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Mosquito-repellent activities of a north central Nigeria local *Hyptis suaveolens* Essential oil and its toxicity evaluation in mice

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ABSTRACT: **Aim**: Mosquito-repellent activities of *Hyptis suaveolens* essential oil (EO) obtained from Kwara State, north central Nigeria; and its toxicity in mice were evaluated. **Materials and Methods**: *Hyptis suaveolens* plants were collected from University of Ilorin premises. Fresh leaves were weighed, pound, hydrodistilled and the EO characterised using GCMS. Mosquitoes (female *anopheles and culex)* 150 were bred from larva stage in the laboratory against which the repellency activities were determined. Fifteen (15) adult mice with the average weight of (25 ±2.21 g) were randomly assigned into three (3) groups (A-C), of five (5) mice each. Daily administration of distilled water, EO 100 mg/kg body weight and 500 mg/kg body weight were done oropharyngeally for seven days to groups A, B and C respectively. The mice were sacrificed, and the blood, liver and kidney of the animals were collected. Blood, tissues, and serum parameters were assayed for in the mice. **Results**: Caryophyllene oxide, caryophyllene, spathulenol, alloaromadendrene, benzaldehyde and bornanone were some of the compounds confirmed present in the EO. The EO in water (1:99) is 100% efficacious, for up to 60 minutes. The EO induced significant increase ($p<0.05$) the blood levels of WBC, RBC, HCT, HGB in all treated groups. Serum albumin, total and direct bilirubin, and the total protein in all the treated groups were significantly reduced while no significant difference in the activities of ALP, ALT and AST in the liver, kidney and serum of treated groups occurred when compared with the control. The levels of the serum urea and creatinine, increased significantly in all the treated groups ($p<0.05$). **Conclusion:** The *Hyptis suaveolens* essential oil possesses mosquito-repellent activities but may cause adverse on the enzymatic and haematological, liver and kidney functions at 500 mg/kg body weight in mice.

Key words: *Hyptis suaveolens*, essential oil, GC-MS, insect-repellent, mosquito

Introduction

Mosquitoes (*Diptera: Culicidae)*

Mosquitoes (*Diptera: Culicidae*) are one of the oldest enemies to humans and represent a significant threat to human health because of their ability to vector parasites that cause diseases that afflict millions of people worldwide (WHO, 2010) and they constitute a major public health problem as vectors of serious

human diseases (El Hag *et al*.,1999). The World Health Organisation (WHO) has declared mosquitoes "Public enemy number one" because they are responsible for the transmission of various dreadful diseases (Vinson, 1997). Different species of mosquitoes belonging to the genera *Aedes, Anopheles* and *Culex* are vectors for the pathogens of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encepahalitis and filariasis (Gubler, 1998). Mosquitoes are the most important vectors of certain human infections and diseases. Mosquito-borne diseases such as malaria, yellow fever, filariasis and dengue contribute significantly to mortality in most tropical countries. Among these diseases, malaria continues to dominate the public health spectrum especially in Africa continent where *Anopheles gambiae sensulatu* (*Diptera: Anophelidae*) complex occurs in endemic regions. This species complex is the vector of *Plasmodium*, the aetiologic organism of malaria fever which annually kills between 1.4 to 2.7 million of the estimated 300 – 500 million clinical cases (Snow *et al.*, 1999). Most of these deaths occur among African children and pregnant women living in endemic countries of sub-Saharan countries of stable malaria transmission. Plant products have been used traditionally by human communities (Darmagadda *et al.*, 2005). The plant world comprises a rich store house of phytochemicals, which are widely used in the place of synthetic insecticides. Use of synthetic insecticides has been reported to cause side effects to non-target organisms and develop insecticide resistance against mosquitoes (Rajkumar, 2004).

The control of mosquito-borne diseases is however becoming increasingly difficult because the effectiveness of vector control has declined due to development of resistance by vectors against the currently used but toxic and environmentally persistent organochlorine (DDT and Lindane), organophosphorus (malathion), carbamates (carboxyl) and pyrethroid insecticides (Sadasivaiah *et al.*,2007). In the absence of effective prophylactic vaccine against most of the mosquito-borne diseases, it is desirable to find compounds that can effectively control mosquitoes with minimal damage to the environment. Naturally occurring phytocompounds that are rich sources of bioactive chemicals appear to be the most likely candidates for the environmentally safe and degradable products targeted specifically against mosquitoes. So far research workers have identified a wide variety of plant species from various ecosystems that have produced a range of acute and chronic toxic effects against mosquitoes (Ghosh et al, 2012). Currently more than 2000 plant species have been identified as having insecticidal properties and about 344 plant products are known to possess anti-mosquito characteristics (Sukumar *et.al.,* 1991). Several researchers have also demonstrated ovicidal and larvicidal activities of various plant extracts (Lee, 2000; Kabaru and Gichia, 2001; Prajapati, 2005; Chansang *et al.*, 2005). The increasing resistance of malaria parasites and mosquitoes to available drugs and insecticides, respectively has created a growing interest in herbal remedies in the hope of identifying new leads for antimalarial and repellent drug development.

