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Terpenoid constituents, insecticidal and antimicrobial activities of *Xylopia aethiopica* fruits found in Ondo State, Nigeria.

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Abstract

Chemical composition of the essential oil obtained by hydro-distillation from the fruits of *Xylopia aethiopica* growing in Ondo State, Nigeria was analyzed by GC/MS. Toxicity using antifeedant and filter paper methods of the essential oils against *Callosobrchus maculatus* was carried out. The essential oil (EO) was also tested against ten pathogens among which are six bacterial (*Escherichia coli, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus, Klebsiella Pneumonia and Streptococcus pneumonia) and four fungal (Aspergillus niger, Aspergilus flavus, Candida tropicalis and Fusarium solani*). Twenty-three components were identified representing 94% of the total oil composition with β-pinene (31.41%), α-pinene (21.7%), 1,8-cineole (8.69%), Δ-3-carene (7.17%) and α-phellandrene (5.0%) as major components. Toxicity was found to be100% in anifeedant method and ranged between 80 and 100% in filter paper methods after 6 and 24 hours of application. The results of the zone of inhibition obtained showed that X. *aethiopica* EO inhibited the growth of all the pathogens in order of *S. paratyphi* (1.86 mm) > *S. aureus* (1.73 mm) > *S. pneumonia* (1.53 mm) > *K. pneumonia* (1.40 mm) > *B. subtillis* (1.23 mm) > *E. coli* (0.46 mm), respectively for bacterial pathogens and *F. solani* (1.93 mm) > *A. niger* (1.66 mm) > *A. flavus* (1.60 mm) > *C. tropicalis* (0.86 mm) for fungal pathogens.

Keywords: *Xylopia aethiopica*, essential oil, *Callosobrchus maculatus* hydrodistilation, toxicity, pathogens

Introduction

Xylopia aethipica (Dunal) A. Rich (Annocaceae) is a tree of 20 m high or more and 60—75 cm of diameter which grows in the forest zone and especially along the rivers in arid areas. The fruit is a slightly hooked cylindrical pod reaching 2-3 mm in width. The mature fruits having green colour take a brown

In the present study, volatile oil composition of the fruits of X. *aethiopica* found in Ondo-state was revealed. The bioactivity such as insecticidal and antimicrobial was also carried out.

Materials and Methods:

Plant Materials

The fruits of ***X. aethiopica*** were collected in September, 2016 at Oka town, Ondo State of Nigeria around 8 am in the morning. The plants were authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba- Akoko, Ondo State.

Isolation of the volatile oil

Fresh fruits of the plants were washed free of sand and other impurities and cut into small pieces. Five hundred gram was hydrodistilled for four hours using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate and kept in a sealed sample bottle at 4^oC until analysed.

Gas Chromatography/Mass Spectrometry Analysis

The analysis of the Volatile compounds was carried out on a Hewlet Packard 6890 GC/MS system equipped with quartz capillary column; 30 m x 0.25 mn i.d x 0.25 µm film thickness. The carrier gas was helium (1 ml/min); oven temperature, 40^oC to 300^oC at a rate 5^oC/min then held isothermal for 2 min. The injector port temperature was 250^oC. The ionization of the sample components was performed on E.I mode (70ev). The identification of different constituents was performed by comparison of their retention time and mass spectra with those of the library.

Insect rearing and maintenance

The initial stock of cowpea bruchid (C. *maculatus*(F)) used for the study was obtained from an already infested cowpea seeds purchased from a local market, Okusa Food Market in Akungba-Akoko, Ondo State of Nigeria in February, 2017. From this stock, new generation was reared on cowpea in the laboratory at room temperature. Freshly emerged adults of C. *maculatus* were then subsequently sub-cultured on the same variety of cowpea over four generations before they were used for experiment.

Antifeedant Test

Four concentrations of the oil (0.01, 0.02, 0.03, and 0.04 ml) were dissolved separately in 0.5 mL of analytical grade of acetone. Each of the concentrations for each oil was admixed with 10 g of cowpea contained in 50 mL glass jar. The admixture was stirred thoroughly with a glass rod to ensure adequate coating of seeds with oil and until the acetone completely evaporated according to the method of Lale, [15]. Twenty mixed sex adults of C. *maculatus* (3-5 days old) were introduced into each jar and the lid was replaced. Control seeds were treated with 0.5 ml pure acetone and second control was only cowpea without any treatment. Each treatment and control were replicated three times. Mortality record was taken at six and twenty four hours interval after introducing insects on the seeds. Insects which did not respond to the gentle touch of a small probe were considered dead [16].

