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Repellent Potential of *Tageteserecta L.* and *Callistemon brachyandrus Lindl.* Against Mosquito Larvae for Formulation of Herbal Repellent Compounds

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ABSTRACT: To avoid harmful effects of commercially available mosquito repellent creams in market, an attempt has been made to prepare herbal formulations from natural products such as flowers of *Tagetes erecta L.* and leaves of *callistemon brachyandrus Lindl.* These are ornamental plants, flourishing in India. All parts of the plant, from root to seed possess a multitude of phytochemical secondary metabolites and also responsible for repellent activity. To evaluate the repellent potential, phytochemical studies, larvicidal activity, smoke toxicity and repellent activity tests were performed against *Anopheles stephensi, Culex infulus and Aedes agepyti.* Water extract of *Tagetes erecta L.* showed of mortality rate of 40%, 40%and 50% and *Callistemon brachyandrus Lindl.* water extract showed 20%, 13.33% and 40% mortality rate against *Anopheles stephensi, Culex infulus and Aedes agepyti* respectively. These plant extracts were used to formulate herbal repellent compounds such as incense log and cream. Repellent activity test for cream formulation showed 89.87%, 87.5% and 90% protection and smoke toxicity test for incense log showed 66.25%, 70% and 67.5% protection against *Anopheles stephensi, Culex infulus and Aedes agepyti respectively*

Keywords: Tagetes erecta L., Callistemon brachyandrus Lindl., larvicidal activity, formulation repellent compound, repellent activity, smoke toxicity.

I. INTRODUCTION

Mosquito borne diseases are the major sources for death in developing countries. *Anopheles stephensiliston* is a major insect vector in India as well as some other countries in the world has been shown directly responsible for about 40-50% of the annual malarial incidence [1],[2].Control of mosquitoes is something important in the present day with increase in number of mosquito borne illnesses. Malaria kills 3 million people each year, including one child every 30 second [3]. Although insect borne diseases are responsible for major health problems in tropical and subtropical climate level.

Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors responsible for the pathogens of various diseases like malaria, filariasis, Japanese Encephalitis, Dengue fever and yellow fever. Most parasitic disease is the tropical, indentifying globalization and climatic changes are increasing the risk of contracting arthropod-borne illnesses [4],[5].One example is West Nile virus; current federal statistics indicate that it will be responsible for thousands of cases of neuroinvasive disease in North America over the next several years [6].

DEET (N,N-diethyl-meta-toluamide) is the active ingredient in many insect repellent products. DEET has been found to inhibit the activity of a central nervous systemenzyme, acetylcholinesterase, in both insects and mammals. This enzyme is involved in the hydrolysis of the neurotransmitter acetylcholine, thus playing a role in the function of

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Vol. 3, Issue 5, May 2014

the neurons which control muscles[7]. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. There are a number of suitable alternatives to using topical pesticides on human skin [8]. These types of products are completely non-toxic, with no side effects, and are just as effective as dangerous chemical pesticides. The only difference is that they may need to be applied more often.Many mosquito repellents and insect repellents are used to control the mosquitoes. Unfortunately these are loaded with chemicals like propoxur, dichlorvos and transfluthrin which are harmful to human beings. This is true of any insect repellent, anywhere in the world. Because of this fact; people are seeking natural ways to repel mosquitoes[9]. One natural and effective way to repel mosquitoes is the use of mosquito repellent plants.

II REVIEW OF LITERATURE

In this study of bioassay of herbal mosquito repellent formulated from the essential oil of plants., the repellency activities of four formulated herbal mosquito repellents of some selected MAPPs Nigeria were evaluated. The essential oils were extracted by hydrodistillation method at 50^0C using all glass clevenger apparatus. These were then stored at 4^0C in refrigeration and it graded concentration (6%, 8%, 10% and 12%) of the essential oils in a complex solution of polyethylene glycol, ethanol and water were prepared and their mosquito repellency activities was carried out in the laboratory against standard kisumu strain of *Anopheles gambiae.* This study showed that both 8% and 10% formulation have the most promising activity exhibiting 68-95% repellency activities lasting for 2 hours.The standard exhibited 75-100% repellency activity which also lasted for 2hours as compared to 8% and 10% formulations. This study demonstrated the potential use of essential oils from medicinal plants as mosquito repellent[10].

