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A Search for Cheaper and Eco-Friendly Approaches to Reducing Mosquito Transmitted Diseases Using Smoke Extract of Fresh Shoot Systems of *Cassia Obstusifolia, Hyptis Suaveolens and Striga Hermonthica*

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Abstract

Plants have co-evolved with insects and are equipped with plethora of chemical defenses, which also tend be a powerful tools for reducing the burden of diseases caused by insects through vector control. In this research an ecofriendly approach to managing mosquitoes was studied. Fifteen (15) sucrose fed mosquitoes were subjected to repellence tests in a linear olfactometer. The stimuli were smoke extract from three plants, pyrethroid-based standard insecticide coil (Sasso mosquito coil) and fresh air as a negative control. Comparative repellence tests were also used to assess the repellent potency of the plants. The highest repellent potency due to *Cassia obstusifolia*, *Hyptis suaveolens and Striga hermonthica* were 38%, 85% and 65% respectively using equivalent volumes of smoke extract. Extracts of two of the plants were effectively repellent as the pyrethroid-based mosquito coil. In comparative tests, there were no significant differences (P>0.05) between the activity of smoke of both *S. hermonthica* and *H. suaveolens* compared to that of the pyrethroid-based coil. Extracts of *Striga hermonthica* maintained good repellence close to the synthetic coil for sixty minutes. Difference between the repellence of *Cassia obstusifolia* and Sasso was significant, indicating the lesser repellent potency of the plant sample. Major phytochemicals in fresh aerial parts of the three plants were; terpenes, alkaloids and phenolic compounds. *Hyptis suaveolens* and *Striga hermonthica* require toxicity studies and standardization to be recommended for management of mosquitoes in pro-poor settings.
Keywords: Smoke; Repellence; Olfactometer; Sasso mosquito coil; Insecticidal; Phytochemicals.

1. Introduction

The most important single group of insects in terms of public health importance is mosquitoes. They transmit a host of pathogens that cause numerous life threatening diseases such as malaria, filariasis, dengue fever, encephalitis and many more [1]. The vector's contact with man is a very serious global issue, as malaria alone killed approximately 445,000 people in 2016 worldwide [2]. One of the best ways to deal with this global enemy is to prevent the contact with man by developing repellents which have high repellent efficacy against the vector and also user and ecologically friendly [3].

Plants possess biologically active compounds which they use against predators, infections and for other ecological purposes. These bioactive molecules could be attractants or repellents to many groups of insects. The compounds can be extracted and used to repel insects especially the deadly malaria vector [4].

Mosquitoes depend solely on their olfactory system to spot food and for other ecological needs in order to survive, hence targeting this system is key [5] in their management. Most repellents are toxic and irritant to insects [6]. These repellents usually target one or more of the following; inhibition of cellular respiration, blockage of calcium channels of the nerve cell membrane, hormonal disruption, mitotic poisoning, inhibition of acetyl cholinesterase and alteration in behaviour and memory loss of cholinergic system of insects [7].

Mosquitoes have developed resistance to most synthetic repellents which as well possess public health concerns as they trigger allergy, skin and eye irritations and respiratory disorders [8]. Even DEET which is a gold standard insecticide has side effects [9-11]. These repellents also contaminate food, water and pollute air as well as release CFCs that deplete the ozone [7, 12]. Also according to Paré Toé, *et al.* [13], there occurs a significant reduction of people's motivation to use mosquito nets and people prefer to spend nights without insecticidal nets. Therefore, the use of mosquito nets is not also sufficient to effectively control the malaria vector, especially in sub-Saharan Africa where urbanization promotes the proliferation of mosquitoes.

Cassia obstusifolia is an annual herb widely distributed and reported to contain over twenty metabolites [14]. The plant contains glycosides, saponins, triterpenoids, tannins, flavonoids, alkaloids and carbohydrates [14, 15] and the ethanol extract is reported to have parricidal and ovipositional deterrence against Anopheles mosquito [16]. The leaves are used to treat ringworm various diseases [17].

Striga hermonthica is a hemi parasitic annual chlorophyllous plant. The aerial parts have anti-diabetic properties and for the treatment pneumonia [18-21]. Essential oils of Striga hermonthica have been found to have mosquito repellence [22].