Filter paper test

Bioassay on the toxicity of *X. aethiopica* essential oil against adult *C. maculatus* was similar to the method described by Ukeh *et al*., [17] in Pyrex glass Petri dishes (10 cm diameter). Different doses of each essential oil (0.01, 0.02, 0.03, and 0.04 ml) were dissolved in 0.5 ml analytical grade acetone and delivered to the Petri dishes pre-lined with Whatman N° 1 filter paper. Pure acetone was used for the filter paper for control. The solvent was allowed to evaporate and 5 mixed pairs of *C. maculatus* adults were introduced into each Petri dish. The Petri dishes were closed and maintained in the laboratory for 6 and 24 hrs at ambient temperature and relative humidity. All treatments were replicated three times for each dose of the essential oil, and account of dead weevils was made at 6 h and 24 h interval

Antimicrobial activity of essential oils

The micro-organism used in this study were isolates collected from out patients ward of the Federal Medical Centre, Owo whose morphological and biochemical characteristics were confirmed. The bacterial cultures were maintained on nutrient broth while the fungal

cultures were maintained on sabouraud liquid medium. The bacteria and fungi used includes three gram negative bacteria *Escherichia coli, Klebsiella pneumonia and Salmonella paratyphi*, three gram positive bacteria *Bacillus subtillis, Staphylococcus aureus, and Streptococcus pneumonia;* and four strains of fungi; *Aspergillus flavus, Candida Tropicalis, Fusarium solani* and *Aspergillusniger.*

Zone of inhibition

Inoculums size containing 10 cfu/mL for bacterial and 10 sfu/ml for fungal were used to seed already solidified Petri plates of Muller-Hinton agar. The antimicrobial activities of the oil was determined using agar well diffusion method. Ten organisms were used in all three gram positive, three gram negative and four fungi. A sterile 6 mm cork borer was used to make well on already solidified agar. The wells were filled with the oil ensuring that they were allowed to stand for about 2 hours to allow absorption of the oil into the medium after which they were incubated at 37^{o} C for fungi for 24 hours for bacterial and 7 days for fungi.

Minimum inhibitory concentration (MIC)

A modified Macro-broth dilution technique was used in this research for MIC. Those recorded as MIC were the lowest concentration of the tested oil that showed no visible growth of the tested isolate. Serial dilutions of the oil were carried out to give a concentration of 0.5, 0.25, 0.125 and 0.0625 mL/ml. 2 mL of each diluted concentration was added to 18 mL of pre-sterilized molten Mueller-hinton and Sabouraud agar mixed properly and allowed to set. After which the standardized inoculums were seeded on the plates. The bacterial plates were incubated at 37^{o}C for 24 hours, while the fungi at 25^{o}C for 7 days. The results were observed and recorded.

Results and Discussion

The qualitative and quantitative essential oil compositions of *Xylopia aethiopica* fruits in Table 1 after 4 hrs of hydro-distillation yielded 5.2% v/w. The composition of the volatile oil revealed the presence of 89.72% monoterpenens (76.11% monoterpene hydrocarbons and 13.61% oxygenated monoterpenes) and 3.95% sesquiterpenes (2.88% sesquiterpene hydrocarbon and 1.07% oxygenated sesquiterpenes). The chemical composition of *X. aethiopica* essential oil showed total 23 components with β-pinene (31.41%), α-pinene (21.71%), 1,8-cineole (8.69%), Δ-3-carene (7.17%), α-phellandrene (5.0%), α-terpineol (3.89%), γ-terpinene (3.02%), Germacrene D (2.17%), Limonene (2.15%) and Myrcene (2.12%) as major component.

Ekong *et al* [18] were the first to report on the chemical composition of *X. aethoipica* and several publications have appeared subsequently on this subject.