Repellent activity of Ageratum houstonianumMill. (Asteraceae) leaf extracts against *Anopheles stephensi, Aedesaegypti*and*Culexquinquefasciatus(Diptera: Culicidae* was determined for hexane, ethyl acetate and methanol crude extracts of *Ageratum houstonianum(A. houstonianum)* leaves against adult *Anopheles stephensi,Aedes: aegypti(Ae. aegypti)* and*Culexquinquefasciatus(Cx. quinquefasciatus)*offixed concentration of Bioassay on laboratory reared Swiss albino mice by topical application at a 0.01 per cent with coconut oil as a base. Crude leaf extracts of *A.houstonianum*in combination with coconut oil repelled vector mosquitoes. Amongst the three extracts, methanol extract gave the maximum protection of 95.0% against *Cx. quinquefasciatus.* Hexane and ethyl acetate extracts gave a maximum of 93.4% protection against *An. stephensi.*finally they conformed The crude extracts of *A. houstonianum*leaves in combination with coconut oil showed repellent activity with repellent quotient ranging from 0.6 to 0.9 [21].

Larvicidal and pupicidal potential of the methanolic extracts from *Moringaoleifera* (*M. oleifera*) plant seeds against malarial vector *Anaphelesstephensi* (*A. stephensi*) mosquitoes at different concentrations such as 20, 40, 60, 80 and 100ppm were studied. They found out larvicidal activity of *M. oleifera* exhibited in the first to fourth instar larvae of the A. stephensi, and the LC_{50} and LC_{90} values were 57.79ppm and 125.93ppm for the first instar, 63.90ppm and 133.07ppm for the second instar, 72.45ppm and 139.82ppm for the third instar, 78.93ppm and 143.20ppm for the fourth instar, respectively. They found out the phytochemicals derived from *M. oleifera* seeds extracts are effective mosquito vector control agents [11].

Essential oil of five plant species *Centuellaasiatica, Ipomoea cairica L., Momordicacharantia L., Psidiumguajava L.* and *Tridaxprocumbens L.* for their topical repellency effect of malarial vector *Anopheles stephensi was studied*. They have used different concentration for repellency test such as 2, 4 and 6%. In this study, clear dose-response relationships were established in all essential oils, with higher concentration of 6% provided high repellency effect [20].

III. MATERIALS AND METHODS

Sample Collection

The plant *Tagetes erecta L.* was collected from Sirumalai, Dindigul District, Tamil Nadu. The plant*Callistemonbrachyandrus Lindl.*was collected from the area around K.S.Rangasamy college of Technology, Tiruchengode, Namakkal District, Tamil Nadu. They were identified at Tamil Nadu Agriculture University,

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Vol. 3, Issue 5, May 2014

Coimbatore. The plants were allowed to dry under shadow condition. The dried plant materials were powdered by an electrical blender. The powder plant material was stored at 4^0C for further use [10].

Mosquito Larvae Collection

The mosquito larvae of *Anopheles Stephensi, Culex infulus* and *Aedes agepyti* larvae of IV pupae were collected from Southern Indian Branch, National Center for Disease Control, Mettupalayam. The mosquito larvae were maintained at room temperature. The larvae were fed with dog biscuits and yeast at 3:1 ratio [11].

