VOLATILE OIL COMPOSITION AND BIOACTIVITY OF *Hyptissuaveolens*(L)SEEDSFOUND IN AKOKO REGION OF ONDO STATE, NIGERIA

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: August 19, 2017 **Accepted:** October 08, 2017

Abstract: The chemical composition of the volatile oil obtained by hydro-distillation using all glass Clevenger apparatus from *Hyptissuaveolens L* seeds grown in Akoko, Ondo State, Nigeria was analysed by GC/MS. The acute toxicity of the oil on *Callosobrochusmaculatus* was carried out using antifeedant and filter paper methods. The oil was also assessed for antimicrobial (antibacterial and anti-fungus) activity against ten pathogens which are three Gram positive; *Bacillus subtillis, Staphylococcus* and*Strepcoccus pneumonia,* three Gram negative; *Escherichia coli, Salmonella paratyphi*and*Klebsiella pneumonia* and four fungal; *Aspergillusniger, Aspergillusflavus, Candida tropicalis* and *Fusariumsolani*using agar diffusion method. Twenty-three volatile compounds were identified representing 93.35% of the total oil composition with 3-Dodecenal (16.41%), hexadecanoic acid (12.11%), 2-decenal (11.56%), linalool (10.78%), geraniol (7.66%), tetradecanoic acid (7.27%), germacrene D (6.48%), β-elemene (5.01%) and geranyl acetate (4.88%) as major components. The result of the insecticidal activity showed that activity is concentration dependent. For antifeedant method, it was 100% mortality at all the concentration used but the percentage mortality for filter paper method ranged between 16 and 40% for 6 h and 30 to 60% for 24 h. The oil inhibited the growth of all the bacterial except *E. coli* and *S. aureus* which showed resistance to the oil and the growth of the fungi were all inhibited at different zones of inhibition. The minimum inhibition concentration (MIC) ranged between 0.125 and 0.5 ml/ml with *K. pneumonia* (0.125 mL/mL) and *S. paratyphis* (0.25 mL/mL) and 0.5 mL/mL for other pathogens. It is concluded in this study that the plant material will serve as natural insecticide and shelf-life preservative for cowpea.

Keywords: Bioactivity, essential oil, *Hyptissuaveolens* seeds, pathogens, toxicity

Introduction

Hyptis is a genus with about 400 species, most of which are native to the tropical and subtropical regions. One of the species named *Hyptissuaveolens* (L.) Poit is naturalized in India and is capable of heavy infestations displacing native flora (Raizada, 2006). *Hyptissuaveolens* (*L*) is herbaceous aromatic plant from tropical America. In tropical Africa, it was widespread in the anthropic formations. This species, high 0.5 to 1.5 m or more, is established of a stem carrying of the opposite leaves in finely toothed edges, to which are grafted an oval limb and an inflorescence in axillaries cymes (Noudogbessi*etal*., 2013).

H. suaveolens is common occurrence along the rail tracks, road sides, foothills of open forests, forest clearing and can heavily infest wastelands particularly arid and rocky substrates. *H. suaveolens* fruits (nulets) are about 1.2 – 1.5mm long and the seeds are protected in spine burr which help in its dispersal and are slightly notched at the end. This species has showed a high variation in composition of volatile constituents and composition of essential oil according to the geographical origin and growth phase (Kodakandla*et al*., 2012; Tonzibo*etal*., 2009). Essential oils isolated from aerial parts of this plant have showed antifugal, antibacterial and wound healing activities (Morreira*et al*., 2010; Iwalokun*et al*., 2012; Chitra*etal* 2009; Sathish*et al*., 2010). The terpene content of distilled volatile oils has been known to different geographical locations and between different species of the same plant. It is well acknowledged that the *H. suaveolens*EO chemical composition and biological activity change as a function of the origin and collecting period of the plants (Tchoumbougnang*et al*; 2005). This is a common feature among secondary metabolities and from essential oils of *Lamiaceae* plants in particular. Several authors have reported that a large variability in the composition of this family is due to genetic, geographical and seasonal factors (Bayda*et al.*, 2004). Since the biological activities of EOs are composition dependent, apparently it is desirable to fully characterize these mixtures from the chemical point of view.

In this present study, the diverse range of its biological activities in relation to its chemical composition had led us to investigate the chemical composition of *H. suaveolens* seed oil obtained by hydrodistillation and the bioactivity (antimicrobial and insecticidal) of *H.suaveolens* volatile oil found in Akoko region of Ondo State, Nigeria as no chemical constituent and pharmacological characteristics of the plant volatile oil of this plant has been reported in the region.

