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COMPARATIVE MOSQUITO REPELLENCY OF DRIED LEAVES OF *HYPTIS SUAVEOLENS, CASSIA OBTUSIFOLIA, STRIGA HERMONTHICA* FROM THE UPPER EAST REGION OF GHANA AND TWO STANDARD REPELLANTS

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ABSTRACT

In this study the ethnobotanical use of mosquito repelling plant leaves as a preventive measure against mosquitoes thus malaria was investigated. Phytochemical screening of ethanol and aqueous extracts, and mosquito repellency tests of powdered dried leaves of *Hyptis suaveolens*, *Cassia obtusifolia* and *Striga hermonthica* in an olfactometer were carried out and compared with two standard repellants. In leaves of *Striga hermonthica*, glycosides, flavonoids, tannins, ketones, alkaloids, reducing sugar and phlobotannins, steroids and terpenoids were present. *Hyptis suaveolens* leaves had glycosides, tannins, alkaloids, reducing sugar, phlobotannins, ketones and coumarins, flavonoids, terpenoids and steroids. In *Cassia obtusifolia* leaves, flavonoids, anthraquinone, ketones, coumarins, terpenoids and phlobotannins, glycosides, saponins, tannins, steroids, alkaloids and reducing sugar were also present. *S. hermonthica* was the most repellent among the three plant leaves, and repelled 11.50±0.50, 6.50±0.63 and 8.33± 0.82 mean number of mosquitoes when it was compared to empty chamber, *Hyptis suaveolens* leaves and *Cassia obtusifolia* leaves respectively. Leaves of *H. suaveolens* were the second efficient repellant sample. It repelled 10.17±0.52 and 8.00±0.79 mean number of mosquitoes when it was compared with empty chamber and *C. obtusifolia* leaves respectively. *Cassia obtusifolia* leaves were the least repellent and mean numbers of mosquitoes repelled were 6.58±0.42 and 5.42±0.54 respectively compared to empty chamber and *Hyptis suaveolens*. The repellence efficiency of *Striga hermonthica*, *Hyptis suaveolens* and *Cassia obtusifolia* were 83.4, 78.3 and 70.4 % respectively compared with the standard, 90.0 %. *Striga hermonthica* compared well with the standard repellent and could be used to repel mosquitoes to avoid their bites.

Contribution/ Originality: This work contributes to new ways of reducing mosquito bites, hence incidence of malaria. A new approach, the olfactometer, was used to investigate the mosquito vector. A direct comparison of naturally growing mosquito repellents, *Striga hermonthica* and *Hyptis suaveolens*, was established, for possible use to formulate herbal mosquito repellents.

1. INTRODUCTION

Malaria continues to be a major public health problem in the tropical world. It is the principal cause of morbidity and mortality in all sub-Saharan countries accounting for 10% - 30% of all hospital admissions, responsible for 15% - 25% of all deaths in children under the age of 5 years and for a substantial number of miscarriages and underweight births [1]. According to a WHO report released in 2014, an estimated number of 437000 African children died before their fifth birthday due to malaria. The report stated that there were about 198 million cases of malaria in 2013 and an estimated 584000 deaths with most victims being children [2]. It is estimated that over a million deaths are caused annually by malaria (mainly by *P. falciparum* malaria) across the globe [3].

Four (4) mosquito parasite species including *Plasmodium falciparum*, *P. vivax*, *P. ovale and P. malariae* are known to infect humans [4]. *Plasmodium knowlesi* is an emerging fifth species [5]. Of the four species, *P. falciparum* is the leading cause of malarial illness in sub-Saharan Africa and causes the most severe form of malaria, cerebral malaria, which is characterized by neurological dysfunction and coma. There is a perennial and intense transmission (holoendemicity) of the parasite among all age groups of the population in the sub-tropics, including Ghana [6].