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Hyptis suaveolens is also commonly distributed in the tropics with the exception of the colder regions [23]. It is commonly found along road sites, waste grounds and water course areas [24]. It is an aromatic herbaceous annual shrub [25]. Ethno botanically, the plant has anti-cancer virtues as well as stomach ache and fever remedies [26]. Hyptis suaveolens has previously been cited to have many phytochemicals [25, 27, 28], and several identified compounds [23, 29]. There are several reports that, aerial parts of *Hyptis suaveolens* are used to repel mosquitoes in Africa and South America [30, 31]. Recent studies also showed that extracts of the plant have larvicidal and mosquito repelling effects, and can reduce malaria incidence [27].

Sasso mosquito coil is one of the common and reliable standard insecticide coils sold in the Ghanaian market. The coil contained 0.08% esbiothrin as active ingredient. Esbiothrin is an insect repelling compound which is derived from pyrethroid with a fast knock down of household arthropods [32].

Interestingly, there are abundant plants with mosquito repellent potency in malaria endemic regions especially in the sub-Sahara [16, 22, 30, 33, 34] especially in rural poor communities. Exploring these plants is worthwhile in communities where they could be harvested for free instead of buying of the synthetic coils. It has therefore become necessary to investigate and incorporate the bio repellents for the control of mosquitoes. These natural repellents will be cheap and available.

This study sought to review the presence of phytochemicals of *Hyptis suaveolens, Cassia obstifolia* and *Striga hermonthica* and to determine mosquito repellent potency using smoke from the fresh shoot systems of the three plants. Smoke of both *Striga hermonthica* and *Hyptis suaveolens* are ethno botanically used in the repellence of mosquitoes and require scientific studies to be refined for their use to have significance in reduction of the burden mosquito related public health challenges especially in poor sub Saharan communities where the plants grow freely.

2. Materials and Methods

The research materials were; linear olfactometer, Sasso insecticide mosquito coil, arial parts of *Cassia obstusifolia*, *Hyptis suaveolens* and *Striga hermonthica*.

The experiments were conducted in the Chemistry and Entomology laboratories of the University for Development Studies, Faculty of Applied Sciences in the Navrongo campus.

2.1. Collection of Plant Samples and Authentication

Fresh shoot systems of *Striga hermonthica* were collected from a millet field in Navrongo. Samples of both *Hyptis Suaveolens* and *Cassia obstusifolia* were collected from Navrongo campus of the University for Development Studies. These plants were authenticated by a botanist in the Department of Applied Biology, Faculty of Applied Sciences in the University for Development Studies.

2.2. Collection and Rearing of Mosquito Larvae

Different unsorted mosquito larval species of the first and second stages were collected from ditches, ponds and septic tanks in the Navrongo municipal.

The collected larvae were transferred into trays containing water. powdered biscuits were used as food for the larval development covered with net [35]. Newly emerged mosquitoes were transferred into the cage using an aspirator. 10 % sucrose was soaked in cotton and placed in the cage to feed the mosquitoes [36].

2.3. Sample Processing and Phytochemical Screening

For the phytochemical screening, the fresh shoot systems of the plants were washed, ground and macerated in distilled water for 24 hours. The extracts were filtered and concentrated via a rotary evaporator and further dried over the water bath. The aqueous extracts were then stored in the freezer [37]. Standard methods previously used by Bhandary, *et al.* [38] and Jaradat, *et al.* [39], were used to qualitatively screen for the various class of phytochemicals in the aqueous extract.

For the repellency tests, 15 g of the fresh shoot system of each plant was put in the tray containing blazing charcoal and allowed to smoke. A glass funnel was inverted to direct the smoke into a 50 mL syringe till it filled, approximated as one unit volume of smoke.

2.4. Repellence Tests

A linear olfactometer previously used by Abagale, *et al.* [30], a modification of that used by Paixão, *et al.* [40], was used. It was constructed using plastic containers. It has three chambers; the middle chamber called the test chamber and the opposite chambers called response chambers. The chambers have diameters of 15 cm, heights of 20 cm and connect to each other by a 3cm diameter PVC tube at a height 5cm from the base of each chamber.

Fifteen (15) sucrose-fed adult mosquitoes were drawn from the rearing cage into the test chamber of the olfactometer. Into one of the response chambers, 2, 4 and 6 respective units (1 unit of smoke is equivalent to 50 ml) of smoke generated from the individual plants was introduced and the other response chamber left empty (contains clean air). The number of mosquitoes in each chamber was counted and recorded after 15 minutes. The most effective repellent volume of smoke was further used to determine the repellent activity over an hour.

