Composition, Insecticidal and Antimicrobial Activity of *Hyptis suaveolens (L)* Aerial Parts

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ABSTRACT

Chemical composition of the essential oils (EO) obtained by hydro distillation from aerial parts of *Hyptis suaveolens* grown in Akoko region of Ondo State, Nigeria, was analyzed by gas chromatography-mass spectroscopy technique. Toxicity tests of the essential oil using antifeedant and filter paper methods against *Callosobruchus maculatus* were carried out. The essential oil was also tested against ten pathogens among which were six bacteria; *Escherichia coli, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia and Streptococcus pneumonia* and four fungi: *Aspergillus niger, Aspergilus flavus, Candida tropicalis and Fusarium solani*. The results showed that the total essential oil composition were 91.61% with β-elemene (39.71%), γ-elemene (8.82%), bicyclogermacrene (8.52%), Germacrene D (7.02%), α-cadinol (Torreyol) (4.22%), β-caryophyllene (3.63%), α-cadinene (2.31%) and δ -elemene (2.02%) as prominent compounds. The toxicity and repellency were found to be concentration dependent after 6 h and 24 h of applications. 100% mortality was recorded for antifeedant method at all concentration except at 0.01 mL/g. The mortality ranged between 16 and 66% at 6 h; and 27 to 70% at 24 h in filter paper method. The results obtained also showed that *H. suaveolens* EO only inhibited the growth of three bacteria in the order of *B. subtilis* (1.60 mm) > K. *pneumonia* (1.36 mm) > S. *paratyphi* (0.63 mm) and also showed inhibition against all the four fungi in order of *F. solani (1.10 mm)* > *A. niger* (0.83 mm) > *C. tropicalis* (0.80 mm) > *A. flavus* (0.73 mm). The MIC ranged between 0.5 mL/mm and 0.125 mL/mm for the ten pathogens except for *E.coli* and *S.aureus* which showed resistance to the oil.

Keywords: *Callosobruchus maculatus*, chemical composition, hydro-distillation, *Hyptis suaveolens*, pathogens, toxicity.

INTRODUCTION

Chemical constituents in the plant are responsible for their medicinal as well as their toxic properties [1]. They are inorganic and organic substances that could be obtained in both primary and secondary metabolic processes. They also provide a source of traditional medicine since the earliest time. The bioactive constituents of plants such as tannins, flavonoids [2], terpenoids [3] and alkaloids [4] have great antimicrobial activity. Plants in all facets of life have served as valuable starting material for drug development [5]. In traditional medicine the use of medicinal plants still play a vital role to cover the basic health needs in the developing countries. Also, the use of herbal remedies has risen in the developed countries in the last decade.

Many internal and external factors can compromise the qualitative and quantitative characteristics of stored grains even after drying. Among these factors, pest insects stand out, which, besides attacking many crops at developmental stages at the field, also damage the stored grains. These are cross-infestation pests [6]. The mean quantitative losses caused by pests in Brazil are estimated at approximately 10 % of the total produced annually. This represents about 9.8 million tons per year, according to FAO and the Brazilian Ministry of Agriculture livestock and supply [7].

Different spice and herbal plant products in the form of essentials oils, powders, pellets, extracts or distillates could be harnessed as potential toxicants, deterrents, antifeedants, repellents and anti-fumigants for exclusion of stored-product pests from grains. The present study therefore seeks to determine the chemical composition and the bioactivity such as insecticidal and antimicrobial of *Hyptis suaveolens* aerial parts leaves found in Ondo State, Nigeria.

MATERIALS AND METHODS

Plant materials

The aerial parts of *H. suaveolens* aerial parts for this study were collected in September, 2016 at the premises of Adekunle Ajasin University, Akungba-Akoko, Ondo State of Nigeria. The plants were authenticated in the Department of Plant Science and Biotechnology.

Isolation of the volatile oil

Fresh aerial parts of the plants were washed free of sand and other impurities and cut into small pieces. 500 g was hydro-distilled for three hours using a Clevenger type apparatus. The oils were dried over anhydrous Na_2SO_4 and kept in a sealed sample bottle at 4 ^oC until analysis.

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 of 30 m length x 0.25mm i.d x 0.25µm 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 electron ionization mode (70 eV). 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*) 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 the experiments.