Mosquitoes are most often thought of as blood-thirsty pest that spread disease and ruin outdoor activities. All mosquitoes go through four distinct lifecycle stages made up of egg, larval, pupa and adult stages. Some mosquito species lay their eggs singly while others glue 100-200 eggs together forming miniature rafts. Within 24 to 48 hours, these eggs hatch into lavae. The larval stage displays a great variation in morphology between different mosquito species and is used for identification purpose. Within seven to ten days, larvae enter the pupal stage. Mosquito pupae do not feed but are mobile and use a tumbling motion to escape predation. Within 24 hours the pupae moult into adults [7].

Adult female and male mosquitoes both require carbohydrate source throughout their life to maintain energy for flying, mating, and seeking host for bloodmeals. But, only the female takes bloodmeal because she needs extra protein to develop eggs. Through the process of taking bloodmeal the mosquito is able to vector viruses, protozoans, and helminthes (worms) to humans and animals. Male mosquitoes tend to live only for a week or two while female mosquitoes can live for up to a month and produce multiple batches of eggs [7]. It is only the adult stage that adversely affects human and animal health. Therefore, man-vector contact of the adult mosquito must be circumvented.

Cutting off or breaking the link between mosquito vectors and human hosts consequently disrupts the life cycle of the malaria parasite. Intervention methods in that direction involve the use of insecticides, larvicides, topical repellents, and treated bed nets among others, to intercept the vector-host interactions or contact [1]. These interventions are achieved by the use of diverse synthetic chemicals or natural repellants. Mosquito repellants therefore have an important place in protecting man from the bites of the insects. Effective repellants play an important role in disrupting the interaction between mosquitoes and human beings by reducing bites. Repellant compounds should be non-toxic, non-irritating and long lasting [8]. Majority of commercial repellants are prepared using chemicals like allethrin, N-N-diethyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM). DEET is generally considered to be the "gold standard" repellant, providing long-lasting protection of up to 8 h from time of application. However, there are some rare reports of severe reactions in people. Additionally DEET melts plastics causing spoilage of equipment, such as glasses and mobile phones, and many consumers find the odour and sensation on the skin unpleasant. For these reasons, many potential users prefer natural alternatives such as those based on plant extracts which have good repellant properties. Continuous use of a particular insecticide, also leads to the development of resistance in the target insect.

The use of traditional repellants is widespread among the different cultures and communities of Africa and beyond [9]. Most households in the developing world rely on personal protection measures of limited effectiveness, such as burning mosquito coils or leaves [10]. The first methods used by man to repel insects were with smoke, covering the skin with mud, or by applying a variety of animal fats and greases [11]. Smoke is a common method used throughout the world in repelling biting insects. In remote areas in Africa, people have used various types of plant-leaves either raw and into powdered form or burnt to release smoke [12]. Fresh or dried plants are also frequently added to fires to enhance the repellant properties of the smoke [13]. Several field evaluations, where plants were burned to repel mosquitoes, have shown good reduction in mosquito landings [12, 14, 15].

The use of plants in mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment [16]. Generally, natural products are safe for human use compared to synthetic compounds [17, 18]. Repellants of plant origin are therefore likely not to pose hazards of toxicity to human and domestic animals and are easily biodegradable. Plants often possess some bioactive components known as phytochemicals which play many ecological functions such as repelling insects, pests and animals. The repellent activity of plants might be due to the presence of certain chemicals that are able to irritate the olfactory senses of the mosquitoes. Some of these chemicals are non-volatile and release insecticidal smoke when the plant materials or the mosquito coil containing the active ingredients are burnt. According to Ghosh and Chandra [19] botanicals have widespread insecticidal properties and will obviously work as a new weapon, and in future may act as suitable alternative products to fight against vector mosquitoes. In-depth laboratory studies and field bioassays are needed to achieve that. They emit volatile organic compounds at different concentrations. Some volatile compounds such as aldehydes (octanal, nonanal and decanal), and ketones (6-methyl-5- hepten-2-one and geranylacetone) have been found in olfactometer trials, to interfered with attraction of mosquitoes [20]. A mixture of 6-methyl-5-hepten-2-one and geranylacetone in a 1:1 ratio was found to even exceed the repellency of DEET when presented at low concentrations [20]. The draw-back of using plant-based repellants is that, many of them are made up of relatively volatile constituents and making them generally not effective over long periods of time and as such require frequent reapplication [21]. Various plants including *Azadirachta indica*, *Eucalyptus sp.* (*Myrtaceae*), *Lantana camara (Verbanaceae)*, *Cymbo- pogon spp. (Gramineae)*, *Mentha piperita (Labiatae)*, *Tagetes minuta (Compositae)* have been reported to possess repellent activity against mosquitoes.