Table 1: Chemical Composition (%) of *X. aethiopica* Essential oil

S/N	Compound Name	Retention Time (min)	Composition (%)
1	Cymene	4.95	1.87
2	Camphene	5.85	0.33
3	Limonene	7.71	2.15
4	α-pinene	9.87	21.71
5	β-pinene	10.95	31.41
6	Δ-3-carene	11.35	7.17
7	Benzyl Alcohol	11.80	0.31
8	α-phellandrene	12.40	5.00
9	Cis Ocimene	12.94	1.33
10	Myrcene	13.04	2.12
11	Gama Terpinene	14.90	3.02
12	Camphor	15.02	0.39

13	Neral	15.33	0.33
14	1,8-cineole	16.56	8.69
15	Citronellol	17.56	0.31
16	α-terpineol	18.70	3.89
17	β-bisabolene	22.22	0.35
18	α- bergamotene	22.89	0.36
19	Germacrene D	24.11	2.17
20	Spathulenol	26.61	0.38
21	Caryophyllene oxide	27.17	0.38
22	Germacrene D-4-ol	28.62	0.31
23	Tetra Decanoic acide	30.52	0.34

Ekundayo [19], published a review of the volatiles in a number of *Annocaceae* species including *X. aethiopica* and reported that they consist mainly of mono and sequiterpernoids with typical constituents being α and β-pinene, myrcene, P-cymene, Limonene, Linalool and 1, 8–cineole.

Reports abound in literature that pinene which is the most predominant among the other compounds appears to be an important compound in the essential oil found in other regions such as Mali, Sudan, Benin Republic, Guinea and Cameroon [20,21,22,23,24]. Other compound found to be prominent in other part is 1,8-cineole.

Table 2: Anti feedant Test for showing mean mortality at different concentrations and time intervals.

Conc. (mL/g)	6hrs	24hrs
0.01	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
0.02	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
0.03	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
0.04	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}

Mean mortality (%) ± SD

Table 3: Filter Paper Test for showing mean mortality at different concentrations and time intervals.

Conc. (mL/g)	6hrs	24hrs
0.01	80.0 ± 0.6^{b}	100.0 ± 0.0^{a}
0.02	86.7 ± 1.2^{b}	100.0 ± 0.0^{a}
0.03	99.0 ± 0.0^{a}	100.0 ± 0.0^{a}
0.04	100.0 ± 0.0	100.0 ± 0.0^{a}
P	0.001	0.001
LSD (0.05)	4.33	-

Mean mortality (%) ± SD

The result in table 2 shows percentage mean mortality of acute toxicity of the essential oil of *X. aethiopica* which revealed that, the oil was toxic to *C. maculatus* after 6 and 24 hours with

100% mortality at all concentration used (0.01 – 0.04 mL/g). The percentage mortality in the filter paper method contained in Table 3 ranged between 80 and 100% after 24 hours in all the concentration.

It has been reported that mortality was due to the biologically active components in the plant products, for instance, eugenol has been found to possess high insecticidal efficacy against stored product coleopteran and the presence of this compound in Z*zgium aromaticum* as its major constituents show this insecticidal characteristic [25,26]. It has also been reported that major components of oil when in combination with other compounds of diverse structure could exhibit different mode of action against organism [27]. Therefore, pinene (α and β) and other compounds in the oil may be responsible for the activity.

Table 4: Antimicrobial Activity of *X. aethiopica* Essential Oil.

Name of Organism	Zoning	Negative Control (Distilled Water)	Positiive Control (Chloramphenicol) 50µg/ML
Escherichia coli	0.46 mm	0.0 mm	2.0 mm
Salmonella paratyphi	1.86 mm	0.0 mm	3.1 mm
Bacillus subtillis	1.23 mm	0.0 mm	3.5 mm
Staphylococcus aureus	1.73 mm	0.0 mm	3.3 mm
Klebsiella pneumonia	1.40 mm	0.0 mm	2.1 mm
Streptococcus pneumonia	1.53 mm	0.0 mm	2.1 mm
Aspergillus niger	1.66 mm	0.0 mm	2.7 mm
Aspergillus flavus	1.60 mm	0.0 mm	2.8 mm
Candida tropicalis	0.86 mm	0.0 mm	2.0 mm
Fusariumsolani	1.93 mm	0.0 mm	2.9 mm

Table 5: Minimum inhibition Concentration (MIC) of X. aethiopica Essential oil

Name of Organism	05 mL/mm	0.25 mL/mm	0.125 mL/mm	0.0625 mL/mm
Escherichia coli	+	-	-	-
Salmonella paratyphi	+	+	+	-
Bacillus subtillis	+	-	-	-
Staphylococcus aureus	+	+	+	-
Klebsiella pneumonia	+	+	-	-
Streptococcus pnuemonia	+	+	+	-
Aspergillu sniger	+	+	+	-
Aspergillus flavus	+	+	+	-
Candida tropicalis	+	-	-	-
Fusarium solani	+	+	+	-