Phytochemical Screening

Dried and powder form of leaf of *Callistemon brachyandrus Lindl*. and flower of *Tagetes erecta L.* were subjected to quantitative phytochemical screening for the identification of various classes of active chemical constituents such as tannins, alkaloids, cardiac glycosides, saponins, reducing compounds and anthraquinones by the methods used in [12]-[14]

IV. FORMULATION OF HERBAL REPELLENT COMPOUNDS

Formulation of Repellent Compounds

Flower of *Tagetes erecta L.* and leaves of *Callistemon brachyandrus Lindl.* ethanol extracts were allowed to evaporation at room temperature and residue was collected that was used to formulate insense log. This product was used to smoke toxicity assay. The mosquito repellent insense log were prepared following the method, 10g of charcoal powder, 3g of white wood powder, 4g of joss powder. All the three were thoroughly mixed with distilled water to form a semisolid paste. Insense log (approximately 3.48±0.2 g weight) was prepared manually by using moulding techniques and shade dried. The control insense log was prepared without the plant ingredient. Pure petroleum jelly (22.37 g) was accurately weighed into a 250 ml wide –mouth glass beaker and heated in water bath at temperature of about 50^0C. At this stage, 1.73 g of *Tagetes erecta L.* and *Callistemon brachyandrus Lindl.*powder (residue) obtained in ethanol extraction was added and mixed well. The mixture, in a screwed, covered bottle, was stirred properly to ensure that the sample was uniformly mixed with the molten stage of cream. The product is then taken for repellency test [15] & [16], [23].

V. CHARACTERIZATION STUDY

Test for Larvicidal Activity

Anopheles Stephensi, Culex infulus and *Aedes agepyti* larvae were used to test the larvicidal activity of Leaves of *Callistemon brachyandrus Lindl.* and flowers of *Tagetes erecta L.* 30^{ty} IV instar larvae of all three mosquito family were kept in 50ml glass beaker containing 40 ml of dechlorinated water (control) and different concentration of both sample was prepared (20,40,60,80 and 100ppm) from stock solution. Three replicates for each concentration were set up. The control mortality rate was corrected by using Abbott's formula [17] – [19].

Abbott formula is,

$$\text{Mortality rate \%} = \left(1 - \frac{\text{No. of dead larvae after treatment}}{\text{No. of larvae in control after treatment}}\right) \times 100$$

Repellent Activity

Repellent activity of mosquito repellent cream was tested with human volunteers in South Indian Branch, National Centre for Disease Control. For the repellent activity of plant extracts percentage protection in relation to dose method was adopted. Three to four days old blood starved female adult mosquitoes (40) were kept in a net cage of 60cm×40cm×100cm and window 20cm×20cm. The arms of tested person were cleaned with ethanol. After air drying the arm only of the dorsal side of the skin on each arm was exposed and the alcohol served as control. The plant extract containing cream was applied on arms skin of volunteer. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 5 min every 60 min, from12.50pm to 6:50pm. The experiment was conducted three times. The percentage protection was calculated by the following formula [20] & [21].

$$\text{\% protection} = \frac{\text{No. of bites received by control arm} - \text{No. of bites received by treated arm}}{\text{No. of bites received by control arm}} \times 100$$

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International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 3, Issue 5, May 2014

Smoke Toxicity Test

The natural repellent incense log (control I) was prepared by the above procedure and that was used for smoke toxicity test. The experiments were conducted in a glass chamber of 80cm X 40 cm X 60 cm. A window of 30 cm X 10 cm was situated at mid bottom of one side of the chamber. Hundred of three or four days' old blood starved adult female mosquitoes, fed with sucrose solution, were released into the chamber. The experimental chamber was tightly closed. The experiment was repeated three times on separate days, including control mosquitoes of the same age groups. The data were pooled and average values were subsequently used for calculations. Controls were maintained in two sets. One set was run with incense log lacking the active ingredient of plant powder (control II), the other was a commercial coil which was used for positive control to compare the effectiveness of plant coils. After the experiment got over, fed and unfed (active and dead) mosquitoes were counted. The protection given by the smoke from incense log against the biting of adult mosquito was calculated in terms of percentage of unfed mosquitoes due to treatment. The % of protection was calculated for unfed mosquitoes by using the following formula,[22] & [11].

$$\%\text{Protection} = \frac{\text{No. of treated mosquito} - \text{No. of mosquito in control}}{\text{No. of mosquito in control}} \times 100$$

VI. RESULTS

Phytochemical Analysis

The phytochemical and elemental analysis of leaf of *Callistemon brachyandrus Lindl.*and flower of *Tagates erecta L.*showed the presence of useful classes of chemical compounds suchas saponins, tannins, alkaloids and reducing compounds. Table 1 shows the phytochemical results of *Tagetes erecta L.* and *Callistemon brachyandrus Lindl.*

Table 1.Phytochemical constituents of *Tagates erecta L.* and *Callistemon brachyandrus Lindl*.

