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EXPERIMENTAL INVESTIGATIONS OF NAGAPATTINAM INDIGENOUS MEDICINAL PLANT EXTRACTS AGAINST DENGUE, MALARIA AND FILARIAL DISEASE

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ABSTRACT

To determine phytochemistry, ovicidal and repellent activity of *Sesamum indicum* extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. Phytochemistry properties of flavonoids, tannins, terpenoids, triterpenoids, anthraquinones, protein, phenol and phytosteroids present in the methanol extract of *S. indicum*. The ovicidal activity was firm against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* to varied concentrations starting from 50-300 ppm underneath the laboratory conditions. The repellent activity of *S. indicum* tested at concentrations of 5.0 mg/cm² was evaluated in an exceedingly net cage (45 cm × 30 cm × 45 cm) containing 100 blood starved female mosquitoes of *A. aegypti, An. stephensi and Cx. quinquefasciatus* using the protocol of WHO (2009). Phytochemical screen of methanol extracts of *S. indicum* plant contain flavonoids, saponins, tannins, terpenoids, tri-terpenoids, anthraquinones, phenol, protein and phytosteroids. Similarly, the methanol extract of *S. indicum* was found to be most effective against *Cx. quinquefasciatus*. For methanol extract exerted 250 and 300 ppm, respectively. The repellent activity of methanol extract of *S. indicum* was most effective and maximum activity was observed at 5.0 mg/cm² concentration provided 100% protection up to 120, 150 and 180 minutes against *Cx. quinquefasciatus*. In this study was undertaken to assess the phytochemistry, ovicidal and repellent potential of methanol extracts from the *S. indicum* against *Cx. quinquefasciatus*.

Keywords: Sesamum indicum, Phytochemistry, Ovicidal, Repellent activity, Vector mosquitoes.

INTRODUCTION

Mosquitoes represent a major threat to human health due to their ability to vector pathogens that cause diseases that afflict millions of folks worldwide (WHO, 2010). A. aegypti L. is mostly called a vector for an arborvirus answerable for infectious disease and chikungunya that is endemic to South Asia, the Pacific island space, Africa, and also the Americas. In terms of dengue, 2.5 billion folks live in danger of infection with one or a lot of the four serotypes of the virus, that cause an calculable 390 million infections a year (Bhatt et al., 2013), and also the affected space has exaggerated speedily within the past 30 years (Guzman et al., 2010). Chikungunya is unfolding Tiger mosquito, A. albopictus. Chikungunya occurrences in Europe have drawn the attention of the western world to the current

disease; unfold by the Asian tiger mosquito, A. albopictus (Abramides et al., 2013; Carrieri et al., 2011; Rogers et al., 2014). Malaria is one in all the grave scourges inflicted upon human beings. It causes human mortality and morbidity alongside giant economic loss. Roughly all tropical regions of the planet area unit expertise the recovery and reoccurrence of 1 of the world's an outsized quantity deadly diseases, ie., malaria and India is not any omission. Malaria afflicts one year of the planet folks i.e. 2020 million in 107 countries and territories placed within the tropical and semitropic regions (Panneerselvam et al., 2013). In line with the newest estimates, there have been regarding 198 million cases of malaria in 2013 and a calculable 584,000 deaths. Most deaths occur among youngsters living in continent, wherever a baby dies each minute from malaria. Malaria mortality rates among

youngsters in continent are reduced by Associate in Nursing calculable fifty eight since 2000 (WHO, 2014).

The dipteran Cx. quinquefasciatus is a crucial feature inflicting filariasis, West nile virus, Avion malaria and St. louis encephalitis. Cx. quinquefasciatus, besides known as the southern house dipteran, is extensively studied because it transmits crucial diseases (Samba et al., 2015). In 2014, estimate is impure with lymphatic filariasis parasites and more than 20 for every penny of the planet populace is at danger of getting roundworm disease. In Asian nation it's calculable that regarding 554.2 million folks area unit at hazard of humor disease unhealthiness in a pair of 43 districts (Ghosh et al., 2013). Worldwide, twenty five million men clumsy person with sex organ sickness and over 15 million folks are afflicted with lymphoedema (WHO, 2014). In India, the south site used to smear with oil the body and hair. It is a meal the first-rate feed for poultry and livestock. Sesamum indicum is an ancient spice, one of the initially recorded plants utilized for its seeds. It has been utilized for a large number of years are still an oil seed of overall essentialness. Sesamolin has insect repellent material and is used as a synergist for pyrethrum pesticides (Morris, 2002). Now a day, Sesame oil is a pharmaceutical aid used as a solvent for intramuscular injections and has nutritive, demulcent and emollient properties (Tyler et al., 1976) and has been used as a laxative and mostly used for cosmetics, medicinal industries in manufacturing proprietary branded oils and medicines and less used as a cooking and culinary oil in India (Shah, 2013).