Plants are known to produce secondary metabolites that are found to be physiologically active and have been used for medicinal purposes for centuries. It is also known that the activity of aromatic repellent plants is due to essential oils present in the plant material and that are used as flavor in food products, odorants in flagrances, pharmaceuticals and as insecticides. Essential oils are natural volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes (Chansang et al.,2005). Mosquito repellent activity has been found in various plant extracts; among them *Azadirachta indica* A. Juss (neem tree); *Ocimum basilicum* L. (basil oil) and *Citronella* species (Kweka *et al.*, 2008). Phytoproducts on account of minimal hazardous effect on the environment and wide range of availability offer promises in future mosquito control programmes. They have revolutionized the fields of vector control as they possess different bioactive components and can be used as general toxicants against various larval stages. Lamiaceae have traditionally been used in developing countries for their insecticidal and repellent properties against several insect species (Ngamo *et al.* 2007). *Rosmarinus officinalis* L. and *Lavandula angustifolia* Millller (Lamiaceae) EOs showed moderate larvicidal activity (Conti *et al.*, 2010) but a noticeable repellent and ovicidal effect against several mosquito species (Prajapati *et al.* 2005). Lamiaceae species of the *Hyptis* genus which included more than 400 species are highly aromatic and grow in tropical regions, mainly in Africa and America. Several studies have shown that Hyptis suaveolens (L.) Poiteau EO has useful insecticidal properties against mosquitoes (Amusan *et al.* 2005) and many stored products pests (Conti *et*

al. 2010). Moreover, its chemical composition and biological activity change as a function of plants origin and their collecting period (Rates, 2001). As further studies are important to improve the knowledge of new plant extracts and their pure constituents for their use against mosquito species, this study investigates the chemical composition of H. suaveolens EO, extracted from plants obtained in the University of Ilorin premises, its mosquito-repellent activities against *culex* and female *anopheles* mosquites and toxicity evaluation in mice were determined.

Materials and Methods

Hyptis suaveolens plants were collected around University of Ilorin premises. The plants were identified at the Herbarium Unit of Department of Plant Biology, University of Ilorin, Ilorin Kwara State, Nigeria, and the Voucher number UILH/001/610 was issued.

Experimental Animals

Fifteen (15) adult mice with the average weight of (25 ± 2.21 g) were obtained from the Animal Holding unit of Biomedical Nigeria Limited, Ilorin, Nigeria. The mice were kept in well-ventilated housing with the temperature of 28-30°C, photo period; 12 hours light, 12 hours dark; humidity: 45-55% and were fed with standard pelletized feed and water before and during administration of extract. They were acclimatized for two weeks prior to the experiment.

Animal Grouping and Administration of Extract

Fifteen (15) adult mice were randomly assigned into three (3) groups (A-C), of five (5) mice each. Daily administration of distilled water (to the control) and the essential oil of *Hyptis suaveolens* was done oropharyngeally by using oral cannula for seven days as follows: -

Group A- (Control mice) received distilled water. Group B- received 100 mg/kg body weight of essential oil (100 x dilution). Group C- received 500 mg/kg body weight of essential oil (100 x dilution in distilled water).

Collection of Blood

At the end of the experimental period, the mice were individually weighed and then anaesthetized in a jar containing cotton wool soaked with diethyl ether. The neck area was quickly cleared of fur and skin to expose the jugular veins. The jugular vein was slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The mice were held with the head downwards and allowed to bleed into a clean anticoagulant-free sample bottle.

Isolation of Organs

Under light anaesthesia, the mice were quickly dissected using clean and sterile dissecting sets. Organ of interest (liver) was excised, cleaned of blood strains, weighed again and transferred into 0.25 M sucrose solution (an isotonic solution).

