British Journal of Pharmacology and Toxicology 3(2): 43-48, 2012
ISSN: 2044-2467

Submitted: October 26, 2011 Accepted: Jaurauy 10, 2012 Published: April 25, 2012

Mosquito Repellent Activity and Phytochemical Characterization of Essential Oils From *Striga hermonthica, Hyptis spicigera* and *Ocimum basilicum* Leaf Extracts

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Abstract: The main aim of this study is to screen the phytochemicals and compare the mosquito repellent activities of essential oils from *Hyptis spicigera, Striga hermonthica* and *Ocimum basilicum* (Basil) against *Anopheles gambiae* and *Culex quinquefasciatus* under laboratory conditions. The global threat of malaria to human race and the need to control its advances is on the focus. Mosquito is the target being the primary host in the spread of malaria. Alkaloids,saponnins, steroids, tannins and terpenoids were present in all the 3 oils. Cardiac glycosides were detected in both *H. spicigera* and *Striga hermonthica*, while anthraquinone and phlobatin were present in *Striga hermonthica* and *Hyptis spicigera*, respectively. The FTIR spectrum revealed the presence of C = O, C-l, C-O-C, OH, C-N, S = O and NO_2. These correlate with the functional groups in the identified phytochemicals. At 50% concentration, *O. basilicum* and *Hyptis spicigera* oil exhibited higher repellant potential on *Anopheles gambiae* with protection time of 183 and 120 min, respectively, while *H. spicigera* and *S. hermonthica* had protection time of 180 and 175 min, respectively against *Anopheles gambiae.* At 100% concentration, *O. basilicum* oil exhibited the highest protection time against the two species of mosquito tested and at all the concentrations. *O. basilicum* was equally potent against *Culex quinquefasciatus* with 180 min protection time. *S. hermonthica* had more repellent potential against *Culex quinquefasciatus*. The essential oils of *Striga hermontica*, *Ocimum basilicum and Hyptis spicigera* leaf extracts have been confirmed to have potentials as mosquito repellent agents against *Anopheles gambiae* and *Culex quinquefasciatus*.

Key words: *H. spicigera, O. basilicum*, oil, malaria, mosquito, repellent, *S. hermonthica*

INTRODUCTION

Mosquito is the primary host in the spread of malaria. Mosquito repellents are substances that are designed to make surfaces unpleasant or unattractive to mosquito. They typically contain one active ingredient that repels mosquito as well as secondary ingredients which aid in delivery of cosmetic appeal WHO (2006). Traditionally, various types of substances have been used to repel mosquitoes. These include smoke, plant extracts, tars and Citronella oil (MIM, 2004). *Ocimum gratissimum*, *Ocimum basilicom* oils (Coker *et al.*, 2000) are plant materials that are traditionally used and speculated to have mosquito repelling potentials. However, certain characteristics such as volatility, limited its effectiveness. Active ingredients, such as terpenes (secondary metabolite) (Coker *et al.*, 2000) and oil components are responsible for the plants mosquito repellent activity. For a material to be valuable as a mosquito repellent it must effectively discourage insect attack on the treated area for many hours and on many different types of surfaces, it must work in different environmental conductions, it must be environmental friendly when applied to human or animal skin, it must be cosmetically acceptable having a pleasant odour, taste and feel, it should also be harmless to clothing, it should have a relatively low cost and be effective against other common types of insects, such as flies (WHO, 2008).

Following the high level of threat malaria poses to humanity, its increased level of mortality and morbidity rate as index of its threat; it has become very necessary to look further for an alternative measure for malaria prevention or control. Mosquito as a primary host to the spread of malaria is an important target. Its control can improve the status of malaria free environment. This study is therefore designed to screen and compare the mosquito repellent activities of *Hyptis spicigera*, *Striga*, *hermonthica* and *Ocimum basilicum* (Basil).

Hyptis spicigera is species of scent leaf, widely distributed in tropical and warm temperate region. About 50 species of the plant are found in Nigeria (Coker *et al*., 2000). It is used in treatment of various kinds of ailment such as upper respiratory tract infections, diarrhea, headache, pneumonia, fever and cholera (Onajobi, 1986).