Antifeedant test

Four concentrations of each oil (0.01, 0.02, 0.03, and 0.04 mL) were dissolved separately in 0.5 mL of analytical grade acetone. Each concentration 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 until the acetone completely evaporated according to the method of Lale, [8]. 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 (solvent control) and the second control was only cowpea without any treatment (normal control). The solvent control and the normal control were prepared in triplicate. Mortality was taken at 6 h and 24h

interval after introducing insects on the seeds. Insects which did not respond to the gentle touch of a small probe were considered death [9].

Filter paper test

Bioassay on the toxicity of *H. suaveolens* essential oils against adult *C. maculates* was similar to the method described by Ukeh *et al* [10] 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 No. 1 filter paper. Pure acetone was used for the filter paper as control. The solvent was allowed to evaporate and 5 mixed pairs of *C. maculates* adults were introduced into each petri dish. The petri dishes were closed and maintained in the laboratory for between 6 h and 24 h at ambient temperature and relative humidity. All treatments were done in triplicate for each dose of all essential oils, and account of dead weevils was made at 6 h and 24 h intervals.

Antimicrobial activity of essential oils

The micro-organism used in this study were isolates collected from Out-patients' ward at 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 included 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 *Aspergillus niger.*

Zone of inhibition

Inoculam size containing 10 cfu/mL for bacteria 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 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 there were allowed to stand for about 2 h to allow absorption of the oil into the medium after which they were incubated at 37^{o}C for 24 h for fungus and bacteria 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 concentrations of 0.5 mL/mL, 0.25 mL/mL, 0.125 mL/mL, 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 which were mixed properly and allowed to set. After which the standardized Inoculam were seeded on the plates. The bacterial plates were incubated at 37 ^{o}C for 24 h, 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 *Hyptis suaveolens* leaves in Table 1 after 5 h of hydro-distillation yielded 1.0% v/w. The chemical composition of the volatiles revealed that the oil is rich in sesquiterpenes with the presence of 91.29% (86.41% sesquiterpene hydrocarbon and 4.88% oxygenated sesquiterpene). The chemical composition of *H. suaveolens* essential oil showed total 16 components with β-elemene (39.71%), γ-elemene (8.82%), bicyclogermacrene (8.52%), Germacrene D (7.02%), α-Humule (7.38%), Germacrene B (5.47%), α-cardinol (torreyol) (4.22%), β-caryophyllene (3.63%), α-cadinene (2.31%) and δ -elemene (2.02%) as major components.

H. suaveolens is a chemotype, because there are differences in components and compositions of its essential oils from several results of researches conducted in different areas and countries [11].

Table 1: Chemical composition (%) of *H. suaveolens* essential oils

S/N	Compound Name	Retention time	Composition (%)
1	Camphor	15.04	0.32
2	Germacrene B	19.23	5.47
3	β-bisabolene	21.67	0.30
4	β-caryophyllene	22.01	3.63
5	α-Humulene	22.36	7.38

6	β-elemene	22.60	39.71
7	α-farnesene	22.99	0.31
8	δ-cadinene	23.41	0.92
9	α-cadinene	23.62	2.31
10	δ-elemene	23.83	2.02
11	Gama Elemene	23.96	8.82
12	α-cadinol (torreyol)	24.18	4.22
13	Germacrene D	24.47	7.02
14	Bicyclogermacrene	24.79	8.52
15	Spathulenol	26.28	0.33
16	Caryophyllene oxide	27.16	0.33

In India, the major components of essential oils from H. *suaveolens* were 1, 8 cineole (44%), β-caryophyellene, β-pinene and camphene [12]. Van Hac *et al* [13] investigated chemical composition of essential oils from Vietnam with eugenol and Germacrene-D as the main constituents identified. These notable variations maybe as a result of different characteristics of the plants, species chemotype, climatic, soil conditions, geographical locations and the methods of extraction [14 - 17].