Preparation of serum and tissue homogenates

The organs were homogenized separately in ice cold 0.25M sucrose solution (1:5 w/v) according to procedure described by (Akanji and Ngaha, 1989) [20]. The homogenates were frozen overnight to allow cell lysis and ensures maximum release of enzymes, then further centrifuged at 33.5 revolutions for 15 minutes to obtain the supernatants which were then carefully collected into samples and used for the various biochemical assays. For the blood sample, they were also centrifuged at 22.5 revolutions for 15 minutes to get the supernatant (serum). The serum was also used for various biochemical assays.

Analytical Techniques

Biochemical analyses were carried out to determine the serum concentrations of total protein, albumin, conjugated and total bilirubin, and the activities of liver enzymes-AST, ALT and ALP using diagnostic kits (Randox kit USA). Total protein was determined by the Biuret method (Gornall et al., 1949) [21], albumin by the bromocresol green method (Doumas *et al.*, 1971) [22], bilirubin was estimated by the method described by Jendrassik and Grof (1938) [23]. Alanine and aspartate aminotransferases were determined based on the colourimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957) [24], alkaline phosphatase by the phenolphthalein monophosphate method (Wright *et al.*, 1972) [25]. Haematological analysis were performed using an automated haematological analyser (SYSMEX-KX21) supplied by SYSMEX Corporation, Hyogo, Japan) at the University of Ilorin Teaching Hospital (UITH).The parameters of the blood samples were measured such as: hematocrit (Hct), hemoglobin (Hb), red blood cells (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), % neutrophil (%N), % lymphocyte (%L), % monocyte (%M), % eosinophil (%E), % basophil (%B) and platelet

Essential oil extraction and analysis

Fresh leaves were weighed, pound and hydro distilled in a Clevenger type apparatus for two hours as described by the British Pharmacopoeia (2008) [26]. The oil collected was stored at 4ºC to 6ºC until it was used.

The GC-MS analysis of the Essential oil of *Hyptis suaveolens* was conducted at the Chemical Engineering Department of University of Ilorin, Nigeria. A gas chromatogram from Agilent USA hyphenated to a mass spectrophotometer (5975) with triple axis detector equipped with an auto injector (10µl syringe) was used. Helium gas was used as carrier gas. All chromatographic separation was performed on capillary column having specification: length; 30m, internal diameter 0.2µm, thickness; 250µm, treated with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 250ºC interface temperature; 300ºC, pressure;16.2 psi, out time, 1.8mm, 1ul injector in split mode with the split ratio 1:50 with injection temperature of 300ºC the column temperature started at 35ºC for 5minutes and changed to 150ºc at the rate of 4ºC/min. The temperature was raised to 250ºC at the rate of 20ºC/min and held for 5mins.The total elution was 47.5 minutes. Ms solution software provided by the supplier was used to control the system and to acquire the data; identification of the compound was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NISTII), the name, molecular weight, and structure of the components of the test materials were recorded. The Retention indexes of the compounds obtained were calculated using the following formula: -

$$RI = \left(\frac{RI_{unknown} - RT_{alkane\ before} + \text{Number of Carbon atom in alkane before}}{RT\ alkane\ after - RT\ alkane\ before}\right) \times 100$$

Where RI is the calculated Retention Index and RT is the Retention Time

Statistical analysis

Experimental data are presented as Mean ± Standard error of mean (SEM). Statistical analysis was implemented using software SPSS 2017 full version statistical package program (SPSS, Chicago, IL) and Graphpad prism six. One way analysis of variance was used to compare variables among the different groups. Level of significance (Post hoc comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at $P<0.05$.

Mosquitoes rearing conditions

The Mosquitoes larvae were obtained from Ikokoro area, Niger Road in Ilorin South area of Ilorin, Kwara State, Nigeria between September, and December 2016. All test mosquitoes were reared into mature mosquitoes viz. *Anopheles stephensi* and *Culex quinquefasciatus* free of exposure to insecticides

and pathogens. Cyclic generation of vector mosquitoes was maintained at 25-29ºC and 80-90 percent relative humidity (RH). In the laboratory, larvae were fed on larva food (i.e., powdered dog biscuits and yeast in the ratio 3:1).

Mosquito-repellent activity determination

H. suaveolens EO repellency was evaluated using the human bait technique to simulate the condition of human skin on which repellents will be applied, as reported by Schreck and Mc Govern (1989) [27], Gleiser *et al.* (2011) [28] and Chaithong *et al.* (2006) [29]. Tests were conducted in January 2017. Groups of over 100 nulliparous, non-blood-fed, starved Female *Anopheles* and *Culex* mosquitoes (7–10 days old) were placed, to facilitate viewing in a cage. Each cage had a cotton stockinet access sleeve on the front. *Anopheles* and *culex* is a night-biting mosquito; therefore, testing period was conducted between 9:00 am and 11:00 am in the day; 6:00 pm and 11:00 pm in the night.