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Striga hermonthica (witch weed) or *wutawuta* as it is called in Hausa language is a major biotic constraint in savanna zone of west and central Africa. It is glabrous in appearance and the genus *striga* is of the family *Scrophulariaceae*. Members of this genus are obligate annual hemi parasites and are *chlorophylous*, but require a host to complete their life cycle, where it devastates cereals like maize, sorghum, pearl, millet, finger millet, hungry rice and upland rice (Mussel-Man, 1987). Dried plants of *S. hermonthica* are traditionally used for various purposes such as treatment of skin diseases (Eczema), jaundices, malaria and leprosy. It is also used as contraceptive and as insect repellent. *S. hermonthica* has been used for medicinal purposes, but its mosquito repellent activity is not yet known.

Basil (*Ocimum basilicum*) is an aromatic, low growing herb. The plants have a bright green to purple ovate colour leafs and are grown in warm, tropical climate. It belongs to the botanical family of *Ocimum basilicum*, which is commonly known as mint. The basil leaves are known to have many medicinal and other healing properties (Grieve *et al*., 1967).

Studies have shown that basil plant extracts and oils can serve as anti-anxiety and anti-depressant due to its ethanolic composition (Edris and Farrag, 2003). Essential oil of basil, obtained from its leaves, has demonstrated the ability to inhibit the growth of *Staphylococcus*, *Enterococcus*, *Shigella* and *Pseudomonas* (Elgayyar *et al*., 2001). The eugenol is reported as an important component of basil's volatile oils and can block the activity of *Cyclooxygenase* (COX) and hence acts as an anti-inflammatory agent. The oil helps in the relief of rheumatoid arthritis and inflammatory bowel conditions (Suppakul *et al*., 2003).

The presence of active agent in some herbs used traditionally in the treatment of many diseases affecting majority of African communities, particularly in Nigeria have been documented (WHO, 2006, 2002; Odetola and Bassin, 1986).

Malaria is a public health problem, most especially in the tropical and subtropical countries where majority bears the burden of the disease. It is one of the six killer diseases in the world today and it has been estimated that 40% of the world population is at risk (WHO, 2006). 500 million people are reported to have being suffering from the disease annually (MIM, 2004). About two million children, mostly less than five years and pregnant women die from malaria related illness annually and nine out of ten cases are found in Sub-Sahara Africa (WHO, 2001). Most vulnerable group in the endemic areas constitutes people in the rural environments who often had little or no access to modern medicine. This situation has been complicated by the emergence of multi-drug resistant strains of plasmodium falciparum coupled with the rapid spread of mosquito resistance to insecticides (Coker *et al*., 2000). This therefore calls for an urgent need to find alternative means of controlling the spread of the disease.

The main aim of this study is to screen the phytochemicals and compare the mosquito repellent activities of essential oils from *Hyptis spicigera, Striga hermonthica* and *Ocimum basilicum* (Basil) against *Anopheles gambiae* and *Culex quinquefasciatus* under laboratory conditions.

MATERIALS AND METHODS

This study was conducted at Kaduna Polytechnic, Kaduna, Nigeria in 2010.

Plant material: Fresh leaves of *Ocimum basilicum* (sweet basil) plant were collected from the premises of college of science and technology main campus at Kaduna Polytechnic, Tudun Wada, Kaduna state. Fresh leaves of *Striga hermonthica* (*Wuta-Wuta)* were obtained from a herbalist at Tudun Nupawa (small community opposite the college of science and technology main campus). Fresh leaves of *Hyptis spicigera* were collected from Kakuri in Kaduna South Local Government Area, in Kaduna state. The plant samples were obtained in March, 2010 and identified by Suleiman Bakori of Biological Sciences unit, Department of Applied Science, Kaduna Polytechnic.

Extraction: Two hundred grams of each of the plant samples was weighed on a watch glass and transferred into a porous thimble which was placed in soxhlet extractor. The plants' oils were extracted with n-hexane. The oils were collected in a flask with air tight cover and kept for further tests. The percentage yield of oil was calculated as follows:

$$Percentage\ yield\ (\%\ yield) = \frac{weight\ of\ n-hexane\ extract\ obtained}{Total\ weight\ of\ sample} \times 100$$

Phytochemical screening: Phytochemical screening for the major constituents was carried out using standard methods described by Sofowara (1995). The plant extracts were screened for the presence of Alkaloids, Anthraquinones, Cardiac glycosides, Carbohydrates, Flavonoids, Steroids, Phlobotannins, Tannins, Saponnin and Terpenes.