Laboratory repellency and field trials are usually carried out to test whether chemicals or their sources could provide protection against mosquitoes. These chemicals could be alkaloids, phenolics and terpenoids. Secondary metabolites, such as terpenes [22] and other active ingredients are responsible for mosquito repellent activity in most plants. Alkaloids are basically insecticidal at low concentrations; nevertheless they can be used as repellents. For a material to be valuable as a mosquito repellant 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.

The ethnobotany of *Hyptis suaveolens*, *Cassia obtusifolia* and *Striga hermonthica* has extended their use as mosquito repellants. *Hyptis suaveolens* belongs to the family *Lamiaceae*. It is a herbaceous aromatic plant, 0.5 to 1.5m tall or more, with a stem carrying opposite leaves in finely toothed edges to which are grafted an oval limb and influorescence in axillaries cymes. It is considered as an obnoxious weed distributed throughout the tropics and subtropics [23] but the oil extracts have been reported to be effective against insects [24]. *Cassia obtusifolia*, also called Sicklepod belongs to leguminosae family. It is an annual, wild legume that has been described as an under shrub herbaceous plant. Reports of the use of *Cassia* species for pest management have also been documented [25]. *Striga hermonthica* is a major biotic constraint in the Savanna zone of West and Central Africa. It is glabrous in appearance and belongs to the family *Scrophulariaceae*. *Striga hermonthica* devastates cereals like maize, sorghum, pearl, millet, finger millet and rice [26]. It has also been found to have mosquito repellent activities [27].

The current study investigated the presence of flavonids, saponins, glycosides, tannins, terpenoids, anthraquinones, steroids, alkaloids, reducing sugar, phlobotannins, ketones and coumarin in extracts of dried leaves of *Hyptis suaveolens*, *Cassia obtusifolia* and *Striga hermonthica*. Mosquito repellency potential of the powdered matured leaves of the three plants was also compared using olfactometry.

Use of mosquito repelling plant leaves in rural communities as a preventive measure against mosquitoes thus malaria has the potential to reduce the burden of purchasing anti-malarial drugs and save lives, hence the need for the current study. There have been many earlier studies on repellent and attractant properties of plants products [28-31]. However, the use of raw powdered leaves has several advantages and thus is suitable for rural application where women and children are affected by malaria under impoverished conditions. It would reduce cost of using energy to extract the leaves, the risk of fire outbreak possible in the use of smoke, and nuisance resulting from topical application of repellents. Results and use of the current study would be useful in augmenting the much promoted use of bed nets to restrain the vector especially in rural communities.

2. MATERIALS AND METHODS

Leaf samples of *Hyptis suaveolens*, *Cassia obtusifolia*, *Striga hermonthica* and adult mosquitoes, olfactometer, net-cage and tray.

2.1. Chemicals and Reagents

Chemicals and reagents such as ethanol, sulphuric acid, distil water, ferric chloride solution, hydrochloric acid solution, Wagner's reagent**,** chloroform, concentrated ammonium hydroxide, sodium hydroxide, Fehling solution (A and B) and iodine were used in the phytochemicals screening process. All chemicals were purchased from local agent of BDH Laboratories in Ghana.

2.2. Apparatus, Glassware and Equipment

Conical flasks, pipette, beakers, olfactometer, net-cages, laboratory burner and aspirators were used.

2.3. Sample Collection and Preparation

Fresh matured leaves of *Hyptis suaveolens, Cassia obtusifolia and Striga hermonthica* were collected from Ghana in the surrounding communities of the University for Development Studies, Navrongo campus. The samples were cleaned from dirt by washing with distilled water. They were then dried at room temperature to reduce the moisture content and to facilitate grinding. After drying, they were then ground using electric blender and the fine samples kept in clean, well labeled polyethylene bags and stored in a freezer until needed for use.