Eight volunteers were chosen amongst susceptible to mosquito bites and non-allergic subjects. They had no contact with lotions, perfumes, oils or perfumed soaps on the day of the bioassay. After cleaning their hands in distilled water, they protected their forearms with a thick fabric sleeve and wore a latex surgical glove, in which a dorsal square area 5×5 cm was cut open. All concentrations were replicated five times. Firstly, the control hand was exposed in the cage for 3 min, during which the number of probing mosquitoes was recorded. Immediately after, the hand was withdrawn and treated with repellent formulation; then it was re-exposed to mosquitoes in the same test cage. The number of probing mosquitoes in a 3-min exposure period was recorded. The percentage of repellency obtained from five replicates expressed as percentage protective efficacy, PE % was calculated at each dosage using this formula: PE % = [(number probing untreated hand − number probing treated hand)/number probing untreated hand] × 100 (Fradin and Day 2002) [37].

Results and Discussion

Chemical composition of *Hyptis suaveolens* Essential oil

H. suaveolens is polymorphic in its essential oil composition because many different compositions have been reported (Conti et al., 2012) [17]. Several chemotypes have been described, such as β-caryophyllene type from Nigeria (Chukwujekwu *et al.* 2005) [30], 1,8-cineole/sabinene from India (Azevedo, et al., 2001) [31], three 1,8-cineole, α-terpinolene and fenchone/ fenchol types from El Salvador (Conti et al, 2012) [17].

The results of several studies have indicated better insecticidal activities of *H. suaveolens* compared to EO from other plants. Furthermore, most recent research had reported the occurrence of several metabolites in *H. suaveolens*. *H. suaveolens* have been found to contain high level of monoterpenes, whose toxicity mechanism (inhibition of metamorphosis of insects), have been corroborated in literature by several authors (Ohimain et al, 2015). Monoterpenes could stimulate the cellular leakage of potassium (Cox *et al.*, 2000) which results to membrane disruption and cell mortality (Sikkema *et al.*, 1994) [34].
The essential oil obtained from the *H. suaveolens* leaves from University of Ilorin (Table 1) cannot be ascribed to a precise chemotype, as it contains high percentages of β-caryophyllene (18.68.%), fenchone (11.29%), fenchol (10.46%) and caryophyllene oxide (5.18%).

Repellent activity

Tables 2, 3 and 4 summarises the result of repellency acivity of *H. suaveolens* EO during day and night expressed as protection efficacy % (PE %) at different dosages for one hour (1 hr) of observation. The results indicated that the EO had a significant repellent activity. At lower dose (2µl/cm^2) of the skin, the EO of *H. suaveolens* offered protective efficacy (100%) for up to one hour and the same thing applied when there was an increase in concentration to (4 µl/cm^2), (10 µl/cm^2) and (20 µl/cm^2) respectively and there was no significance difference between the different concentration for the period of one hour.

Table 1: Chemical Composition of Essential Oil of *Hyptis suaveolens*

S/N	Compound[a]	Area%	RT	R.I[a]	R.I[b]
01	Ethylbenzene	1.26	7.278	878	878
02	P-Xylene	3.76	7.628	883	883
03	O-xylene	3.76	7.628	892	892
04	Benzaldehyde	0.81	11.465	961	1023
05	1-Octen-3-ol	2.44	12.549	942	1031
06	O-Cymene	3.81	14.223	1011	1019
07	Nonanal	1.04	17.553	1104	1102
08	Fenchol	10.46	17.734	1117	1111
09	Bornanone (Camphor)	0.82	18.857	1143	1143
10	Borneol	1.30	19.713	1165	1162
11	Terpinen-4-ol	3.45	20.169	1179	1175
12	Thymol	1.27	20.522	1290	1290
13	Caryophyllene	18.68	28.378	1418	1418
14	Alloaromadendrene	1.85	30.452	1461	1459
15	Spathulenol	4.43	33.154	1575	1574
16	Caryophyllene oxide	5.18	33.287	1573	1580

Where RT is the Retention time, R.I[a] is the retention index obtained while R.I[b] is the calculated retention index. The percentage compositions of the oils were computed in each case from GC peak areas.
The identification of the components was based on comparison of retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature (Jennings and Shibamoto, 1980; Joulain and Kong, 1998; Adams, 2012).