Each repellence experiment was replicated ten times and the mean number of mosquitoes in each chamber was calculated. During each experiment, the response chambers were washed and alternated after five trials to avoid bias. Comparative tests were also carried out between the smoke of the plants and that from the pyrethroid-based mosquito coil to assess the relative efficacy of the plants.

The repellence percentage was calculated using the formula (1) below.

$$\left(\frac{Blank - treatment(smoke)}{total\ number\ of\ mosquitoes} \times 100 \text{---------------------(1)}$$

[36, 41].

2.5. Statistical Analysis

Statistical tools such as mean, standard deviation, and T-test were used at a confidence interval of 95%. This was done using Microsoft excel 2013 version.

3. Results

Table-1. Phytochemical profile, *Cassia obstusifolia*, *Hyptis suaveolens* and *Striga hermonthica*

Phytochemical	*H. suaveolens*	*C. obstusifolia*	*S. hermonthica*
Phenols	++	+	+
Tannins	+	++	+
Reducing sugars	+	+	+
Saponins	++	+	+
Flavonoids	++	++	+
Alkaloids	+	+	++
Terpenes	++	+	+
Glycosides	+	+	+
Steroids	-	+	-
Cummarins	+	+	+

Key: (+) = present in lower levels, (++) = present in higher levels and (-) = absent

Table-2. Table of repellence

Plants	Units* of smoke	Mean number of mosquitoes repelled	Mean repellence (%)
H. suaveolens	2	4.2 ±0.2	28
	4	10.7±0.4	71
	6	12.4± 0.3	83
S. hermonthica	2	2.8±0.5	18.7
	4	7.5±0.2	50
	6	9.7 ±0.4	65
C. obstusifolia	2	1.9 ± 0.1	12
	4	3.8 ± 0.01	25
	6	5.7 ± 0.1	38