Plant sample	Phytochemical constituents	Results
Tagates erecta L.	Tannins	+ +
	Alkaloids	+ +
	Cardiac glycosides	+ +
	Saponins	+ +
	Reducing Compounds	+ +
	Anthraquinones	_ _
Callistemon brachyandrus Lindl.	Tannins	+ +
	Alkaloids	+ +
	Cardiac glycosides	+ +
	Saponins	+ +
	Reducing Compounds	+ +
	Anthraquinones	_ _

++ Positive result, -- Negative result

Mortality rate

The results of mortality rate of *Anopheles stephensi, Culex infulus* and *Aedes agepyti* at different concentration of water extract were presented in Table 2. The 100ppm concentration solution showed % mortality of 40%, 40% and 50% in *Anopheles stephensi, Culex infulus* and *Aedes agepyti* respectively. Table3.shows The 100ppm concentration of solution showed % mortality of 20%, 13.33% and 40% in *Anopheles stephensi, Culex infulus* and *Aedes agepyti* respectively.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 5, May 2014

Table 2. Mortality rate of IV instar larvae of *A.stephensi, C.infulus* and *A.agepty* mosquitoes at different concentration of water extract for *Tagetes erecta L.*

Concentration of sample (ppm)	Mosquito larvae family	Exposure time (min)	Total no. of live mosquito larvae before exposure time	Total no. of dead mosquito larvae after exposure time	Mortality rate (%)
20	*Anopheles stephensi*	180	30	-	100
40		180	30	6	80
60		180	30	12	60
80		180	30	12	60
100		180	30	18	40
20	*Culex infulus*	180	30	-	100
40		180	30	9	70
60		180	30	12	60
80		180	30	18	60
100		180	30	21	40
20	*Aedes agepyti*	180	30	-	100
40		180	30	3	90
60		180	30	9	70
80		180	30	9	70
100		180	30	15	50

Table 3. Mortality rate of IV instar larvae of *A.stephensi, C.infulus* and *A.agepty* mosquitoes at different concentration of water extract of leaves of *Callistemon brachyandrus Lindl.*

Concentration of sample (ml)	Mosquito larvae family	Exposure time (min)	Total no. of live mosquito larvae before exposure time	Total no. of dead mosquito larvae after exposure time	Mortality rate (%)
20	*Anopheles stephensi*	180	30	-	100
40		180	30	2	93.33
60		180	30	7	76.67
80		180	30	18	40
100		180	30	24	20
20	*Culex infulus*	180	30	4	86.67
40		180	30	9	70
60		180	30	12	60
80		180	30	23	23.33
100		180	30	26	13.33
20	*Aedes agepyti*	180	30	-	100
40		180	30	-	100
60		180	30	5	83.33
80		180	30	17	43.33
100		180	30	18	40

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 5, May 2014

Formulation of Repellent Compounds

Repellent compounds were prepared such as incense log and cream as said in the procedure. The compounds were tested to assess their efficacy against mosquito by repellent activity and smoke toxicity tests.