Characterization procedure: The routine procedure described by Egwaikhide and Gimba (2007), for the preparation of plant extracts for infrared analysis was adapted using Fourier Transform Infrared (FTIR)

spectrophotometer model 8400s. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin film. The cell was mounted on the FT IR spectrophotometer and scanned through the infra red region.

Acute toxicity studies (Dermal irritation test): For the Primary irritation test, the back hairs of albino rats were shaved using a shaving stick to expose a skin area of about 5 cm. The albino rats were grouped in replicates of three for each extract. The exposed area of the albino rat was treated with 0.5 g of the extracted oil and covered with a gauze pad (bandage). The groups were labelled A, B and C respectively for the different oil extracts. After 24 h the tape and gauze were removed and the treated area evaluated for erythematous lesions (Redness of the skin produced by congestion of the capillaries) and edematous lesions (accumulation of excess fluid in skin and tissue). The results were expressed on a numerical scale. After additional 24 to 48 h, the treated areas were further evaluated.

Repellency assay test (Hand in cage method): The mosquito repellency of the essential oils was evaluated by using an arm-in-cage test as described by (Schreck and McGovern, (1989) and WHO (1996) with little modification. The technique involves counting of the number of mosquitoes biting a volunteer‘s hands introduced into a 14×14×17 cm mosquito cage containing 50-60, 3-5 days old, male and female mosquitoes. Counting was done for the first three minutes of every half-hour exposure. Approximately 0.1 cm^3 of 50 or 100% concentrations of the extracted oil was applied to the forearm of each volunteer. Each test was repeated three times for each concentration. Four different human volunteers were used per test after obtaining their consent to participate in the experiment. The repellency test was carried out against two species of mosquito, *Culex quinquefasciatus* and *Anopheles gambiae* which were identified by their physical characteristics. Control readings were obtained by placing hand inside the repellent chamber without applying any repellent oil before experiment.

RESULTS

The % yield of oil extracted from *Ocimum basilicum* (5.60%) was higher than the yield of oil from *H*yptis *spicigera* (3.23%) and *Striga hermonthica* (1.7%) (Table 1).

Phytochemical screening: The phytochemical screening of the extracts revealed the presence of alkaloids, saponins, streroids, tannins and terpenoids in all the three oils and flavonoids in *H. spicigera* and *O. basilicum*. Cardiac glycosides were found in oils from *S. hermonthica* and *H.* spicigera. Anthraquinones and phlobatanins were detected only in oils from *S. hermonthica* and *H. spicigera*, respectively (Table 2).

Table 1: The percentage yield of oil from the plants

Plant%	Yield of extracted oil
Hyptis spicigera	3.23
Ocimum basilicum	5.60
Striga hermonthica	1.70

Table 2: Phytochemicals in oils extracted from *Hyptis spicigera, Striga hermonthica and Ocimum basilicum* leaf

Phytochemicals	*Striga hermonthica*	*Hyptis spicigera*	*Ocimum basilicum*
Alkaloids	+	+	+
Anthraquinones	+	-	-
Flavonoids	-	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Terpeniods	+	+	+
Cardiac glycoside	+	+	-
Phlobatanins		+	

+: Present; -: Absent

FTIR characterization: The FTIR data is shown in Table 3.

- ***H. spicigera*:** The spectra analysis of *H. spicigera* indicated the presence of functional groups, C-H, C = C, C-N, O-H and C-O-C identified as aromatics, alkenes, amines, alcohols and ethers.
- ***S. hermonthica*:** The spectra analysis of the oil of S. *hermonthica* revealed the presence of peaks corresponding to the functional groups, C-H (aromatic), C-O-C (ether), C-O (phenyl ether), C = O (carboxylic acid) and O-H (alcohols).
- ***O. basilicum*:** The spectra analysis of the *O. basilicum* oil revealed the presence of peaks corresponding to the functional groups, C-H (aromatics), NO_2 (aliphatic/Aromatic), C = C (Aromatic), C = O (Aldehyde/Ketone) and O-H (Alcohols) (Table 3).

