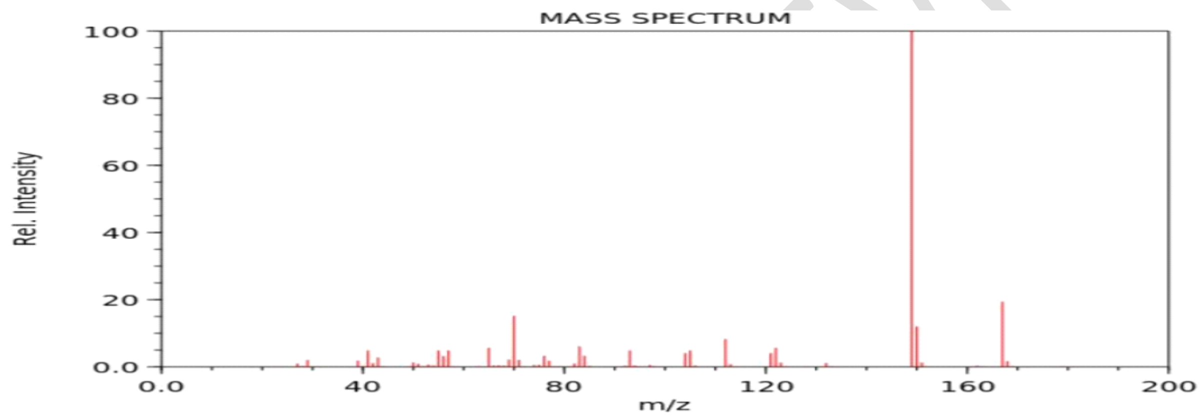


Fig 11: Structure of Squalene, identified in methanoic extract of *E. camaldulensis*

Table 4**:** Mortality of *C. maculatus* treated with *E. camaldulensis*n-hexaneleaf extracts

Dosage (%)	Percentage Mortality at h post-treatment			
	24	48	72	96
1.0	15.20±0.45^{c}	18.20±0.81^{d}	20.40±0.72^{d}	24.34±1.14^{d}
2.0	17.35±0.33^{c}	22.15±1.25^{c}	28.15±1.34^{c}	37.15±2.20^{c}
3.0	26.18±1.18^{b}	38.35±2.15^{b}	57.22± 2.13^{b}	77.25±2.34^{b}
4.0	42.25±2.31^{a}	73.20±2.39^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.0 (control)	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}

Means in the same column followed by the same alphabet(s) are not significantly different at p < 0.05 using Tukey's test.

Table 5: Effect of *E. camaldulensis*n-hexaneleaf essential extract on oviposition and adult emergence of *C.maculatus.*

Dosage (%)	mean number of egg laid	Percentage adult emergence
1.0	24.25 ± 1.13^{b}	23.25 ± 2.14^{b}
2.0	18.15 ± 1.35^{c}	10.40 ± 0.62^{c}
3.0	6.42 ± 0.62^{d}	0.00 ± 0.00^{d}
4.0	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
0.0 (control)	65.25 ± 1.85^{a}	87.30 ± 3.17^{a}

Means in the same column followed by the same alphabet(s) are not significantly different at p < 0.05 using Tukey's test.

Mortality of *C. maculatus* treated with *E. camaldulensis*n-hexane leaf extracts

The extract of *E. camaldulensis* leaves is effective in controlling *C. maculatu*as it caused a significantly high mortality of the weevils as revealed by Table 4. Weevil mortality increased with increased number of days in which they were exposed to the extract and the dosage level. There was no significant different (P < 0.05) in weevil mortality when exposed to 1 and 2 % extract concentration by 24 h of exposure.One hundred percent (100 %) weevil mortality was achieved by 72 h when treated with 3 % dosage of the extract.

Effect of *E. camaldulensis*n-hexaneleaf extract on oviposition and adult emergence of *C.maculatus.*

The extract significantly (P < 0.05) caused reduction in oviposition and adult emergence by the weevils as revealed by Table 5.Oviposition and adult emergence decreased with increased in the dosage level of the extract. Oviposition was totally suppressed when insects were exposed to 4 % extract dosage concentration. While there was no adult emergence on exposure to 3 and 4 % extract dosage concentrations.