MATERIAL METHODS

Plants assortment

Fully developed leaves of *S. indicum* were collected from Pagasalai village, Nagapattinam District, Tamil Nadu, India, and washed methodically, blotted and shade dried. It had been genuine by plant taxonomer from the Department of botany, Annamalai University. A voucher example is that the stored at the herbarium of plant Phytochemistry division, Department of zoology, Annamalai University, Tamil Nadu, India.

Extraction

The solid leaves were washed with sterile refined water, shade dried, and finely ground. the finely ground leaf powder (500 g/ml) was extracted with hexane, chloroform, diethyl ether, ethyl acetate and methanol exploitation Soxhlet extraction equipment, and therefore the extraction was continued until visibly no more extraction is feasible (by perceptive the colour of the extracted portion). The solvent from the extract area unit removed employing a rotary vacuum evaporator to gather the crude extract and keep at 4°C. Normal stock solutions were ready at 1 % by dissolving the residues in plant product. From this stock solution, totally different concentrations were ready and this solution is employed for larvicidal activity.

Phytochemical screening

The phytochemical take a look at for the screening bioactive chemical constituents within the medicinal plants under study were applied in extracts exploitation the quality procedure (Kokate, 1994; Sathish Kumar et al., 2013). By this analysis, the presence of many phytochemicals test for flavonoids, alkaloids, saponins, steroids, terpenoids, tri-terpenoids, anthraquinones, amino acid, phenol, glycosides, carbohydrate, protein, and phytosteroids.

Mosquito Rearing

The mosquitoes, *A. aegypti, A. stephensi* and *C. quinquefasciatus* were procured from the Centre for research in Medical entomology (ICMR), Viruddhachalam, reared within the laboratory, Department of zoology, Annamalai University. The larvae were gobbled dog biscuits and yeast powder within the 3:1 magnitude relation. Adults were supplied with 100 percent sucrose solution and one week previous chick for feed. Mosquitoes were controlled at 28±2 °C temperature, 70%-85% ratio RH, with a photo amount of 14 h lightweight and 10 h dark.

Ovicidal activity

The method of Su and Mulla (1998) was followed to check the ovicidal activity. The leaf extracts was diluted with the several solvent to attain completely different concentrations (50, 100, 150, 200, 250 and 300 ppm). The freshly ordered egg raft containing 100 eggs of *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* were exposed to every dose of leaf extract till they hatched or died. Every concentration was replicated six times. Eggs exposed to several solvents in water served 48 h post treatment by the subsequent formula.

$$\begin{tabular}{ll} Number of hatched larvae \\ \hline We Hatchability = & $------ \times 100$ \\ \hline Total number of eggs \\ \hline \end{tabular} \begin{tabular}{ll} Number of hatched larvae \\ \hline \end{tabular} \begin{tabular}{ll} Number of hatched larvae \\ \hline \end{tabular} \begin{tabular}{ll} Number of eggs \\ \hline \en$$

Repellent activity

The minutes of protection in admiration to measurement method was utilized (WHO, 2009). Three-day-old bloodstarved female A. aegypti, An. stephensi and Cx. quinquefasciatus mosquitoes (100) were unbroken during a net cage (45 cm \times 30 cm \times 45 cm). The volunteer had no contact with lotion, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, solely 25 cm2 dorsal facet of the skin on every arms was exposed and therefore the remaining space lined by rubber gloves. The crude extracts were applied at 5 mg/cm2 singly within the exposed area of the fore arm. The time of the check enthusiastic about whether or not are the target mosquitoes day or night biters. An. stephensi, Cx. quinquefasciatus are testing throughout the getting dark from 20:00 to 4:00, while A. aegypti was tested throughout the day time 8:00 to 16:00. The management and treated arm were introduced at the same time in to the experimental cages, the mosquitoes were activated. Every check concentration was continual six times. The volunteer conducted their check of every concentration by inserting the treated and management arm in to a similar cage for one full minute for each 5 minutes. The mosquitoes that landed on the hand were recorded so agitated off before imbibing any blood; creating out a five minutes protection. The proportion of repellency was calculated by the subsequent formula.