Table 2: Protection Efficacy of *Hyptis suaveolens* essential oil during day

Concentration of Essential oil (100 x dilution) (µl/cm^2 of skin)	15 minutes	30 minutes	60 minutes
2.00	100±00^a	93.74±00^a	81.25±00^b
4.00	100±00^a	100±00^a	100±00^a
10.00	100±00^a	100±00^a	100±00^a
20.00	100±00^a	100±00^a	100±00^a

Protection by *Hyptis suaveolens* essential oil at different dosages against *Anopheles* and *Culex* during 60 minutes of observations. Efficacy protection (% ± SE) after 5 different observations.

Table 3: Protection Efficacy of H. suaveolens essential oil during night

Concentration of Essential oil(100 x dilution) (µl/cm^2 of skin)	15minutes	30minutes	60minutes
2.00	100±00^a	100±00^a	100±00^a
4.00	100±00^a	100±00^a	100±00^a
10.00	100±00^a	100±00^a	100±00^a
20.00	100±00^a	100±00^a	100±00^a

Protection of *Hyptis suaveolens* essential oil at different dosages against *Anopheles* , *Culex* during 60 minutes of observations .Efficacy protection (% ± SE) after 5 different observations.

Table 4: Protection efficacy of *H. suaveolens* essential oil at very low concentration

Concentration of Essential oil (1:99 water) (µl/25 cm^2 of skin)	15minutes	30minutes	60minutes
0.200	83.33±006^a	66.67±021	33.33±006^a
0.400	91.66±006^a	75.00±039	58.33±006^a
0.600	91.66±006^a	75.00±039	50.00±033^a
0.800	100.00±0^a	66.67±021	41.67±003
1.00	91.66±006	75.00±039	33.30±006

Protection of *Hyptis suaveolens* essential oil at different dosages against *Anopheles* , *Culex* during 60 minutes of observations .Efficacy protection (% ± SE) after 5 different observations.

Table 4 shows protection time for the five concentrations at an exceptionally low dosage, (0.2 µl/cm^2) offered 83.33% for the first 15minutes, after 30minutes, PE % reduced to 66.67% and in 60minutes the PE % have reduced to 33.33%. (0.4 µl/cm^2), (0.6 µl/cm^2), (0.8 µl/cm^2) and (1.0µl/cm^2) almost gave complete protection (91.66%, 91.66%,100% and 91.66% respectively) for the first 15minutes. At 30minutes the PE % reduced for all the concentrations while at 60minutes the PE % reduced to the lowest. *H. suaveolens* EO efficacy as repellent improve previous evidence from several studies, in which the repellent activity of *H. suaveolens* was proven through different application methods. In fact, it is known that placing *H. suaveolens* branches or whole plants in houses was one of the most effective methods, in western Kenya, to repel malaria vector *Anopheles gambiae s.s*. Giles (Seyoum *et al.* 2002b) [35]. By contrast in semi-field conditions, it was observed that *H. suaveolens* potted plants did not significantly repel *A. gambiae* mosquitoes (Seyoum *et al.* 2002a) [36]. Moreover, studies performed in Guinea Bissau, West Africa showed that smoke produced by burning whole plants of *H. suaveolens,* indoors at night,

significantly repelled mosquitoes (Pålsson and Jaenson 1999a, b) [38, 39]. Pålsson and Jaenson (1999b) reported that fresh or smouldering whole plants of *H. suaveolens* were used in Guinea Bissau, to reduce the number of mosquitoes indoors at night, with a repellent activity ranging from 85.4% to 66.5% (for smouldering and fresh plants, respectively). Similar results were obtained with the same method in western Kenya, against *A. gambiae* (Seyoum *et al.* 2002b) [36].

However, any significant repellent effect was recorded against this latter mosquito species when *H. suaveolens* flowers and leaves were tested through thermal expulsion method (Seyoum *et al.* 2002b) [36]. The EO of *H. suaveolens* have shown great repellent activity against the *Aedes* (Pålsson and Jaenson 1999) [40]. Also, ethyl acetate extracts of *H. suaveolens* from Guinea Bissau strongly reduced the probing activity of *A. aegypti* (Jaenson *et al.* 2006) [41].