2.4. Extraction Plant Samples

96% ethanol was used to extract each of the powdered leaf samples. 25 g of the powdered leaves sample was shaken in 250 ml of the solvent. The mixtures were allowed to stand for 24 hours to extract. The procedure was also repeated using distilled water as a solvent. They were then filtered and the extracts were used in phytochemical screening. Screening was done according to procedures found in Magadula and Tewtrakul [32] and Trease and Evans [33].

2.5. Breeding of Mosquitoes

Adult mosquitoes were obtained by breeding them from the egg stage using modified procedures indicated in Gerberg [34]. Ovitraps were set to collect mosquito eggs in an area of high mosquito population. This was done by putting pieces of filter pater into small containers with the inside coated dark. Each container with the filter paper was half filled with water. Four days later, when eggs were laid by mosquitoes attached to the inserted filter papers, the filter papers with the eggs, were removed and placed in trays under laboratory conditions of suitable humidity and temperature. Direct sunlight was avoided. The trays were then flooded with water and covered with a net. After two days, the eggs hatched into larvae. The larvae were fed each day with yeast up to seven days, when they had developed into pupae which were then transferred into the net-cage. The next day, the pupae metamorphosed into young mosquitoes which were fed with sugar solution made available in the cage. They became fully grown in three days and ready to be used in the experiment.

2.6. The Olfactometer

A three-chamber linear olfactometer, a modified form based on Wasserberg, et al. [35] was constructed and used in the repellency experiment. The olfactometer was constructed using three plastic containers, PVC pipe and a rubber. It has two response chambers and one test chamber between them. Each response chamber had an opening for light penetration and aeration. The openings are covered with fine mosquito netting. All the three chambers were connected by means of a 5.5 cm diameter polyvinylchloride tubes 6 cm long between the chambers. The connecting channels offered test mosquitoes the choice to either remain in the test chamber or move through the channel into the adjoining chamber.

Figure-1. Olfactometer
Source: Constructed personally (Modified from Wasserberg, et al. [35])

The top opening of each of the chambers was covered with a transparent rubber to enable viewing into the chambers. A small hole was created in the top covering rubber of the test chamber through which mosquitoes could be transferred into the chamber.

2.7. Tray and Net-cage

Constructed Trays and Net –cages, were used in the mosquitoes breeding process.

Figure-2. The Tray and the Net-cage
Source: Constructed personally for collection of mosquitoes

The tray was made from a plastic container and a fine mosquito net. It was fabricated by covering the plastic container with the fine net held together using glue. The net-cage was also constructed using a plastic container and fine mosquito net. An opening was created on one side of the plastic container around which was glued the net in such a way to create a circular aperture for passing the pupae through and for removing developed adults. The top was also covered with net.

2.8. Olfactometry

About 25g of powdered leaves of *Striga hermonthica*, was placed in one response chamber while the other response chamber was left empty to serve as a control. Twenty (20) adult mosquitoes were transferred from the net-cage using the aspirator into the test chamber through the small hole in the top transparent rubber-cover. The hole was closed back with a small piece of the rubber just after the transfer. Opaque lids were then used to cover each chamber.

Each test was timed for 15 mins and the number of mosquitoes in each chamber was noted by viewing through the transparent rubber cover. The experiment was repeated twelve (12) times and the mean number of mosquitoes in each test was determined. The experiment was also repeated using powdered leaves of *Hyptis suaveolens* and *Cassia obtusifolia*. Comparative tests were also carried out where different samples were placed in the response chambers in one test. Two strongly repelling leaves samples were identified and used in comparative tests with standard repellants (powdered form of Holy black mosquito coil and Medisoft mosquito repellant).

Results from each test were recorded as the number of mosquitoes counted in each chamber at the end of the test. The mean number of mosquitoes, standard error (SE) and level of significant difference at 95 degrees of confidence were calculated in each test.