The presentstudies on the evaluation of leaf extract of *E. camaldulensis*on *Callosobruchusmaculatus*showed that the extract adequately protected the cowpea seeds from the weevils' attack. The results obtainedis in consonant withAdedire*et al*. (2011) who obtained 100 % weevil mortality and total suppression of oviposition and and emergence on treatment of stored cowpea seeds with Cashew kernel oil at the dosage rate of 1.0 mL/20g. The high mortality of the extract-treated weevils may be ascribed to the extract blocking the spiracleswhich must have led to difficulty in gaseous exchange and consequent respiratory activities which caused suffocation and death of insects. (Adedire*etal*., 2011)

"The fact that extract caused the complete inhibition of oviposition by female coleopteran pests and mortality of the developmental stages had been reported by a number of authors and fairly well documented" (Boeke*et al*., 2001). "The ability of the extract to reduce oviposition by *C. maculatus* could be linked with respiratory impairment, which probably affects the process of metabolism and consequently other systems of the body of the beetles" (Onolemhemhem and Oigiangbe 1991)."The extract possibly inhibited locomotion; hence the beetles were unable to move freely thereby affecting mating activities and sexual communication" (Ogunleye and Omotoso 2011). "The inability of the eggs to stick to the cowpea seeds due to the presence of the extracts which affected egg hatchability and survival subsequently reduced adult emergence" (Adedire*etal*., 2011). "It has been reported that one of the main mechanism of action of plant extracts is their ability to penetrate the chorion of bruchid via micropyle and cause the death of developing embryos through asphyxiation" (Annie Bright., 2001).

It has been reported by various authors that most plant species possess phytochemicals such as terpenoids, tannins, saponins, flavonoids and alkaloids among others which are reasonably toxic to insect pests (Fernando *et al*., 2005)). The insecticidal activities of saponins have been connected with its cholesterol which results in impaired steroid hormones (ecdysteroid) production in insects. Yang *et al*. (2006) reported the insecticidal activities of alkaloids against stored product insects.

"Apart from the insecticidal activities of the phytochemical, they are also of medicinal benefits.Alkaloids have been used for several hundreds of years in medicine and even today it is still a prominent drug. In most of the human history, alkaloids from plant extracts have been used as ingredients in liquid medicine and poison. Ancient people used plant extracts containing alkaloids to treat a large number of ailments including snake bite, fever, and insanity"(Molyneux*et al.,* 1996)."Flavonoids are important class of natural products, particularly they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits and vegetables" (Burak and Imen, 1999). "They have favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), and atherosclerosis", (Lee *et al.,* 2009). "Saponins have antifungal, antihelminthic, immune stimulant, cytotoxic, anti-inflamatory, hypocholesterolemic, hypoglycemic, and abortifacient properties" (Francis *et al*., 2002). "Saponinspossess foaming, pharmacological, medicinal, and hemolytic properties and also find a place in cosmetic, beverage, and confectionery industries. In pharmaceutical industries, saponins are widely considered as precursors for the synthesis of steroidal drugs" (Waheed*et al.,*2012). "Terpenoid is a natural compound with various medicinal properties, and found in both plants and animals. They have antimicrobial properties, which has the ability to ll or stop growth of microorganisms and are commonly used in traditional and modern medicine" (Himejima*et al.,* 1992)."Phenols exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects" (Arts and Hollman,2005). "They have antioxidant properties due to their high redox potential, which allows them to act as reducing agents and singlet oxygen quencher" (Ignat, 2011).

"Tannins have shown remarkable antioxidant properties that justify their use as food additives to improve food shelf-life and safety, an issue that has made several tannins undergo trials for their legal approval as such additives. Furthermore, their precipitation accounted for their decade-long use as clarification agents in the beverage industry" (Sharma *et al.*, 2019).

E. camaldulensis contains phytochemicals which are used as insecticides. These phytochemicals are eco-friendly, easily biodegradable and non-toxic to non-target species. The botanical insecticides are having certain bioactive compounds which act against the major pests of wheat, rice, maize and soyabean. These active compounds act on the system of the pests and can kill the pests and at the same time medicinal.