Table 2: Anti feedant test for *H. suaveolens* aerial parts against *C. maculatus*

Conc. (ml/g)	**6h**	**24h**
0.01	90.0 ± 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 *H. suaveolens* aerial parts against *C. maculatus*

Conc. (ml/g)	**6hrs**	**24hrs**
0.01	16.7 ± 0.7^{c}	27.7 ± 0.67^{d}
0.02	23.3 ± 0.3^{b}	36.7 ± 1.2^{c}
0.03	30.0 ± 1.2^{b}	53.3 ± 0.9^{b}
0.04	66.7 ± 1.2^{a}	70.0 ± 0.0^{a}
P	0.001	0.001
LSD (0.05)	9.67	6.67

Mean mortality (%) ± SD

The result in Table 2 shows percentage mean mortality of acute toxicity of the essential oil of *H. suaveolens* which revealed that the oil was toxic to *C. maculatus* after 6 h and 24 h exposure times with 100% mortality at all concentration used (0.01- 0.04 mL/g) except for 0.01 mL/g at 90 % mortality after 6 h exposure time. In the filter paper method in Table 3, the mortality ranged between 16.7 and 70% after 6 h exposure and 24 h exposure in all concentrations.

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 *Syzgium aromaticum* as its major constituents shows this insecticidal characteristic [18, 19]. It has also been reported that major components of oil when in combination with other compounds of diverse structure in the oil could exhibit different modes of action against organism [20]. Therefore, elemene (β and γ) and other compounds in the oil may be responsible for the activity.

Table 4: Anti bacteria activity of *H. suaveolens* essential oils against some pathogens (zone of inhibition (mm))

Name of Organism	Zone of inhibition	Negative control (Distilled Water)	Positiive Control (Chloramphenicol) 50µg/ml
Escherichia coli	0.00	0.00	2.20
Salmonella paratyphi	0.63	0.00	3.10
Bacillus subtillis	1.60	0.00	3.50
Staphylococcus aureus	0.00	0.00	3.20
Klebsiella pneumonia	1.36	0.00	2.70
Streptococcus pneumonia	0.00	0.00	3.00
Aspergillus niger	0.83	0.00	3.00
Aspergillus flavus	0.73	0.00	2.70
Candida tropicalis	0.80	0.00	2.10
Fusariumsolani	1.10	0.00	2.90

Table 5: Minimum inhibition concentration (MIC) of *H. suaveolens* essential oil

Name of Organism	0.5 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	-	-	-	-
Aspergillus niger	+	-	-	-
Aspergillus flavus	+	-	-	-
Candida tropicalis	+	-	-	-
Fusarium solani	+	-	-	-

The antimicrobial activity of *H. suaveolens* against ten pathogens among which are six bacteria and four fungi is summarized in Table 4. The results revealed that the essential oils inhibited the growth of the test organisms at varying degrees with the inhibition of the pathogens growth in the order of *Bacillus subtillis* (1.60 mm) > *Klebsiella pneumonia* (1.36 mm) > *Salmonella paratyphi* (0.63 mm) against bacterial with *Escherichia coli* and *Streptococcus pneumonia* showing resistance against the oils. For fungi, it was *Fusarium solani* (1.10 mm) > *Aspergillus niger* (0.83 mm) > *Candida tropicalis* (0.80 mm) > *Aspergillus flavus* (0.73 mm).

Mozhiyarasi and Anuradha [21] reported that the aqueous extract of *Hyptis suaveolens* (L.) Poit showed 6 mm in inhibitory zone against both *Escherichia coli* and *Staphylococcus aureus* and showed no inhibitory activity against both *Aspergillus niger* and *Aspergillus flavus*. Also, the ethanolic extract zone of inhibition was found to be 13 mm and 10 mm against *Escherichia coli* and *Staphylococcus aureus* in their report. Table 5 shows the MIC of the oil, which range between 0.5 mL/mm and 0.125 mL/mm for the seven pathogens, the remaining organisms- E *coli*, S. *aureus* and S. *pnuemonia* show resistance to the oil

CONCLUSION

β-elemene (39.71%), ϒ-elemene (8.82%), bicyclogermacrene(8.52%), α-humulene(7.38%) were found to be the major components of *H. suaveolens* in Akoko, Ondo State, Nigeria. The oil's acute toxicity using antifeedant and filter paper test methods were found to be concentration dependent and the antifeedant test showed 100% mortality against *C. maculatus* in all concentrations except at 0.01mL/g at 6 hr exposure time. The percentage mortality of filter paper method ranged between 16.7 and 70%. The results obtained also showed that H. *suaveolens* EO inhibited the growth of three bacteria pathogens: B. *subtilis*, K. *pneumonia*, S. *paratyphi* and all the four fungi. The present study therefore showed that the essential oils from the leaves of *H. suaveolens* in Akoko, Ondo State, Nigeria have the potential to be used in pest control and also as preservative for stored grains as they inhibited some pathogenic organisms.

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