Table 5: Effect of the essential oil of H. suaveolens (100 and 500 mg/kg bw) on haematological parameter in mice treated orally for 7 days

S/No	Haematological parameter	Control	100 mg/kg b.wt	500 mg/kg b.wt
1	WBC(x 10^3/µl)	7.70±0.07[a]	7.70±0.07[a]	7.90±0.05[b]
2	RBC (x 10^6/µl)	6.80±0.06[a]	8.34±0.05[b]	8.23±0.06[c]
3	HGB (g/dl)	8.00±0.06[a]	10.40±0.01[b]	10.37±0.01[b]
4	HCT (%)	34.07±0.00[a]	43.30±0.01[b]	45.00±0.03[c]
5	MCV (fl)	48.67±0.04[a]	52.00±0.24[b]	55.07±0.03[c]
6	MCH (pg)	12.07±0.03[a]	12.47±0.03[b]	12.60±0.05[c]
7	MCHC(g/dl)	25.27±0.02[a]	24.07±0.04[b]	23.20±0.03[c]
8	PLT (x10^3µl)	900.50±0.01[a]	1063.00±0.07[b]	846.67±0.01[c]
9	LYM	78.20±0.04[a]	74.67±0.01[b]	82.13±0.01[c]

Results are means of 5 determinations ± SEM. Mean along the same column with different superscripts are significantly different ($p<0.05$).

Haematology and toxicity

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound in the blood. It can also be used to explain blood relating functions of biochemical compound (Yakubu *et al.*, 2007) [42]. Haematological parameters can also be used to investigate and determine blood diseases that affect the production of blood and its component such as PCV, RBC, WBC, Neutrophils, and Lymphocytes and so on. The haematological profiles of the experimental and control groups are shown in Table 5. There was no change in the haematological profile of groups treated with the essential oil of *Hyptis suaveolens* (EOHS, 100 and 500 mg/kg) in either sex, this shows that there was no deleterious effect on the blood. There was no observed significant difference($P<0.05$) in the level of WBC in both the control and 100 mg/kg bw(Table 5) though there was significant increase($P<0.05$) in the level of WBC in 500 mg/kg bwgroup, white blood cells function mainly to fight infection, defend the body by phagocytosis against invasion of foreign organisms, and to produce, transport and distribute antibodies in the immune response, the observed increase in the level of white blood cells in 500 mg/kg bwgroup may be as a result of stimulation of the immune system against infections.

Table 6: Concentration of Liver and Kidney Function indices in Serum of Mice administered with *H. suaveolens* essential oil (100 and 500 mg/kg bw) orally for 7 days

Groups	Creatinine(mg/dl)	Urea(mmol/L)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Total protein
Control	0.66±0.05^{a}	20.81±0.77^{a}	7.19±0.39^{a}	1.76±0.33^{a}	5.50± 1.24^{a}	4.02±0.40^{a}
100 mg/kg bw	0.79±0.06^{ab}	21.22±0.31^{a}	5.55±0.19^{b}	1.65±1.10^{a}	4.73±1.26^{b}	4.97±0.40^{a}
500mg/kg bw	1.09±0.17^{b}	22.41±2.69^{a}	1.15± 0.28^{c}	1.45±1.76^{a}	4.16± 0.78^{c}	5.30±0.56^{a}

Results are means of 5 determination ± SEM. Mean along the same colum with different superscripts are significantly different ($p<0.05$).