Conclusion

The extracts of *E.camaldulensis*used in this finding were effective in the control of cowpea weevil, *Callosobruchusmaculatus.* The potency of the extracts caused adult mortality, suppressed oviposition and adult emergence. Therefore, these extracts can be used as sustainable substitute to conventional insecticide in the control of *C. maculatus.*

References

Adebayo, R.A. and Anjorin, O.O. (2018). Assessment of entomocidal effect of solar radiation for the management of cowpea seed beetle, *Callosobruchusmaculatus*(F) (Coleoptera*:* Chrysomelidae)in stored cowpea. *Global Journal of Science Frontier Research* 18: 21-26

Adedire, C.O., Obembe, O.M., Akinkurolere, R.O., Oduleye, O. (2011) Response of *Callosobruchusmaculatus* (Coleoptera: Chrysomelidae: Bruchidae) to extracts of cashew kernels. *J Plant Dis Protect*, 118(2):75–79

Adedire, C.O., Obembe, O.M., Akinkurolere, R.O. and Oduleye, O. (2011). Response of *Callosobruchusmaculatus* (Coleoptera: Chrysomelidae: Bruchidae) to extracts of cashew kernels. *Journal of Plant Disease and Protection*, 118(2): 75-79.

Akinkurolere, R.O., Adedire, C.O. and Odeyemi, O.O. (2006). Laboratory evaluation of the toxic properties forest anchomanes, *Anhomanesdifformis* against pulse beetles, *Callosobruchusmaculatus*(Coleoptera:Bruchidae). *Insect Science,* 13: 25-29.

Annie Bright A., Babu A., Ignacimuthu, S., Dom, S. (2001).Efficacy of *Andrographispeniculata*Nzes on *Callosobruchuschinensis* L. during post-harves storage of cowpea.*Indian JExp Biol*., 39:715–718

Arts, I.C.W. and Hollman, P.C.H. (2005). Polyphenols and disease risk in epidemiologic studies. *American Journal of Chemical Nutrition*, 81: 317-25.

Ashamo, M. (2007).Evaluation of contact toxicity and fumigant effect of some plant powder against *Sitophiluszeamais*(Mots.). Proceeding of the AKure Humboldt Kelllong (3rd SAAT Annual Conference: *Medicinal Plant in Agriculture*, The Nigeria Experience, pp. 64-67

Boeke S. J., Van Loon, J.J.A., Van Huis, A. Kossou, D. K. and Dickle, M. (2001). The use of plant materials to protect stored leguminous seed from beetles: a review. Wageningen University papers 2001- 2003.Backhuys publishers.The Netherlands.108pp .

Boukar, O., Abberton, M., O., Oyatomi, A., Togola, A., Tripathi, L. and Fatokun, C. (2020). Introgression breeding in cowpea (*Vignaunguiculata* (L.)Walp).*Frontier in Plant Science,* 11, 567425

Burak, M and Imen, Y. (1999).Flavonoids and their antioxidant properties.*TurkiyeKlin Tip BilDerg* 19: 296–304

Carlos, G. (2000). Cowpea Post Harvest Operation, Food and Agriculture Organisation of United Nations, Rome, Italy,

Fernando, A. C., Silva, K.F.S.D, Santos, K.K.D., Junior, K.A.L.R., Ana, A.E.G.S. (2005). "Activities of some Brazilian plant against larvae of the mosquito Aedesaegypti". *Fitoterapiai*, 76: 234 - 239.

Francis, G., Kerem, Z., Makkar, H.P.S., and Becker, K. (2002) The biological action of saponins in animal systems: a review. *Br J Nutr* 88(6):587–605. https://doi.org/10.1079/BJN2002725

Hiama, P.D., Ewusi-Mensah, N. and Logah, V. (2019). Nutrient uptake and biological nitrogen fixation in cowpea under biocharphosphorus interaction.*Journal of Animal and Plant Siences.*29: 1654-1663.

Harborne, J.B (1973). Phytochemical Methods. Chapman and Hall, Limited, London. (1973) 49-188.

Himejima, M. (1992) Antimicrobial terpenes from oleoresin of ponderosa pine tree, *Pinusponderosa*: a defense mechanism against microbial invasion. *J ChemEcol* 18(10):1809–1818.

Huang, Y., Ho, S., Lee, H. and Yap, Y. (2002).Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophiluszeamais* (Motsch.) (Coleoptera: Curculionidae) and *Triboliumcasteneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal ofStored Products Research*, 38: 403–412.