The antimicrobial activity of *X. aethiopica* against ten pathogens among which are six bacterial and four fungi is summarized in Table 4. The result revealed that the essential oils inhibited the growth of test organisms at varying degrees with the inhibition of the pathogens growth in the order of *Salmonella paratyphi* (1.86 mm) > *Staphylococcus aureus* (1.73 mm) > *Streptococcus pneumonia* (1.53 mm) > *Klebsiella pneumonia* (1.40 mm) > *Bacillus subtillis* (1.23 mm) > *Escherichia coli* (0.46 mm) for bacterial and *Fusarium solani* (1.93 mm) > *Aspergillus niger* (1.66 mm) > *Aspergillus flavus* (1.60 mm) > *Candida tropicalis* (0.86 mm) for fungal. Hassan *et al* [28] reported that the oil of *X. aethiopic a* dissolved in methanol (1:10) showed high activity (21mm) against *S. aureus*, *P. aeruginosa* and 19 to 21mm against *A. niger* and *C. albicans*, moderate activity (15 mm) against *F. coli*, 16 mm against *B. subtillis.*

Conclusion

β-pinene (31.41%) and β-elemene (39.71%) have been found to be the major component of *X. aethiopica* in Akoko, Ondo State, Nigeria. The oil toxicity (antifeedant and filter paper) test were found to be concentration dependent and the antifeedant test showed a total mortality of 100% against *C. maculatus* for all the dosage with an exposure time of 6 and 24 hours, respectively. The present study showed that the essential oil from the plant has the potential to be used in pest control.

References

1. F. Irvine (1961). Woody plants of Ghana. Crown Agent for Overseas Admistration, London, pp. 23-24.
2. D. Fall, M. Badiane, D. Ba, P. Losieau, C. Bories, C. Gleye, A. Laurens and R. Hocquemiller (2003). Antiparasitic acivities of Senegalese Annonaceae used in traditional medicine, *Dakar* m*ed*., 48(2), 112-11.
3. A.T.S Ogunkunle and T.A Ladejobi (2006). Ethnobotanical and phytochemical studies on some species of *Senna* in Nigeria, *African Journalof Biotechnology*, 5 (21), 2020-2023.
4. N. Nadia, H. Hatil, M. Manal, Z. Kadry, H. Abdel Rasik (2013). *Xylopia aethiopica* volatile compounds protect against Panadol-induced hepatic and renal toxicity in male rats, *World Applied Science Journal*, 27 (1), 10-22.
5. A.O. Tairu, T. Hofmann and P. Schieberle (1999), Identification of the key Aroma compounds in dried fruits of *Xylopia aethiopica*. In: J. Janick (Ed.), Perspectives on New Crops and New Uses ASHS Press, Alexandria, VA. Pp. 474-478.
6. L. Lajide, P. Escoubas and J. Mitzutani (1995), Termite antifeedant activity *in Xylopia Aethiopica*, *Phytochemistry*, 40 (4), 1105-1112.
7. L.N. Tatsadjieu, J.J.E. Ngang, M.B. Ngassoum and F.X Etoa (2003), Antibacterial and antifungal activity of *Xylopia aethiopica, Monodora myristica, Zanthoxylum oxanthoxyloides* and *Zanthoxylum leprieuri* from Cameroon, *Fitoterapia*, 74: 469-472.
8. O.T. Asekun, and B.A. Adeniyi (2004), Antimicrobial and Cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria, *Fitoterapia*, 75 (3-4), 368-370.
9. G.H. Konnings, C. Agyare, and B. Ennison (2004). Antimicrobial activity of some Medicinal plants from Ghana, *Fitoterapia*, 75 (1), 65-67.
10. A. Del-Rio, B.G. Obdulio, J. Castillo, F.R. Main and A. Ortuno (1997). Uses and Properties of *Citrus flavonoids. Journal of Agriculture and Food Chemistry*, 45, 4505-4515.
11. M. Iwu (1993), Handbook of Africa Medical Plant, CRC press, New York, PP. 435.
12. P. Shanmugam, W.D. Anura, D.N. Karunaratne, G. Carr, D.S.A. Wijesundara, R. Andersen and V. Karunaratne (2008). Antioxidant