% Repellency = $[(Ta-Tb)/Ta] \times 100$

Where Ta is the number is that the variety of mosquitoes within the management group and Tb is that the number of mosquitoes within the treated group.

Statistical analysis

Values obtained were subjected to means and standard deviation through to the one-way ANOVA using Statistical Package for Social Sciences 16.0 software. Results with p<0.05 were considered to be statistically significant (Finney, 1971).

RESULTS

Phytochemical screening of hexane, chloroform, diethyl ether, ethyl acetate and methanol extracts of S. indicum plant studied showed that the leaves were rich in alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, triterpenoids, anthraquinones, amino acid, phenol, glycosides, carbohydrate, protein and phytosteroids (Table 1). In the present study, the toxicity of different solvent extracts of S. indicum was experimented against A. aegypti, An. stephensi and Cx. quinquefasciatus. The denoted percent hatchability of A. aegypti, An. stephensi and Cx. quinquefasciatus in Table 2. The methanol extract establish to be extra effective than the other extract against Cx. quinquefasciatus eggs, the 100% mortality at 250 and 300 ppm. The repellent activity of S. indicum was established to be the majority effective for repellent activity against Cx. quinquefasciatus followed by An. stephensi and A. aegypti and a superior concentration of 5.0 mg/cm2 provided 100% protection up to 150 and 180 minutes against Cx. quinquefasciatus (Table 3). From the result, it can be accomplished the extracts of *S*. indicum as an outstanding possible agent for controlling chosen mosquitoes species.



Figure 1. Phytochemical screening of Sesamum indicum extracts.

Table 1. Phytochemical screening of plant extract of Sesamum indicum.

| S. No. | Phyto constituents | Methanol | Ethyl acetate | Acetone | Benzene |
|--------|--------------------|----------|---------------|---------|---------|
| 1 | Alkaloids | | + | + | |
| 2 | Flavonoids | ++ | ++ | | + |
| 3 | Saponins | + | | | |
| 4 | Steroids | | + | | |
| 5 | Tannins | + | | + | |
| 6 | Terpenoids | + | ++ | +++ | ++ |
| 7 | Tri-terpenoids | ++ | +++ | ++ | + |
| 8 | Anthraquinones | ++ | ++ | | + |
| 9 | Amino acid | | | | |
| 10 | Phenol | + | | + | |
| 11 | Glycosides | | | | |
| 12 | Carbohydrate | | + | + | |
| 13 | Protein | +++ | ++ | + | + |
| 14 | Phytosteroids | +++ | + | | + |

^{+++ -} Strongly positive phytochemical group, ++ - Positive phytochemical group, + - Trace phytochemical group, -- Absence of phytochemical group.

Table 2. Ovicidal activity of Sesamum indicum different extracts tested against selected mosquitoes.