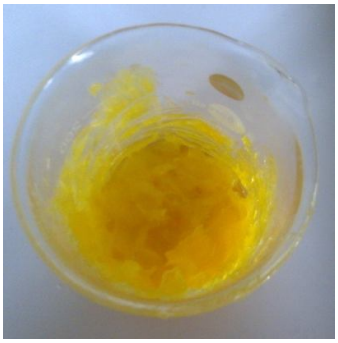

Fig. 1. Herbal Repellent Cream

Fig. 2. Herbal Incense log

Repellent activity and smoke toxicity test

Table 4 shows the repellent activity test conducted with mosquito repellent with sample (Control I), cream without sample (Control II) and already available commercial cream against *Anopheles stephensi*, *Culex infulus* and *Aedes agepyti*. The control I showed effective result of % protection of 89.87%, 87.5% and 90% against *Anopheles stephensi*, *Culex infulus* and *Aedes agepyti.* and it is comparable with available cream in market. Table 5 showed smoke toxicity of three types of repellent products used in smoke toxicity test such as commercial coil, insense log with sample (Control I) and incense log without sample (Control II). Control I showed 66.25%, 70% and 67.5% protection against *Anopheles stephensi*, *Culex infulus* and *Aedes agepyti* respectively. \

Table 4.Repellent activity and % protection of mosquito repellent compounds

Mosquito repellent Product	Observation time (hours)	Mosquito family	Total No. of mosquitoes in cage	Total No. of bites received	% Protection
Control I	6	*Anopheles stephensi*	40	4	89.87
		Culex infulus	40	5	87.5
		Aedes agepyti	40	4	90
Control II	6	*Anopheles stephensi*	40	39.5	-
		Culex infulus	40	40	-
		Aedesagepyti	40	40	-
Commercial product	6	*Anopheles stephensi*	40	4	89.87
		Culex infulus	40	4	90
		Aedes agepyti	40	3	92.5

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(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 5, May 2014

Fig.3. Repellent Activity Test

Fig. 4. Repellent Activity (Volunteer)

Fig.4. Smoke toxicity test

Table 5. Smoke toxicity test of varies mosquito repellent compounds

Mosquito repellent product	No. of mosquitoes tested	Mosquito family	Fed mosquitoes	Unfed mosquitoes	% Protection
Control I	40	*Anopheles stephensi*	8	33	66.25
		Culex infulus	5	36	70
		Aedes agepyti	8	33	67.5
Control II	40	*Anopheles stephensi*	34	6	-
		Culex infulus	33	7	-
		Aedes agepyti	35	5	-
Coil	40	*Anopheles stephensi*	6	34	70.5
		Culex infulus	3	37	74.25
		Aedes agepyti	4	36	75

VII. CONCLUSION

To overcome the harmful effects of commercially available mosquito repellent formulations, herbal formulations are prepared using the flowers of *Tagetes erecta L.* and leaves of *Callistemon brachyandrus Lindl.*

Phytochemical such as alkaloids, flavonoids and monoterpenes are known their mosquito repellent activity. The phytochemical study for *Tagetes erecta L.* showed positive results for tannins, saponins test, alkaloids test, reducing compound test and cardioglycosides test and showed negative result on anthraquinones. *Callistemon brachyandrus Lindl*. Showed positive result on tannins test, saponins test, alkaloids test, reducing compound test and cardioglycosides test and showed negative result on anthraquinones. *Tagetes erecta L.* showed 40% of mortality rate against *Anopheles stephensi* and *Culex infulus*. So, this plant source has maximum larvicidal activity against *Anopheles stephensi* and *Culex infulus*. *Callistemon brachyandrus Lindl.*showed maximum mortality rate 13.33% against *Culex infulus.* So, this plant source has maximum larvicidal activity against *Culex infulus.*

The formulations such as incense log and cream showed better repellent activity and the smoke toxicity test also supports the efficacy of the products in terms of percentage protection. Incense log (Sambrani) showed maximum protection of 90% against *Aedes agepyti* and 89.87% against *Anopheles stephensi* and it is comparable with the commercial cream. Smoke toxicity test of incense log showed maximum percentage protection of 67.5 against *Aedes agepyti*.

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Vol. 3, Issue 5, May 2014

The results of present investigation revealed that flowers of *Tagetes erecta L.* and leaves of *Callistemon brachyandrus Lindl.* showed potential larvicidal activity against *Anopheles stephensi*, *Culex infulus* and *Aedes agepyti.* These herbal products are eco-friendly, non-toxic and possess no side effects; the formulation process is also quite simple.

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