Ignat I, Volf I and Popa, V.I. (2011). A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4): 1821-1835.

Kpoveisi, A.D., Agbahoungba, S., Agoyi, E.E., Assogbadjo, D.C. (2019). Resistance of cowpea to Cowpea bruchid (*Callosobruchusmaculatus* Fab.): Knowledge level on the genetic advance. *Journal of Plant Breeding and Crop Science* 11: 185-195

Lee, Y., Yuk, D., and Lee, J. (2009) Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of β-amyloid generation and memory deficiency. *Brain Res* 1250: 164–174.

Mishra, S.K., Panda, M.L.R. and Panigrahi, J. (2018).Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annal of Agricultural Sciences*,172(1):196-205.

Mobolade, A.J., Bunindro, N., Sahoo, D. and Rajashekar, Y. (2019).Traditional methods of food grains preservation and storage in Nigeria and India. *Annals of Agricultural Sciences*, 64 196-205.

Molyneux, R.J., Nash, R.J., and Asano, N. (1996) Alkaloids: *Chemical and BiologicalPerspectives*. Vol. 11, S.W. Pelletier (ed.), Pergamon: Oxford

Nkhoma, H., Shimelis, H., Laing., M.D., Shayanowako A., and Mathew, I. (2020). Assessing the genetic diversity of cowpea (*Vignaunguiculata*(L.)Walp) germplasm collections using phenotypic traits and SNP markers, BMC genetics, 21: 1-6

Ntoukam, G.L, Murdock, L.L. and Shade, R.E. (2000). “Managing insect pest of cowpea in storage, Midcourse 2000 Research meeting, Senegal, pp.3-4,

Odeyemi, O.O. and Daramola, A.M. (2000). Storage practices in the tropics: food storage and pest problems. First Edition, Dave Collins publication, Nigeria, Vol. 1, 235.

Ogunleye, R.F., Omotoso, O.T. (2011) Comparative effectiveness of the latex and extracts of six botanicals in the control of *Callosobruchusmaculatus* (F.) (Coleoptera: Bruchidae). *J PhysBiolSci* 4(1):24–26

Omoigui, L.O., Kamara, A.Y., Kamai, N., EKeleme, F. and Aliyu, K.T. (2020). Guide to cowpea production in Northern Nigeria. IITA, Ibadan, Nigeria. 48.

Oni, M.O., and Ileke, K.D. (2008).Fumigant toxicity of four botanical plant oils in survivl, egg laying and progeny development of the dried yam beetle, *Dinoderusporcellus*(ColeopteraBostrichidae).*Ibadan Journal of Agricultural Research* 4(2):31-36.

Onolemhemhem, O.P., Oigiangbe, O.N. (1991) The biology of *Callosbruchusmaculatus* (F.) on cowpea (*Vignaunguiculata*) and pigeon pea (*Cajanuscajan*(L.)Millsp.)treated with vegetable oil of Thioral samaras. *J Agric Res*., 8:57–63.

Profit, M. (1997). Bruchid Research at Royal Holloway, University of London,

Sharma, K., Kumar, V., Kaur. J., Tanwar. B., Goyal. A., Sharma. R., Gat. Y., and Kumar. A.(2019). Health effects, sources, utilization and safety of tannins: *A critical review. Toxin Rev.*:1-13.

Varma, J. and Dubey, N.K. (2001).Efficacy of essential oils of Caesuliaaxillaris and MenthaArvensis against some storage pests causing biodeterioration of food commodities.International.*Journal of Food Microbiology*, 68: 207–210.

Varma, J. and Dubey, N.K. (1999).Prospectives of botanical and microbial products as pesticides of tomorrow *,Current Science Association*, 76(2):172-179.

Waheed, A., Barker, J., Barton. S.J., Owen, C.P., Ahmed, S., and Carew, M.A. (2012) A novel steroidal saponin glycoside from Fagoniaindica induces cell-selective apoptosis or necrosis in cancer cells. *Eur J Pharm Sci* 47(2):464–473.*https://doi.org/10.1016/j.ejps.2012.07.004*

Yang, Z., Zhao, B., Zhu, L., Fang, J., Xia L. (2006). Inhibitory effects of alkaloids from *Sophoraalopecuroids* on feeding development and reproduction of *Clostera anastomosis*. Front China 1(2):190–195 Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.