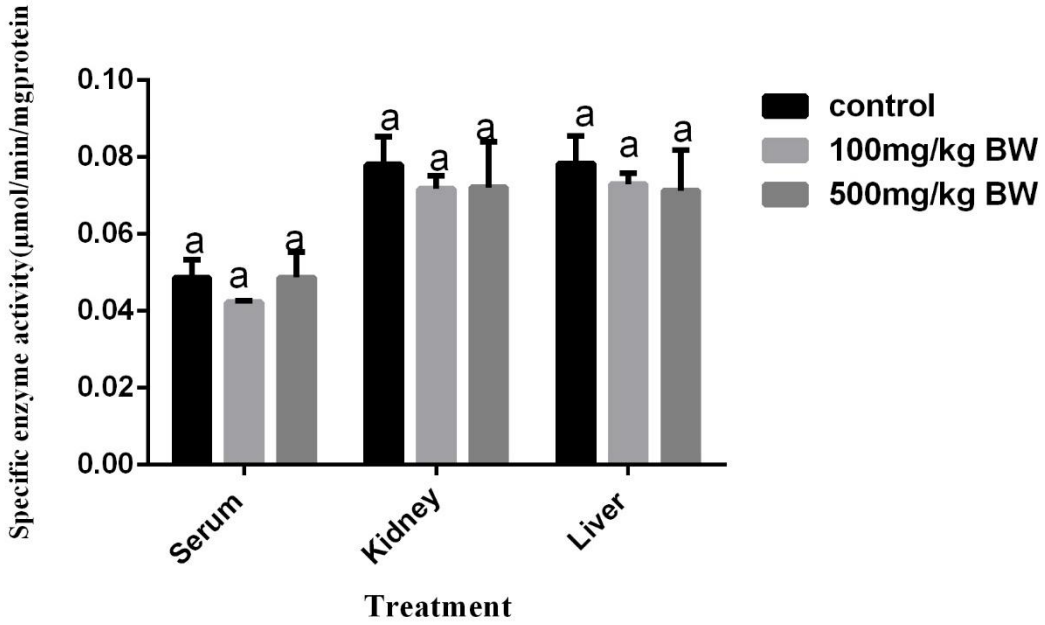

Figure 1: Serum, Kidney and Liver ALT activities in mice administered with *Hyptis suaveolens* EO
Values are means of 5 determinations± SEM. Mean with different superscript letters are significantly different ($p<0.05$).

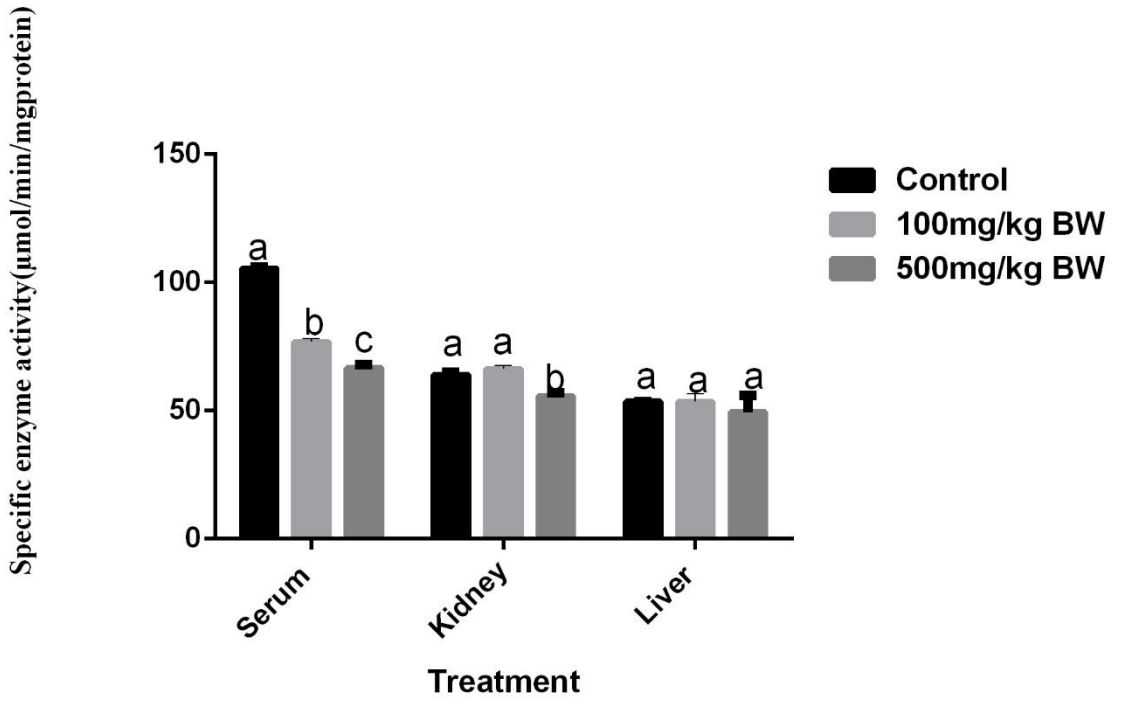

Figure 2: Serum, Kidney and Liver ALP activities in mice administered with *Hyptis suaveolens* EO
Values are means of 5 determinations± SEM. Mean with different superscripts are significantly different ($p<0.05$).