** 1 unit of smoke is equivalent to 50 ml*

Figure-1. Bar graph showing relative repellence (%) against volume of smoke

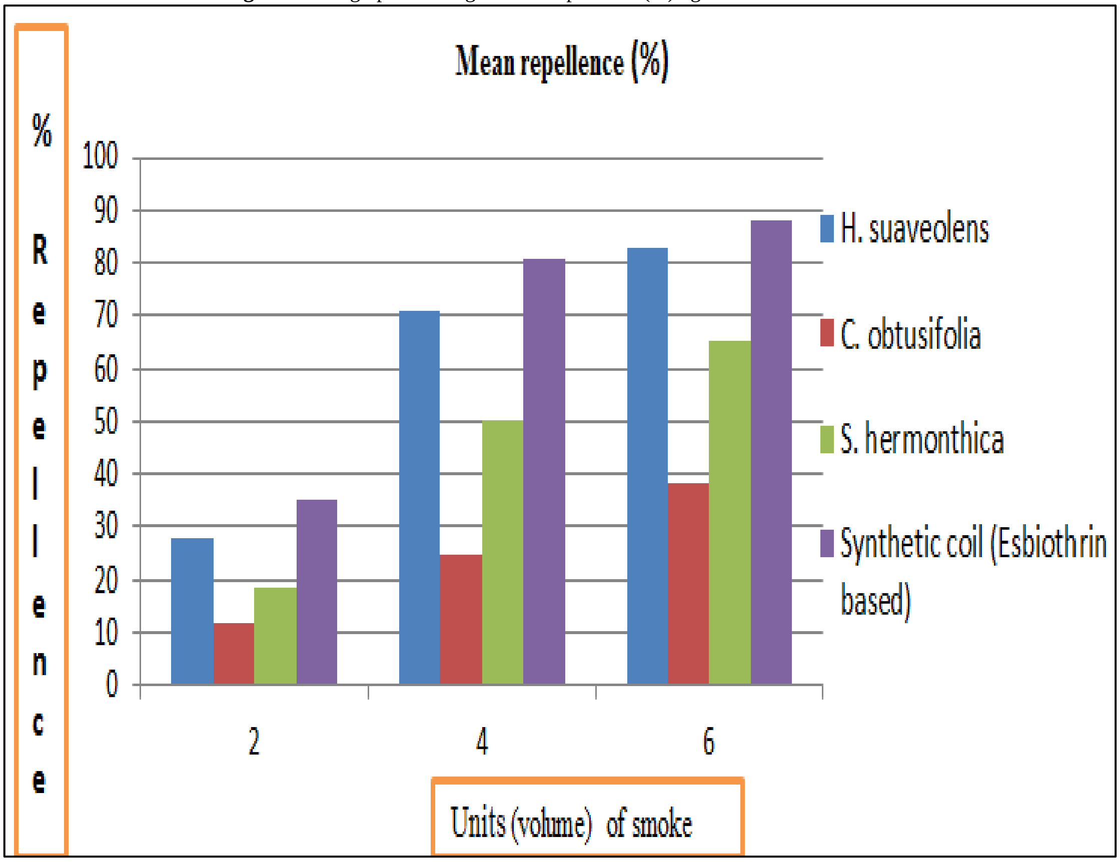

Figure-2. Graph showing mosquito repellence (%) against time

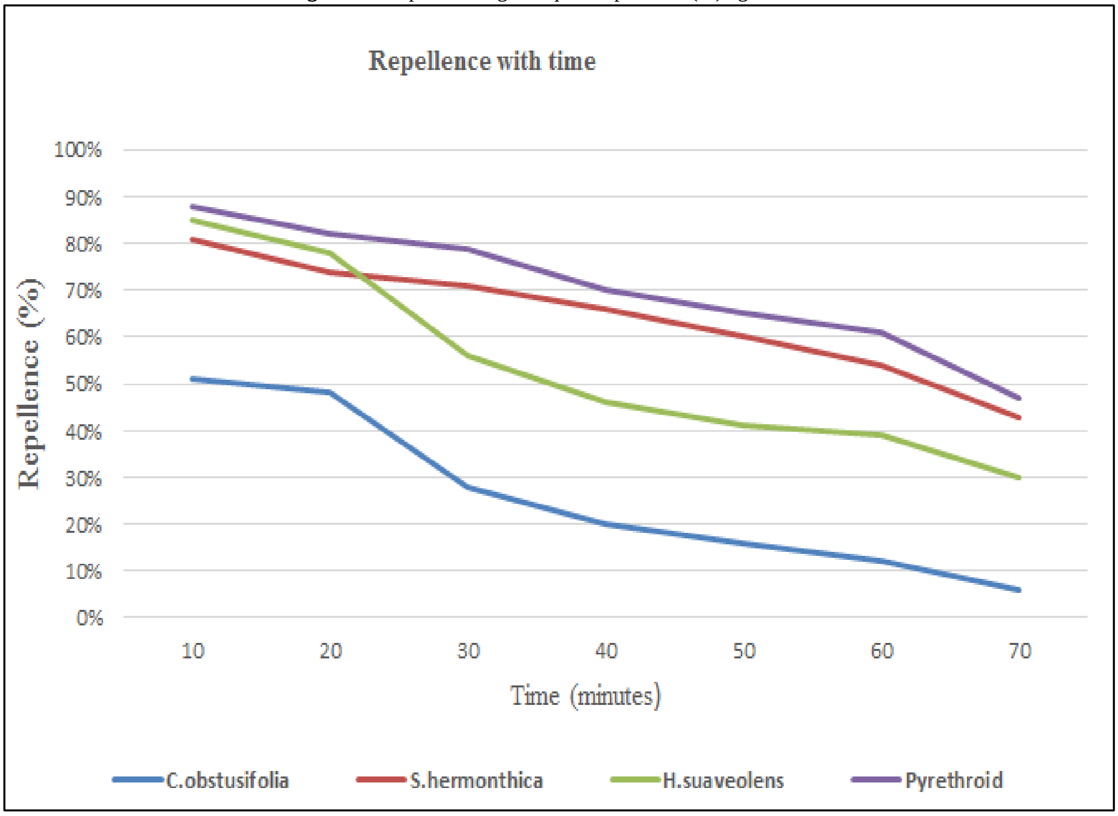

Table-3. Comparative mosquito repellence of plant smoke and pyrethroid-based mosquito coil

Test	Mean ± S.E.M	Repellence (%)	P at 95% Confidence Level
H.Suaveolens against	6.8± 0.3	45	
Pyrethroid-based coil *(Sasso)*	8.2 ± 0.1	55	P > 0.05
S.hermonthica against	6.7 ± 0.4	44	
Pyrethroid-based coil *(Sasso)*	8.3 ± 0.1	56	P > 0.05
C. obtusifolia against	5.0± 0.3	33.3	0.0049 (Significant)
Pyrethroid-based coil *(Sasso)*	10 ± 0.1	66.7	