2.9. Repellent efficiency

Repellent efficiency was calculated using the following relation:

$$\text{Percent repellent efficiency (E)} = \frac{\text{Mean number of mosquitoes repelled}}{\text{Total number of mosquitoes used}} \times 100\% \quad \text{.........................(1)}$$

3. RESULTS

Table-1. Phytochemical composition of *Striga hermonthica*, *Hyptis suaveolens* and *Cassia obtusifolia*

Extract	Phytochemicals	*Striga hermonthica*	*Hyptis suaveolens*	*Cassia obtusifolia*
Aqueous extract	Saponins	_	_	++
	Flavonoids	+	++	+
	Anthraquinones	_	_	+
	Tannins	+	+	++
	Ketones	+	+	+
	Reducing Sugar	+	+	++
	Coumarin	_	+	+
Ethanolic extract	Alkaloids	+	+	++
	Terpenoids	++	++	+
	Phlobotannins	+	+	+
	Steroids	++	++	++
	Glycosides	+	+	++

Key: (+) - present in small amount, (++) - present in considerable amount and (-) -not present.

Table-2. Behavioral responses of adult mosquitoes to dried powdered leaves

Test	Stimuli	Mosquitoes repelled (Mean ± SE)	Repellent efficiency (%)	P
S. hermonthica leaves compared to empty chamber	*S. hermonthica*	11.50±0.50	83.4	<3.9E-6
	Empty chamber	33.00±0.53	16.6	
H. suaveolens leaves compared to empty chamber	*H. suaveolens*	10.17±0.52	78.3	<1. 7E-7
	Empty chamber	4.33±0.36	21.7	
C. obtusifolia leaves compared to empty chamber	*C. obtusifolia*	6.58±0.42	70.8	N S
	Empty chamber	5.83±0.39	29.2	
Holy black mosquito coil compared to empty chamber	Powdered Holy black mosquito coil	13.00±0.51	90.0	<7.8E-9
	Empty chamber	2.00±0.28	10.0	

Source: From determination of number of mosquitoes repelled in the olfactometer

Table-3. Comparative behavioral responses of adult mosquitoes to dried powdered leaves

Test	Stimuli	Mosquitoes repelled (Mean ± SE)	Repellent efficiency (%)	P
S. hermonthica leaves compared to *H. suaveolens* leaves	*S. hermonthica*	6.50±0.63	67.5	NS
	H. suaveolens	5.42±0.70	32.5	
S. hermonthica leaves compared to *C. obtusifolia* leaves	*S. hermonthica*	8.33±0.82	58.3	<0.0009
	C. obtusifolia	3.91±0.48	41.7	
H. suaveolens leaves compared to *C. obtusifolia* leaves	*H. suaveolens*	8.00±0.79	60.0	<0.0314
	C. obtusifolia	5.42±0.54	40.0	

Source: From determination of number of mosquitoes repelled in the olfactometer

Table-4. Comparative behavioral responses of the plant samples with the standard repellent

Test	Stimuli	Mosquitoes repelled (Mean ± SE)	Repellent efficiency (%)	P
Dried powdered *S. hermonthica* leaves compared to that of Holy black mosquito coil	*S. hermonthica*	4.50±0.47	22.5	NS
	Powdered Holy black mosquito coil	5.25±0.58	77.5	
Powdered *H. suaveolens* leaves compared to that of Holy black mosquito coil	*H. suaveolens*	3.92±0.57	19.2	NS
	Powdered Holy black mosquito coil	5.33±0.63	80.5	
Dried powdered *S. hermonthica* leaves compared to that of Medisoft mosquito repellant	*S. hermonthica*	3.5±0.52	7.5	7.29 E-7
	Medisoft mosquito repellant	8.17±0.55	82.5	
Dried powdered *H. suaveolens* leaves compared to that of Medisoft mosquito repellant	*H. suaveolens*	2.67±0.43	13.4	1.36 E-07
	Medisoft mosquito repellant	9.08±0.54	86.6	