constituents from *Xylopia championi. Pharmaceutical Biology*, 46 (5), 352-355.
13. H. Kouninki, E. Haubruge, F.E. Noudjou, G. Lognay, F. Malaisse, M.B. Ngassoum, A. Goudoum, P.M. Mapongmetsem, L.S. Ngamo, L.S and T. Hance (2005). Potential use of essential oils from Cameron applied as fumigant or contact insecticides against *Sitophilus Zeamais* motsch. (*Coleoptera: Curculionidae) Commun, Agriculture And Applied Biological Sciences*, 70 (4), 787-792.
14. H. Kouninki, L.S.T. Ngamo, T. Hance, M.B. Ngassoum (2007), Potential use of Essential oils from Cameroon plants for the control of red flour weevil *Tribulium castaneum* (Harbst.) (*Coleoptera: Tenebrionidae), African Journal of Food Agriculture and Nutrition Development*, 7 (5), 1- 15.
15. N.S.E. Lale (1991). The biological effects of three essential oils on *Callosobruchus maculatus* (F), *Journal African Zoology*, 105 (5), 357-362.
16. H.C.F. Su (1976). Toxicity of a chemical component of lemon oil to cowpea weevils, *J. Georgia Entomol*. 11 (4), 298-301.
17. D.A. Ukeh, E.E. Oku, I.A. Udo, A.I Nta, and J.A. Ukeh (2012), Insecticidal effect of fruit extracts from *Xylopia aethiopica* and *Dennettia tripetala* (Annonaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae), *Chilean Journal of Agricultural Research*, 72(2), 195-200.
18. D.E.U. Ekong and A.U. Ogan (1968). Constituents of *Xylopia aethiopica*; Structure of xylopic acid, diterpene acid, *Journal of Chemical Society, C*, pp: 311.
19. O. Ekundayo (1989). A review of the volatiles of the *Annonaceae. Journal of Essential Oil Resarch*, 1: 223.
20. B. Keita, L. Sidibe, G. Frigueredo and J.C. Chalchat (2003), Chemical composition of the essential oil of *Xylopia aethiopica (Dunal) A. Rich* from Mali, *Journal of Essential Oil Research*, 15(4), 267-269.
21. H.H. EL-kamili and H.O. Adam (2009). Aromatic Plants from the Sudan: Part II. Chemical compositon of the essential oil of *Xylopa aethiopica* (Dunal) A. Rich-Existence of chemotype species, *Advance in Natural Applied Sciences, 3(2), 166-169.*
22. A.M. Ayeodun, B.S. Adeoti, P.V. Sossou and P.A. Leclerq (1996). Influence of fruit conservation methods on the essential oil composition of *Xylopia aethiopica* (Dunal) A. Richard from Benin, *Flavour and Fragrance Journal*, 11, 245-250.
23. F. Tomi, J. Casanova and M. Nianga (1996). Identification of the seed oil of *Xylopia aethiopica* from Guinea using ^{13}C-NMR Spectroscopy, *Journal of Essential Oil Research*, 8, 429-431.
24. L. Jirovetz, G. Buchbauer and M.B. Ngassoum (1997), Investigation of the essential oils from the dried fruits of *Xylopia aethiopica* (West African "peppertree") and *Xylopia parviflora* from Cameroon, *Ernahrung*, 21 (7-8), 324-325.
25. P. Schanenberg and K. Paris (1977), Guide to Medical Plants. Butter worth, London, 76-78
26. A. Olonisakin, M.O. Oladimeji and L. Lajide (2006). Bioactivity of steam distilled oils against cowpea bruchid, *callosobruchus maculatus* (F) infesting stored cowpea seeds. *Pakistan Journal of biological Sciences*, 9(7), 1271 – 1275.
27. G. Jilani and H.C.F. Su (1983), Laboratory studies on several plant materials as insect repellents for protection of cereal grains, *Journal of Economic Entomology, 76,154-156.*
28. A.M. Hassan, A.Z. Almagbou and H. Abdelgadir (2014). Antimicrobial activity of the fruits essential oil of *Xylopia aethiopica* (Dun.) A. Rich, *Advancement in Medicinal plant Research*, 2 (4), 58-61.

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