| | | Ovicidal activity | | | | | | |
|---------------------|---------------|-----------------------------|------------------------|------------------------|------------------------|-------------------|------------------------|--|
| Species | Solvents | Concentrations tested (ppm) | | | | | | |
| species | | 50 | 100 | 150 | 200 | 250 | 300 | |
| A. aegypti | Hexane | 70.8±2.28 a | 65.6±1.94 ^a | 57.8±3.03 a | 53.2±2.58 a | 48.6±2.19 a | 42.4±2.96 a | |
| An. stephensi | | 58.3±1.57° | 46.5±1.42° | 37.8±1.81 ° | 32.1±1.28 ° | 23.6±1.72 ° | 18.6±1.62° | |
| C. quinquefasciatus | | 64.2±2.16 ^b | 58.8±1.48 b | 51.6±1.81 b | 46.8±1.92 b | 41.2 ± 2.16^{b} | 35.2±1.92 ^b | |
| Control | Chloroform | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | |
| A. aegypti | | 65.6±1.94 a | 51.4±1.81 a | 47.2±1.48 a | 41.6±2.30 a | 35.2±2.16 a | 30.6±2.19 a | |
| An. stephensi | | 54.8±1.64° | 40.2 ± 2.16^{c} | $32.6\pm2.60^{\circ}$ | 28.4±1.81 ° | 18.6±1.94° | 14.2±2.16° | |
| C. quinquefasciatus | | 60.4±1.81 b | 45.2±1.78 b | 37.2±1.48 ^b | 34.2±1.09 ^b | 25.6±1.51 b | 18.6±1.14 ^b | |
| Control | | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | |
| A. aegypti | Diethyl ether | 59.4±1.81 a | 46.2±1.09 a | 37.4±1.81 a | 31.2±2.16 ^a | 26.4±1.81 a | 20.2±2.16 a | |
| An. stephensi | | $50.4\pm2.30^{\circ}$ | 36.2±1.78° | 27.4±1.51 ° | 24.2±1.64° | 13.4±1.14 ° | 9.6 ± 0.54^{c} | |
| C. quinquefasciatus | | 54.6±1.94 b | 41.4 ± 2.30^{b} | 32.6±1.51 b | 27.6 ± 2.19^{b} | 22.2 ± 2.38^{b} | 16.6±1.94 b | |
| Control | | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | |
| A. aegypti | | 57.8±1.92 a | 48.2 ± 2.58^{a} | 36.2±1.48 a | 30.2±2.16 ^a | 24.6±2.30 a | 17.8±1.92 a | |
| An. stephensi | Ethyl acetate | 46.1±1.38° | 35.4±1.88° | 24.9±1.64° | 19.3±1.75° | 12.8±1.69° | NH | |
| C. quinquefasciatus | | 51.4±2.07 ^b | 42.2 ± 2.16^{b} | 29.2±2.16 ^b | 24.4 ± 2.50^{b} | 18.4±1.81 b | 11.2±1.92 b | |
| Control | | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | |
| A. aegypti | | 51.8±2.16 a | 43.4 ± 2.07^{a} | 31.2±2.16 a | 22.6±0.89 a | 16.2±1.09 a | 12.2±2.16 a | |
| An. stephensi | Methanol | 41.9 ± 1.26^{c} | 31.7±1.65 ° | 21.4±1.43 ° | 12.2±2.74° | NH | NH | |
| C. quinquefasciatus | | 46.8 ± 1.48^{b} | 37.4±1.51 b | 25.4±1.81 b | 16.8±1.64 ^b | 9.8 ± 1.78^{b} | NH | |
| Control | | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | 0.00 ± 0.00^{d} | |

Value represents mean \pm S.D. of five replications. Different alphabets in the column are statistically significant at p<0.05 level DMRT Test. Eggs in control groups were sprayed with no phytochemicals.

Table 3. Repellent activity of the *Sesamum indicum* extract against *Aedes aegypti*, *An. stephensi* and *Anopheles quinquefasciatus* at 5 mg/cm².

| | | % of repellency Time post application of repellent (min) | | | | | | |
|---------------------|-----------------|----------------------------------------------------------|-------------|---------------|---------------|---------------|-----------------|--|
| Species | Extracts | | | | | | | |
| | | 30 | 60 | 90 | 120 | 150 | 180 | |
| | Hexane | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 100±0.0 | 94.6±1.67 | |
| | Dichloromethane | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 97.2 ± 2.04 | |
| A. aegypti | Diethyl ether | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | |
| | Ethyl acetate | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | |
| | Methanol | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | |
| | Hexane | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 84.6±2.77 | 76.6 ± 2.62 | |
| | Dichloromethane | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 93.4 ± 2.9 | 88.4 ± 1.78 | |
| A. stephensi | Diethyl ether | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 93.2±1.48 | |
| | Ethyl acetate | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 95.4±1.92 | |
| | Methanol | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 98.7 ± 2.60 | |
| | Hexane | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 91.2 ± 2.04 | 85.2 ± 1.48 | |
| | Dichloromethane | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 96.6±1.94 | 93.6±2.07 | |
| C. quinquefasciatus | Diethyl ether | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 96.2±2.16 | |
| | Ethyl acetate | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 98.2±1.78 | |
| | Methanol | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | |

Value represents mean \pm S.D. of five replications.