Materials and Methods

Plant materials

The seeds of *H. suaveolens*were collected in September, 2016 at the premises of AdekunleAjasin University, Akungba-Akoko, Ondo State of Nigeria. The plant material was authenticated in the Department of Plant Science and Biotechnology of the University

Extraction of the volatile oil

Five hundred grammes (500 g) of fresh seeds free of sand and other impurities were hydrodistilled for two hours using a Clevenger apparatus. The oils were dried over anhydrous sodium sulphateand kept in a sealed sample bottle at 4ºC until analysis.

Gas chromatography/mass spectrometry analysis

The analysis of the Volatile compounds was carried out on a Hewlet Packard 6890 Gas chromatography system equipped with quartz capillary column; 30 m x 0.25 mni.d x 0.25 µm film thickness. The carrier gas was helium (1 mL/min); oven temperature, 40 to 300ºC at a rate 5ºC/min then held isothermal for 2 min. The injector port temperature was 250ºC. 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 in laboratory in on cowpea 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 each of the oil (0.01, 0.02, 0.03, and 0.04 mL) were dissolved separately in 0.5 ml of acetone. Each of the concentrations for the oil was admixed with 10g 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 (1991). Ten mixed pairs 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 HPLC grade acetone and second control was only cowpea without any treatment. Each treatment and control were replicated three times. Mortality 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 death (Su, 1976b)

Filter paper test

Bioassay on the toxicity of *H. suaveolens*essential oils against adult *C. maculates* was similar to the method described in Ukeh*et al*. (2012) 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 HPLC 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 ten 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 h at ambient temperature and relative humidity. All treatments were replicated three times for each dose of all essential oils, and account of dead weevils was made at 6 and 24 h intervals

Antimicrobial activity of essential oils

The micro-organisms used in this study were isolates collected from out patients ward of the Federal Medical Centre, Owowhose 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 (B*acillus subtillis, Staphylococcus aureus*and *Streptococcus pneumonia)* and four strains of fungi; *Aspergillusflavus, Candida tropicalis, Fusariumsolani*and *Aspergillusniger*.

Zone of inhibition

Inoculum sizes containing 10 cfu/mL for bacterial and 10 sfu/mL for fungi were used to seed already solidified Petri plates of Muller-Hinton agar. The antimicrobial activities of the oil were determined using agar well diffusion method. Ten organisms were used in all the 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 h to allow absorption of the oil into the medium after which they were incubated at 37ºC for 24 h for bacterial and 7 days for fungi.

Minimum inhibitory concentration (MIC)

A modified Macro-broth dilution technique was used in this research for MIC. Serial dilutions of the oil were carried out to give a concentration of 0.5, 0.25, 0.125, 0.0625 mL/mL. Two millilitre 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 37ºC for 24 h, while the fungal at 25ºC for 7 days. The inhibition concentrations were observed and recorded.

Results and Discussion

The volatile constituents identified, their retention time, and concentrations of the seeds are summarized in Table 1. After 3 h of hydrodistilation, the *hyptissuaveolense* seeds essential oil yield was 0.2% and twenty-three major volatile compounds were found with 3-docecenal (16.41%), hexa-decanoic acid (12.11%), 2–decenal (11.56%), linalool (10.78%), geraniol (7.66%), tetra-decanioc acid (7.27%), germacrene D (6.48%), β-elemene (5.01%) and Geranyl acetate (4.88%) as major components. The results also indicated that 24.66% were monoterpenes (0.33% monoterpene hydrocarbon and 24.33% oxygenated monoterpene) and 21.01% sesquiterpenes (17.94% sesquiterpene hydrocarbon and 3.07% oxygenated sequiterpene). Other compounds that are abnormal terpenes/terpenoids or volatile compounds found in the oil are aldehyde (27.97%) like (2-decenal and 3–dodecenal), alcohol (0.33%) (Benzyl Alcohol) and carborxylic acid 19.38% (tetra decanoic acid and hexadecanoic acid).

The results from other countries revealed β-caryophyllene to be major component present in the leaves oil of *H.suaveolens* found in Indonesia (Chatri*et al*., 2014), India (Mandal*et al*., 2007; Sharma, *et al*., 2007), Malaysia (Din *et al*., 1988) and in Australia (Peerzada, 1997) but the results of this study indicates that β-caryophyllene and its oxygenated derivatives has 3.20%.