4. Discussion

Olfactory tests from table 2 show that all three plants under study have mosquito repellent capacity, though the repellence levels were lower under all volumes of the *Casia obtusifolia* test samples. Several reports indicate the capacity of plant metabolites to repel mosquitoes and other insects. According to Debbourn, *et al.* [4], Lawal, *et al.* [42] and Sai, *et al.* [43], classes of phytochemicals such as alkaloids, terpenes, phenolics and tannins have insecticidal and repellence properties in them. Alkaloids are basically insecticidal at low concentration; nevertheless they can be used for insect repellence. They are nonvolatile and release insecticidal smoke when the plant materials or the mosquito coil containing the active ingredients are burnt. They repel mosquitoes through direct toxicity [31]. Other sub phytochemicals such as methone, limonene, beta pinene, alpha pinene and linaliol have been isolated and noted for mosquito repellence [44].

Results from the current study showed that the smoke extract from the fresh aerial parts of *Hyptis suaveolens* has high repellency rates: 2 units of smoke (100 mL) had 28 % repellence, 4 units (200 mL) had 71 % and 6 units of smoke yielded 83 % (Figure 1). Over an hour there was high repellence overall, compared to *Cassia obtusifolia* (Figure 2). These repellence indices may be due to the phytochemical make-up especially the high levels of terpenes (Table 1). Indeed, findings in the present study are consistent with various published articles on the repellent potency of *Hyptis suaveolens*. Extracts of *Hyptis suaveolens* have previously shown significant inhibition on the larvae, pupae and adult stages of mosquitoes [24]. Also, leaves of the plant showed high repellency of 90 % at high concentration and there was significant difference ($P<0.05$) between low and high concentration of the leaf extract [45]. Dried leaf extract of *Hyptis suaveolens* has also been reported to have repellent effects on adult mosquitoes both at low and high concentrations. At low concentrations (50 mg/ml), there were low repellency rates (5.0 %) and at higher concentrations (500 mg/mL), the repellence rate was 53.1 % [46].

Striga hermonthica showed fairly high repellence: 2 units (100 mL) of smoke extract had 18.7 %, 4 units (200 mL) resulted in 50 % repellence and that of 6 units of smoke was 65 %. There was a high repellence over an hour compared to *Hyptis suaveolens* (Figure 2). According to Baba, *et al.* [22], 50 % of the essential oils of this plant showed a protection time of 2 hours.

Cassia obtusifolia, was found with the weakest repellent potency among the three tested plants. This could be due to the low levels of terpenes, alkaloids and phenols (Table 1). 2 units of smoke had a repellence of 12 %, 4 units revealed 25 % and 38 % was repelled using 6 units volume of smoke. There is little research on the repellence reported of *Cassia obtusifolia* but some studies available have showed that the leaves of the plant have strong larvicidal ability at 25 mg/l [16].

In comparative tests between the samples (Table 3), there was no significant difference ($P>0.05$) between the repellence of the extract from the pyrethroid based coil (Sasso) and those from both *S. hermonthica* and *H. suaveolens*. However, there was significant difference between mosquitoes repelled by the coil and *C. obstusifolia*.

Where mosquitoes exist with humans, repellence is a viable option to avoiding bites by the insects [3]. This thus becomes valuable in preventing the transmission of several life threatening diseases [1, 2].

5. Conclusion

Smoke of *Hyptis suaveolens* had the highest repellence (83%), followed by *Striga hermonthica* with fairly high repellence (65 %) and *Cassia obtusifolia* had the lowest repellence (38 %). Both *S. hermonthica* and *H. suaveolens* were as potent as the pyrethroid-based repellent coil. The sample from the synthetic pyrethyroid-based mosquito coil (Sasso) did not have significant difference in repellence compared to *Hyptis suaeolence* and *Striga hermonthica*. Therefore these two plants can be integrated into mosquito management schemes after further investigations and could contribute to reduction of mosquito bites hence its related diseases and improvement of public health. Smoke of the plants is cheaper and eco-friendly alternative to the synthetic mosquito coils commonly sold in Ghana.

The presence of terpenes, saponins, alkaloids, coumarins, and phenols (tannins, flavonoids) in both *Striga hermonthica* and *Hyptis suaveolens* may have contributed to the high repellent potency. Presence of lesser quantities of phytochemicals and nature of interactions of compounds produced from burnt metabolites may have contributed to the lower repellent potency of *Casia obtusifolia*.

The findings in this study also provided scientific backing to the folklore mosquito repellent usage of *Hyptis suaveolens* and *Striga hermonthica*. Further studies regarding quality control and toxicity studies are required to support usage of the two plants.

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