Table 4, 5 and 6 indicate the high protection offered by the plants oils against *Anopheles and culex* mosquitoes. Maximum mean protection time of 303 min was exhibited by the oil of *O. bacilicum* at 100% concentration against *Anopheles* (Table 4). At both 50 and 100% oil concentrations, the mean protection time exhibited by *O. basilicum against Anopheles was higher than culex. O. basilicum* and *Hyptis* s*picigera* exhibited higher repellent potential against *Anopheles* with protection times of 183 and 120 min, respectively

Table 3: The FTIR data for characterization of oils extracted from *S. hermonthica*, *O. basilicum* and *Hyptis spicigera*

	Peak (cm^{-1})			
Vibrational motion	*Hyptis spicigera*	*O. basilicum*	*S. hermonthica*	Functional group
C-I stretch	472.58			Alkylhalids
C-H bend (ortho)			723.33	Aromatics
C-H bend (mono)	730.08	778.3		Aromatic
C-N stretch (alkyl)	1,035.81			Amines
S = O stretch		1057.03		Sulfoxide
C-O-C stretch	1,177.58		1041.6, 1098.5, 1164.08	Ethers
OH bend	1,405.19			Carboxylic acid
C-O stretch			1245.09	Phenyl alkyl ethers
C-H in plane bend			1329.15	Alkenes
NO_2 aliphatic/ aromatic		1395.54		Nitro groups
CH_2 bend	1,457.27	1488.13		Alkanes
C = C stretch		1488.13		Aromatic
C = O stretch		1725.38	1463.06	
			1711.88	
			1,717.67	Aromatics
				Acetic acid
				Aldehyde/ketone
C-H stretch	2848.96, 2918.4	2946.36	2851.85, 2921.29	Alkanes
O-H stretch	3443.05	3400.62	3429.55	Alcohols

Table 4: Repellent activity and protection time of 50 and 100% oil from *Ocimum basilicum* against *Culex* and *Anopheles* mosquito

Ocimum basilicum																
	50%								100%							
	Number of landed mosquitoes				Protection time (min)				Number of landed mosquitoes				Protection time (min)			
Species of mosquitoes	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2n	d3rd	Mean ±SEM
Culex	2	2	3	2±0	140	200	200	180±20	2	3	2	2±0	176	285	285	249±36
Anopheles	3	2	4	3±1	160	188	200	183±12	2	4	3	3±1	300	305	305	303±2

Table 5: Repellent activity and protection time of 50 and100% oil of *Hyptis spicigera* against *Anopheles gambiae* and *Culex quinquefasciatus*

S. hermonthica																
	50%								100%							
	No of landing mosquito				Protection time (min)				No of landing mosquito				Protection time (min)			
Species of mosquitoes	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2n	d3rd	Mean ±SEM
Anopheles gambiae	3	2	2	2±0	90	123	136	116±14	3	3	3	3±0	175	95	175	148±27
Culex quinquefasciatus	2	3	2	2±0	90	150	150	130±20	4	3	4	4±0	164	160	165	163±2

Table 6: Repellent activity and protection time of 50 and 100% oil of *S. hermonthica* against *Anopheles gambiae* and *Culex quinquefasciatus*

Hyptis spicigera																
	50%								100%							
	No of landing mosquito				Protection time (min)				No of landing mosquito				Protection time (min)			
Species of mosquitoes	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2nd	3rd	Mean ±SEM	1st	2n	d3rd	Mean ±SEM
Anopheles gambiae	3	2	2	2±0	120	120	120	120±0	2	2	2	2±0	180	170	180	176±3
Culex quinquefasciatus	2	3	2	2±0	90	120	120	110±10	3	2	2	2±0	160	170	175	170±4

(Table 4 and 5). On the contrary, the oil of *S. hermonthica* had higher mean protection time against *Culex* at both 50 and 100% concentrations (Table 6). Table 7 indicates the results of dermal irritation tests on the plants oils.

Table 7: Effect of the oils of *Ocimum basilicum*, *Hyptis spicigera* and *S. hermonthica* on animal skin (dermal irritation test)

Extract group	*Ocimum basilicum*	*Hyptis spicigera*	*S. hermonthica*
Erythematous lesion	0	0	0
Irritation	0	0	0

0: No effect; 1: Effect

DISCUSSION

The results of this investigation showed the oil of *O. basilicum* was extracted in higher yield (5.6%) than oils from *Hyptis spicigera* (3.23%) and *S. hermonthica* (1.7%). This probably explains why *S. hermonthica* is usually smoked locally, to provide repellency against mosquitoes.