Table 1: Chemical composition (%) of *H. suaveolens* seed volatile oil

S/N	Compound name	Retention time	Composition (%)
1	Benzyl Alcohol	11.55	0.33
2	Trans Ocimene	12.22	0.33
3	Geraniol	14.90	7.66
4	Camphor	15.03	0.35
5	Neral	15.51	0.34
6	Citronellol	17.02	0.32
7	Linalool	17.68	10.78
8	Geranyl Acetate	21.47	4.88
9	β-bisabolene	21.69	0.36
10	β-Caryophyllene	22.05	0.86
11	α-humulene	22.36	1.35
12	β-elemene	22.59	5.01
13	Δ-cadinene	23.44	0.40
14	A-cadinene	23.49	0.54
15	α-cadinol (torreyol)	24.25	0.34
16	Germacrene D	24.40	6.48
17	Bicyclogermacrene	24.80	2.94
18	Spathulenol	26.00	0.39
19	2-Decenal	26.58	11.56
20	3-dodecenal	28.25	16.41
21	Caryophyllene oxide	28.45	2.34
22	Tetra Decanoic acid	30.57	7.27
23	HexaDecanoic acid	31.08	12.11

However, the concentration of Germacrene-D (6.48%) recorded in this present study was also reported by Chatri, *et al*, 2014 to be present in the leaves oil of. *H suaveolens* found in Indonesia with 10.32% concentration and in Vietnam (Van Hac*etal*, 1996). This shows that both the seeds and leaves have some similar components in nature.

The results in Table 2 shows the percentage means mortality of acute toxicity of essential oil of *H. suaveolens* seeds against *C. maculatus* after 6 and 24 h. There was 100% mortality recorded after 6 and 12 hours, respectively. The ability of the

plant oil to cause mortality of adult beetle on grains can be attributed to contact toxicity of the oil on the beetle. It was thus observed from the result obtained in this study that the oil of *H. suaveolens* seed possesses high mortality rate on the stored bean weevil (*C. maculatus*).

Table 2: Acute toxicity of essential oil of *Hyptissuaveolens* against *Callosobrochusmaculatus*after 6 and 24 h of application

Conc. Ml/5g seed	Mean Mortality (%) ± SD		
	Control	6h	24h
0.01	0.00	100 ± 0.0[a]	100.0 ± 0.0 [a]
0.02	0.00	100 ± 0.0 [a]	100.0 ± 0.0 [a]
0.03	0.00	100 ± 0.0 [a]	100.0 ± 0.0 [a]
0.04	0.00	100± 0.0 [a]	100.0 ± 0.0 [a]
P	-	0.001	0.001
LSD	-	-	-

Table 3: Toxicity (filter paper) test of essential oil of *Hyptissuaveolens*oil against *Callosobrochusmaculatus* after 6 and 24 h

Conc. Ml/5g seed	Mean Mortality (%) ± SD		
	Control	6h	24h
0.01	0.00	16 ± 0.7[d]	30.0 ± 1.2[d]
0.02	0.00	23.3 ± 0.3[c]	36.7 ± 1.2[c]
0.03	0.00	33.3 ± 0.7[b]	53.3 ± 0.9[b]
0.04	0.00	40.0 ± 0.6[a]	60.0 ± 0.0[a]
P	-	0.001	0.001
LSD	-	4.00	5.33

The results in Table 3 showed the percentage toxicity of *Hyptissuaveolens* oil against *C.maculatus* using filter paper. The results revealed that the mortality rate is concentration dependent with an increased in mortality rate in the order of 0.01 (16.7 and 30.0%) < 0.02 (23.3 and 36.7%) < 0.03 (33.3 and 53.3%) < 0.04 ml/cm^2 (40.0 and 60.0%) after 6 and 24 hours, respectively.

The oil of hyptis effected insecticidal properties by killing adult *C. maculatus* over the period of exposure and even though the lethal activity seems to be dose dependent. Effects of plant materials as crop seeds protectants have also been observed in the treatment of cowpea and maize weevils where adult insects were killed through contact toxicity (Ofuya and Dawodu, 2002; Asawalam*et al*., 2007). Lale and Mustapha (2000) has reported the reduction in the hatchability of the weevil's eggs laid on cowpea seeds. Previous work on components of some bioactive plant species also showed that they caused mortality, oviposition, deterrence and or ovicidal action resulting in reduced progeny production of stored product insects (Asawalam, 2007; Oni, 2014). Musa (2008) in his study reported that the toxicity of *H. suaveolens* seed extract on eggs and adult emergence varies with concentrations and that the active ingredients present in the test plant may be responsible for the observed reduction in number of eggs and adult emergence. The modes of action of the active ingredients probably blocked the respiratory pore of the eggs and toxic to the immature stages and adults of *C. maculatus*. The toxicity of *H. suaveolens* seed extract probably showed non-persistence with increase in period of exposure. It has been observed that active pesticidal principles of plant origin do not persist in the environment for a long time (Wink, 1993). The result of present study corroborates the findings that *H. suaveolens* possesses insecticidal properties (Rao*etal*., 1990). The compounds present in *H. suaveolens* act by deterring insects from feeding, others kill on contact with insects (Simmond and Blaney, 1992). The insecticidal activities of petroleum ether extract of *H. suaveolens* seeds on second instar larvae of the Diamond back moth, *Plutellaxylostella* (*L*.) was reported (Keita *etal*., 2006).