Source: From determination of number of mosquitoes repelled in the olfactometer

4. DISCUSSION

Research reports by Ansari and Razdam [28] stated that, active ingredients such as alkaloids, flavonoids, saponins, phenolics and tannins, present in plant extracts have influenced behavior of mosquitoes [28]. Also, some oils extracted from plants have been reported to have insect repellence [24] while flavonoids have also been reported to possess cytotoxic effects on insects [36]. The phytochemical results **(Table 1)** indicated that tannins, ketones, reducing sugar, alkaloids, phlobotannins, steroids, glycosides, and flavonoids were present in all three plants but in varying amounts. Saponins and anthraquinones were found only in *Cassia obtusifolia* while coumarins were present only in *Hyptis suaveolens* and *Cassia obtusifolia*. The phytochemistry could therefore play a major role in mosquito repellency of the plants under study. Research has also shown that alkaloids and tannins in plant leaves may interfere with insect nerve impulses and are therefore good insecticides [37]. Therefore, the phytochemical deposits indicated in the leaves under study would contribute to mosquito repellent activity.

Studies have shown that insects respond to volatiles in their environment [38] and the influence of these volatiles may lead to a 'push' of the insect away from their hosts [39, 40]. The assumption in the current tests was therefore that, the mosquitoes would move away from a particular chamber containing if contents in the chamber were noxious. When the individual repellants were tested, the leaves of *Striga hermonthica* repelled a mean of 11.50±0.50 mosquitoes, with a repellence efficiency of 83.4 % **(Table 2)**. *Hyptis suaveolens* also repelled a mean of 10.17±0.52 (repellence efficiency of 78.3 %) mosquitoes compared to empty chamber. Thus, *Hyptis suaveolens* leaves may only be moderately repellent compared to leaves of *Cassia obtusifolia* which repelled only 6.58±0.42 mosquitoes, with a repellence efficiency of 70.8 %. *Cassia obtusifolia* therefore caused the least repellence of the mosquitoes. Except for *Cassia obtusifolia*, the other two plant samples were significantly (P = 3.9*E-6 and 1.7*E-7) repulsive compared to the empty chamber **(Table 2)**. Holy black mosquito coil, a standard mosquito repelling coil was highly repellent as it repelled a mean number of 13.00±0.51 mosquitoes (90.0 %) compared to the empty chamber.

In comparative tests of *Striga hermonthica* and *H. suaveolens* **(Table 3)**, the number of mosquitoes repelled by leaves of *Striga hermonthica* and *H. suaveolens* were 6.50±0.63 and 5.42±0.70 respectively. The difference between the numbers of mosquitoes repelled by the two samples was not significant. The repellence efficiency of *Striga hermonthica* and *Hyptis suaveolence* was 67.5 % and 32.5 % respectively. However, the difference in number of mosquitoes repelled by leaves of *Striga hermonthica* compared with *C. obtusifolia*, 8.33±0.82 and 3.91±0.48 respectively, was significant **(Table 3)**. The results confirmed that *C. obtusifolia* leaves were weakly and the least repellant when each plant was tested alone **(Table 2)**. The results also suggest that leaves of *Striga hermonthica* were also better repellant than *Hyptis suaveolence*. Considering the comparative tests results **(Table 3)**, it could be seen that *S. hermonthica* repelled higher number of mosquitoes when compared to *C. obtusifolia* than when it was compared to *H. suaveolens*. This further indicated that, *C. obtusifolia* had a weak repellency and so in presence of *S. hermonthica* higher number of mosquitoes moved from the test chamber into the chamber containing *C. obtusifolia* (ie. *away from S. hermonthica*). From results of the comparative test between *H. suaveolens* and *C. obtusifolia*, the respective mean numbers of mosquitoes repelled were 8.00±0.79 and 5.42±0.54, and the difference was significant (P = 0.0314). *H. suaveolens* leaves were therefore better repellents to mosquitoes compared to *C. obtusifolia* leaves.