The phytochemical screening of the oil extracts indicated the presence of alkaloids, saponin steroids, terpenoids, anthraquinones, flavonoids, Tannins, Cardiac Glycoside and Phlobatanins.

The spectra analysis of oil from *H. spicigera* indicated the presence of functional groups C-H, C = C, C-N, O-H and C-O-C identified as aromatics, alkenes, amines, alcohols and ethers respectively (Table 3). These functional groups have been linked with alkaloids characterized with nitrogen with heterocyclic rings (Harborne, 1998; Van Wyk *et al*., 2000), terpenoids, saponins, cardiac glycosides and steroids principally characterized with long chain unsaturated hydrocarbon, poly-hydroxyl alcohols, esters, aromatic rings and ether (Heinrich *et al*., 2004; Gurib-Fakim, 2006). Flavonoids have aromatic nucleus with C-OH (Phenols) and central oxygenated heterocyclic ring (Hollam and Katan, 1999) which may also be associated with the function groups revealed by the spectral analysis.

The spectra analysis of the oil of *S. hermonthica* revealed the presence of peaks corresponding to C-H (aromatic) , C-O-C (ether), C-O (phenyl ether), C = O (carboxylic acid) and O-H (alcohols) and these correlate the identified phytochemicals such as flavonoids characterized with phenolic nucleus and oxygenated heterocyclic center, saponin, steroid and tannins. Antraquinone and cardiac glycosides which are characterized with the functional groups like Carboxylic acid, phenols, oxygenated heterocyclic centers coupled with poly-hdroxyl aldehydes or ketones (Bruneton, 1999) were revealed by the FTIR spectral.

The spectra analysis of the *O. basilicum* oil revealed the presence of C-H (aromatics), NO_2 (aliphatic/ Aromatic), C = C (Aromatic), C = O (Aldehyde/Ketone) and O-H (Alcohols) (Table 3). Steroids are complex lipids which are invariably esters with C = R, C = C, C = O and C-OH as the contributing functional groups. These functional groups were confirmed by the absorption bands in the spectra of the O. *basilicum* oil and the other two oils studied in this work. Phytochemicals are the principal active components that are believed to exhibit the medicinal activity of the plants and possibly the repellent activity of the oils. Terpenoids are said to be responsible for the flavour of fruits, the fragrance of the flowers and the quality of agricultural products (Banthorpe, 1991; Heinrich *et al*., 2005). The presence of terpenes is speculated to be associated with fragrance material and repellent activity of oils (Coker *et al*., 2000).

Any agent that is to be employed as insect repellent needs to be well defined in terms of its toxicity to man and non-target organisms before it is recommended. On this premise, skin irritation test was carried out using experimental models with albino rats. The results of this did not indicate any observable reaction. In this study, repellent activities of the oils extracted from the plants on human volunteer were therefore demonstrated. This was given preference over the laboratory animal models as these models may not provide sufficient stimulus to mimic the repellent potential of human skin (Oyewole *et al*., 2008; WHO, 1996).

The results of this work indicated that the repellent potential of the oils is concentration dependent. However it is observed that all the 3 oils indicated protection of at least 2 h at 50% concentration and 2.5-5 h protection at 100%. Further investigation on the other aspect of toxicity and field trial of the plants oils are in progress. The use of plants oil in controlling mosquito bite is expected to reduce the cost and environmental effect of mosquito repellents.

CONCLUSION

The preliminary findings of the laboratory evaluation of the repellent potential of the essential oils of *Striga hermonthica*, *Ocimum basilicum* and *Hyptis spicigera* leaves have confirmed their traditional use as a broad mosquito repellent agents. Further investigations leading to identification of the lead compounds exhibiting the repellent activity is recommended. This would enhance a robust development of plant based oil for protection against mosquito bite.

ACKNOWLEDGMENT

The authors appreciate the technical assistance and facilities provided by National Research Institute for Chemical Technology (NARICT) Zaria and Department of Applied Science, Kaduna Polytechnic.

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