DISCUSSION

In our outcomes demonstrated that the hexane, chloroform, diethyl ether, ethyl acetate and methanol have noteworthy phytochemistry analysis, ovicidal and repellent movement against A. aegypti, An. stephensi and Cx. quinquefasciatus. The investigated that the larvicidal, pupicidal, repellent and adulticidal activity of Citrus sinensis against An. stephensi, A. aegypti and Cx. quinquefasciatus. The most noteworthy concentrations of 450 ppm provided over 180 and 150 min protection in ethanol extracts of Citrus sinensis against Cx. quinquefasciatus. Among three vectors tested, the highest adulticidal activity was observed in high mortality followed by An. stephensi, A. aegypti and Cx. quinquefasciatus (Murugan et al., 2012). The larvicidal, oviposition deterrent and repellent activity of Annona squamosa against A. aegypti, An. stephensi and Cx.. quinquefasciatus. The LC₅₀ and LC90 values of 219.41 and 394.87 ppm, severally. In oviposition deterrent activity the best concentration of 0.1%, Annona squamosa manufacture 92.4% against A. aegypti. Skin repellent check at 0.02 ppm concentration of Annona squamosa offers the entire protection time renges from 50.4 to 271 minutes. The Annona squamosa exerted the best protection time of 126.2 minutes (Vijaya kumar et al., 2011). Krishnamoorthy et al. (2015) examination that the larvicidal potential of *Murraya exotica* essential oil against A. aegypti, An. stephensi and Cx. quinquefasciatus. After 12 h of introduction period, the larvicidal actions are LC_{50} = 74.7 and LC_{90} = 152.7 ppm; after 24 h presentation period were LC₅₀= 35.8 and LC₉₀= 85.4 ppm, respectively against A. aegypti. The most noteworthy mortality was found in acetone extract against Ae. aegypti with LC50 and LC₉₀ estimations of 4.1783 and 9.3884 mg/ml, individually. Smoke poisonous quality was seen at 10 min interim for 40 min, and the mortality information was recorded (Govindaraju et al., 2015). The investigated that the larvicidal, ovicidal and repellent activity of Polygala arvensis benzene and methanol extracts tested against A. aegypti, An. stephensi and Cx. quinquefasciatus with maximum LC50 and LC90 values of methanol extract of Polygala arvensis were 58.21, 46.37 and 42.68 ppm; 208.45, 189.82 and 130.44 ppm, respectively. The maximum ovicidal activity of methanol extracts against A. aegypti, An. stephensi and Cx. quinquefasciatus at 200 ppm concentration. The highest repellent activity of methanol extracts provided 100% protection against A. aegypti, An. stephensi and Cx. quinquefasciatus for 280 minutes (Deepa et al., 2014). Among the different extracts of the plants screened the hexane extract of Limonia acidissima recorded the highest ovicidal activity of 79.2% at 500 ppm concentration against the eggs of Cx. quinquefasciatus. Among the Aegle marmelos, Limonia acidissima, Sphaeranthus indicus, Sphaeranthus amaranthaides and Chromolaena odorata extract screened, the hexane extract of Limonia acidissima noted the 100% oviposition deterrent activity at tested concentrations against Cx. quinquefasciatus and A. aegypti adult females (Reegan et al., 2015). The highest lethal activity was recorded against Gnetum ula extract in the experimental larvae of An. stephensi (LC50 = 82.86 ppm). Ovicidal activity revealed that Spermacoce hispida showed more than 50% activity against A. aegypti, An. stephensi and Cx. quinquefasciatus. Notably, at 200 ppm concentration of all the plants showed 100% ovicidal activity against An. stephensi, followed by A. aegypti and Cx. quinquefasciatus. The selected two plants, Gnetum ula, Spermacoce hispida extract offers 100% protection against An. stephensi, A.

aegypti and Cx. quinquefasciatus adult female mosquitoes as far as repellency up to 120 minutes of presentation periods (Dhanasekaran et al., 2013).

CONCLUSIONS

The present study clearly reveals that the extracts of *S. indicum* seem to be made in phytochemicals, ovicidal and repellent activity, wide utilized in traditional drugs to combat and cure varied ailments. The antispasmodic, anti-inflammatory, antidiuretic drug and antianalgesic is attributed to their high steroids, terpenoids, tannins and saponins and extraction of *S. indicum* has efficiency to manage the eggs and adults of the mosquito, *Cx. quinquefasciatus*. Exploitation of those pharmacologic properties involves more investigation of those active ingredients by implementation techniques of purification, separation, crystallization and identification.

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