The results in Table 4 revealed that the essential oil in the seed inhibited the growth of the test organisms at varying degrees. The order of activity is *Klebsiella pneumonia* (1.7 mm) >*Salmonellaparatyphi* (1.1 mm) > *Streptococcus pneumonia* (0.8 mm) >*Bacillus substillis* (0.3 mm) while *Escherichia coli* and *Staphylococcus aureus* showed no activity for bacteria and the fungal activity ranged between 0.6 and 0.9 mm.

Table 4: Anti-microbial activity of *Hyptissuaveolens* seed oil against some pathogens

Name of Organism	Zone of Inhibition (mm)	Negative control (Distilled Water) (mm)	Positive control (Chloramphenicol) 50 mg/mL
Escherichia coli	0.00	0.0	2.0mm
Salmonella paratyphi	1.1	0.0	3.1mm
Bacillus subtillis	0.3	0.0	3.3mm
Staphylococcus	0.0	0.0	3.1mm
Klebsiella Pneumonia	1.7	0.0	2.6mm
Streptococcus pneumonia	0.9	0.0	2.6mm
Aspergillusniger	0.8	0.0	2.9mm
Aspergillusflavus	0.7	0.0	2.9mm
Candida tropicalis	0.6	0.0	1.9mm
Fusariumsolani	0.8	0.0	3.0mm

Table 5: Minimum inhibition concentration (MIC) of*Hyptissuaveolens* seed oil

Name of organism	0.5 mL/mL	0.25 mL/mL	0.125 mL/mL	0.0625 mL/mL
Escherichia coli	-	-	-	-
Salmonella paratyphis	+	+	-	-
Bacillus Subtillis	+	-	-	-
Staphylococcus aureus	-	-	-	-
Klebsiella pneumonia	+	+	+	-
Strepococcus pneumonia	+	-	-	-
Aspergillusniger	+	-	-	-
Aspergillusflavus	+	-	-	-
Candida tropicalis	+	-	-	-
Fusariumsolani	+	-	-	-

+ = inhibition potential against the microorganism; - = no inhibition potential against the microorganism

The results of the Minimum Inhibition Concentration (MIC) are represented in Table 5. The range values of 0.5, 0.25, 0.125 and 0.0625 mL/mL were carried out on the pathogens with the test oil showing no inhibition against the pathogens at 0.0625 ml/ml. At 0.125 ml/ml, no inhibition was shown except for Gram (-) *K. pneumonia* and at 0.25 ml, inhibition was shown against two Gram (-) *S. paratyphi* and *K. pneumonia* while the highest rate of inhibition against the pathogens was recorded at MIC value of 0.5 mL/mL except for a Gram (+), *E. coli* and Gram (+) S.*aureus*, *E. coli* showed resistance against the oil at all concentrations. Mozhiyarasi and Anuradha, (2016) reported higher values from aqueous, ethanol, methanol and chloroform extracts of *H. suaveolens* (*L*.) Poit from India showed (6 and 9 mm), (13 and 14 mm), (14 and 10 mm) and (15 and 13 mm) in diameter of inhibitory zone against both *E. coli* and *S. aureus*, respectively

Conclusion

From this study, 3-dodecenal (16.41%), hexadecanoicacide (12.11%), 2-decenal (11.56%) and Linalool (10.78%) have been found to be the major components of *H. suaveolens*seeds oil in Akoko, Ondo State, Nigeria. The acute toxicity (anti-

feedant) test showed that *C. maculatus* was susceptible to the oil at each dosage and the filter paper test showed that the toxicity effect on *C. maculatus* is concentration dependent. The present study, therefore shows that essential oil from this plant could have potential application in insect pest control and preservative as it was found to inhibit the growth of some pathogenic organisms.

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