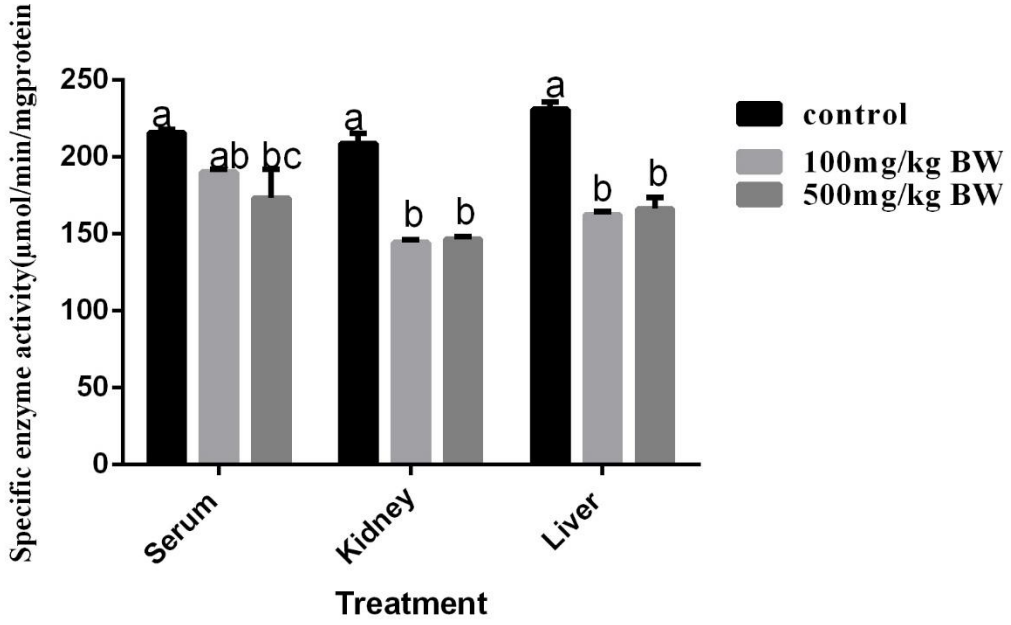

Figure 3: Serum, Kidney and Liver AST activities in mice administered with *Hyptis suaveolens* EO
Values are means of 5 determination± SEM. Mean with different superscript letters are significantly different ($p<0.05$).

There was observed increase in the level of RBC in 100 mg/kg bw and 500 mg/kg bwgroup when compared to the control group and there was also increase in the level of haemoglobin (HGB) and this can be due to increase in the RBC size (microcytes). Furthermore, there was increase in HCT (PCV) and MCV in 100 mg/kg bw and 500 mg/kg bw when compared to control group (Table 5). The Bilirubin is usually increased in relation to the severity of the acute process (Baranono *et al.,* 2002) [43]. There was no significant difference in the Total bilirubin in 100 mg/kg bw and 500 mg/kg bw when compared to the control; however, this shows that the effective dose was not toxic. Conjugated or direct bilirubin is decreased in 100 mg/kg bw and 500 mg/kg bw when compared to the control due to associated hepatocyte dysfunction in the body. Hypoalbuminemia may have resulted from impairment of hepatic function since albumin is synthensized by the liver (Table 6), a rise in blood creatinine levels is observed in those administered with 100 mg/kg bw and 500 mg/kg bw group and rise in blood creatinine only marked damage to functioning nephrons. Alkaline phosphatase was employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji *et al.,* 1993). Results of ALP activity in the serum show elevated level of ALP in the serum and this may be due to leakage in the liver. Also, there was no significant difference in the hepatic and nephrotic ALP in all the groups and this is an indication that there was no leakage in the liver and kidney and that means the structural integrity of the enzyme has been preserved. Similarly, the increase in the hepatic and nephrotic ALT activity in all the groups could be due to complete preservation of the enzyme and a corresponding decrease in the serum activity of the enzyme shows that there was no leakage in the organs. Furthermore, there was an increase in the serum AST and there was increase in control of the liver and kidney AST when compared to 100 mg/kg bw and 500 mg/kg bw and this signify the leakage of the enzyme. Apart from the liver, the kidney is a prominent site of amino acid metabolism because they help in retaining amino groups to form new amino acids during the degradation of amino acids and are also involved in the biochemical regulation of intracellular amino acid pool. Kidneys also help in providing necessary intermediates for gluconeogenesis (Yakubu et al., 2005).

Conclusion

The present study improves the knowledge about the composition of the EO of an important tropical *Lamiacea*, such as *H. suaveolens*. Our data, compared with those reported earlier in literature, confirm that the plant EO can have different chemical contents as a function of the different habitats where plants are grown. Investigation on repellent activity against female *anopheles* and *culex* mosquito demonstrated that *H. suaveolens* EO had repellent properties. The heamatological and toxicological study shows that the EO admistered through oral route might be toxic in large doses 500mg/kgbw. Its insecticidal and/or repellent activity could be used for the development of natural and safer products against A*nopheles* and *Culex* mosquitoes.

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