Throughout the tests, *Striga hermonthica* and *H. suaveolens* leaves repelled higher mean number of mosquitoes and therefore were tested in comparison with two standard repellants, Holy black mosquito coil and Medisoft mosquito repellant. A mean number of 4.50±0.47 and 5.25±0.58 mosquitoes were respectively repelled by leaves of *Striga hermonthica* compared to Holy black mosquito coil. The difference in repellency between the two repellants was not significant. Also, in a comparison of *H. suaveolens* with Holy black mosquito coil, the mean numbers of mosquitoes repelled were 3.92±0.57 and 5.33±0.63 respectively. The difference between the numbers of mosquitoes repelled by the two repellants was not also statistically significant **(Table 4).** However, in the comparison of Medisoft mosquito repellant with *H. suaveolens* and *S. hermonthica* seperately, Medisoft mosquito repellant was found to be significantly repellent compared to the two plant samples. A mean number of 8.17±0.55 and 9.08±0.54 mosquitoes where respectively repelled by the standard product to *S. hermonthica* and *H. suaveolens* **(Table 4).** The calculated repellency efficiency therefore showed that the standard repellant is most repellent **(Table 4)**.

It was reported [15] that burning of repellant plant leaves to produce smoke, is the most widely used means of repelling mosquitoes. Some field evaluations, where plants were burned to repel mosquitoes, have shown good reduction in mosquito landings [15]. Nevertheless, there are earlier reports on repellant activity of the volatile phytochemical extracts application on human body [1]. Though *C. obtusifolia* had low repellency potential, it could become more effective if the leaves were smoldered as it contained high concentration of tannins. Terpenes are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils. Monoterpenes, are major components of many essential oils [41]. They mostly contribute to the odoriferous constituents or *'essences'* of aromatic plants that are used in repelling insect when they are burnt [22, 41, 42]. However, in rural homes there is the need to reduce cost of using energy to extract the leaves, the risk of fire outbreak in the use of smoke, and nuisance resulting from topical application of repellents. Therefore the poor repellency potential of *C. obtusifolia* leaves could be associated to its low level of terpenes. On the other hand, *Hyptis suaveolens* and *Striga hermonthica* have higher terpenes present **(Table 1)**, hence justifies their abilities in mosquito repellence compared to *Cassia obtusifolia.*

Both *Hyptis suaveolens* and *Striga hermonthica* powdered leaves exhibited good repellency potential in comparison to Holy black mosquito coil, a standard product. The two unrefined plant materials were therefore comparably good. Studies have shown that changes in behavioural responses by insects to compounds can occur through forms of 'conditioning' or 'learned behaviours' that are not genetically determined [43]. It may therefore be possible for mosquitoes to later become familiar to the two good plant products, thus resulting in reduced efficiency.

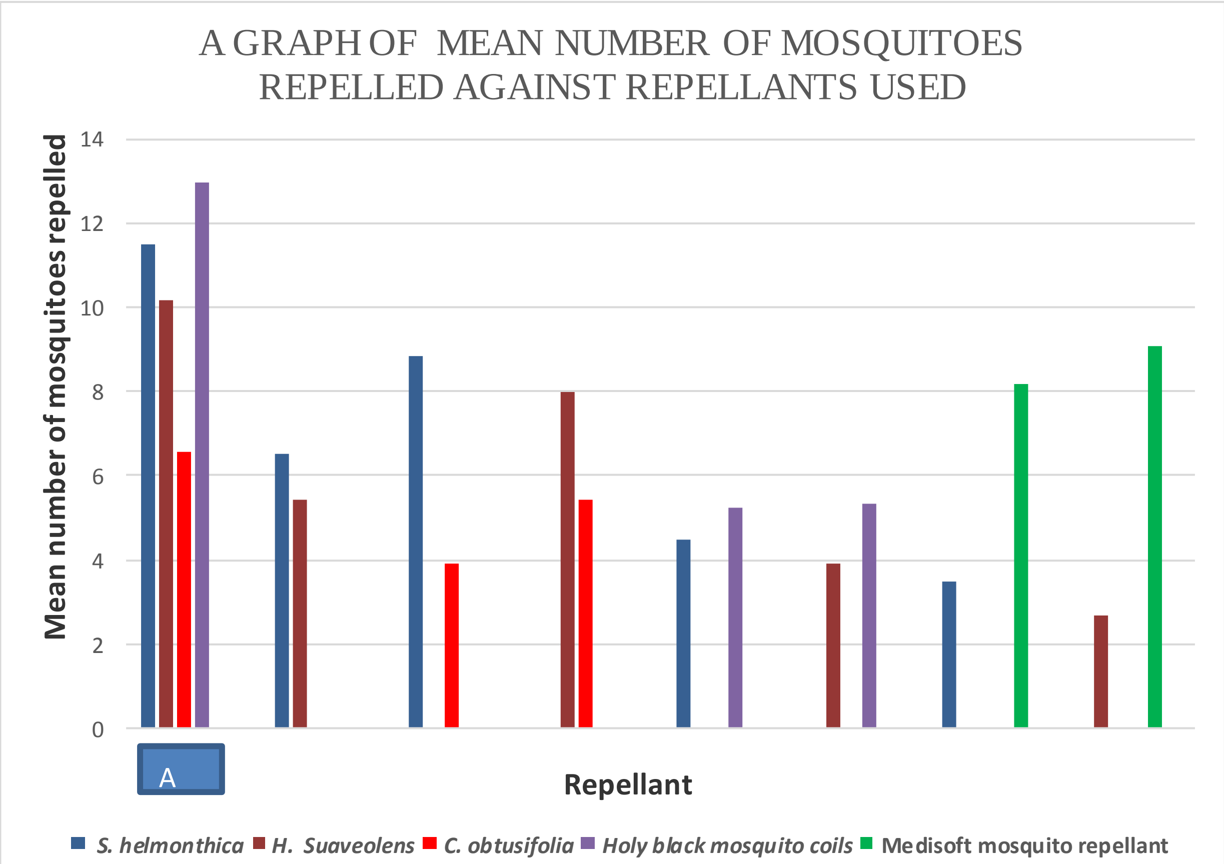

Figure-1. Graph of mean number of mosquitoes repelled by each repellant

Source: Drawn from tables 2-4

From the graph in Figure 1, the relative potential of each of the repellants is shown. "A" represents test of plant sample compared to empty chamber, while the other tests are all comparative tests.

As expected, each of the repellants showed higher repellency potential when they were tested separately, compared to empty chamber, than in the comparative tests. In the comparative tests, some of the mosquitoes remained in the test chamber. Depending on the degree of repellency difference between the two repellants used in the comparative test, mosquitoes may have preferred conditions in the test chamber compared to either response chambers, and thus remained in the test chamber.

5. CONCLUSION

In *Striga hermonthica* leaves, glycosides, flavonoids, tannins, ketones, alkaloids, reducing sugar and phlobotannins were detected in smaller amounts whereas steroids and terpenoids were found in considerable amounts. *Hyptis suaveolens* leaves had glycosides, tannins, alkaloids, reducing sugar, phlobotannins, ketones and coumarin in smaller amount but flavonoids, terpenoids and steroids were identified in larger amounts. In *Cassia obtusifolia* leaves, flavonoids, anthraquinone, ketones, coumarin, terpenoids and phlobotannins were detected in smaller amounts but glycosides, saponins, tannins, steroids, alkaloids and reducing sugar were identified in larger amounts.

The plant leaves with the highest repellant efficiency among the three plant leaves, was *S. hermonthica.* It repelled 11.50±0.50, 6.50±0.63 and 8.33±0.82 mean number of mosquitoes when it was compared to empty chamber, powdered *Hyptis suaveolens* leaves and *Cassia obtusifolia* leaves respectively. Leaves of *H. suaveolens* were found to be the second efficient repellant sample. It repelled 10.17±0.52 and 8.00±0.79 mean number of mosquitoes when it was compared to empty chamber and *C. obtusifolia* leaves respectively. *C. obtusifolia* leaves had the least repellency potential and mean numbers of mosquitoes repelled, 6.58±0.42 and 5.42±0.54 respectively compared to empty chamber and *Hyptis suaveolens.* The standard controls were better repellants compared to all the three plant leaves, Medisoft mosquito repellant being more efficient than Holy black mosquito coil. There was significant difference between the number of mosquitoes repelled by Medisoft and those repelled by powdered leaves of *Striga hermonthica* and *Hyptis suaveolens* while the difference by Holy black mosquito coil was not statistically significant.

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