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Original Article

Evaluating Bioactive Potential of Lichen Secondary Metabolites for the Formulation of Mosquito Repellent

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ABSTRACT

Background: Mosquitoes belong to the major disease transmitters causing millions of deaths every year. The cost of commercially available pyrethroids, environmental regulations and toxicity of commercial repellents force researchers to develop potential repellents of natural origin. Lichens comprise of bioactive compounds exhibiting multifarious biomedical potential. **Objectives:** In this present study, *Parmotrema* species was analyzed for its biomedical potential including antioxidant, anti-inflammatory and repellent activity. **Materials and Methods:** Antioxidant activity was evaluated by DPPH, FRAP assay. Anti-inflammatory activity was assessed by albumin denaturation and protease inhibition assay. The repellent potential evaluated against third and fourth-instar larvae of *Aedes ageypti*. **Conclusion:** Antioxidant activity by DPPH assay and FRAP assay exhibited IC_{50} of 16.08 µg/mL and 24.6 µg/mL respectively. Anti-inflammatory potential via inhibition of albumin denaturation and protease inhibition assay shown maximum inhibition at 100 µg/mL with IC_{50} value of 17.02 µg/mL and 27.5 µg/mL respectively. The repellent test confirmed that at 100 µg/mL, 90% of mortality rate was recorded. The repellent cream formulated form lichen extract exhibited 86.6% protection and smoke toxicity test demonstrated 26.6% protection against *Aedes ageypti*. Thus, the study evidenced the proven biomedical potential of methanolic lichen extract.

Keywords: Mosquito, Lichen, Secondary metabolites, Larvicidal activity, Mosquito repellent activity.

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INTRODUCTION

Mosquitoes causes serious diseases like malaria, dengue, yellow fever, Chikungunya and encephalitis etc., Moreover, 90% of mortality in experienced by young children. It acts as an important vector for causing diseases. A few mosquito-borne pathogens pose potential threats and cause many diseases. So far, no precise antiviral compound has been identified to treat mosquito borne diseases. The existing chemicals which are available to control mosquitoes are very dangerous and may cause irreversible damage to the human health. Further, mosquitoes have developed resistance to the chemical-based formulations[1] which are currently in usage. This mosquito originates in the tropical as well as subtropical region across the world. The female bites humans and animal for blood.

Lichen exhibiting symbiotic relationship and composed of mycobiont (fungus) and photobiont (alga). Lichens are widely distributed in the region contains sufficient moisture, light and altitude region because these will often favour growth of lichen. Lichens produce different range of secondary metabolites which are characterized according to their chemical structure. The mycobiont produces secondary compounds which are accumulated in cortex or in medulla region of the thallus. Lichens produce a wide spectrum of secondary metabolites with diverse biological characteristics. These metabolites are complex, but mostly tiny molecules cconstitute upto 20% dry weight of the lichen thallus.[2] Lichen secondary substances comprises many classes of compounds. Mycobionts are responsible to produce secondary metabolites in lichens.[3] These metabolites have diverse biological potential including larvicidal activity, hence used for the study.

A novel liquid vaporizable formulations using essential oils was developed from the leaves of *Lantana camara* and *Ocimum gratissimum*. These formulations were assessed for their mosquitocidal and larvicidal activities against malaria vectors *Anopheles subpictus, Aedes aegypti*, and *Culex quinquefasciatus*. A combination of essential oils from *L. camara* and *O. gratissimum* leaves, prepared in ethyl alcohol at a 1:5 ratio, demonstrated the highest synergistic mosquitocidal effect against all three species (94-97%).[4] The insecticidal properties of the ether extract obtained from the lichen *Ramalina complanata*, along with an

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isolated metabolite known as divaricatic acid was tested against the *Maize weevil, Sitophilus zeamais*. Ingestion of the extract resulted in a maximum mortality rate of 33.33% (at 12 mg/g), with the extract exhibiting a deterrent effect ranging from mild at 4 mg/g to robust at 12 mg/g.[5] The effectiveness of pine oil against mosquito larvae was also demonstrated.[6] The novel method of in situ azoic dyeing utilizing *Terminalia chebula* and ethyl anthranilate for functional coloration of cotton was reported.[7] Further, this novel method yielded mosquito-repellent cotton with 100% efficacy, maintaining durability through at least 20 washes. Furthermore, antibacterial, antioxidant and UV protection were also confirmed. The repellent efficacy of *Mentha spicata, Ocimum gratissimum*, and *Moringa oleifera* leaves against mosquitoes was reported.[8]

Limited number studied have been performed on larvidical activity of lichen extracts. The insecticidal activities of *Leucodermia leucomelo* against *Aedes aegypti* mosquito larvae in their second and third larval stages was examined. The lichen majorly contains atranorin and salazanic acid as bioactive compounds. The susceptibility was 80% for third instar larvae and 100% for second instar larvae, while at 2000 µg/mL survival rate was 0% indicating 100% mortality, hence 2000 µg/mL is the best concentration to kill second instar larvae of *A. aegypti*.[9]The larvicidal activity of *Rocella montagani* against *A. egypti, A. ephensi* and *C. quinquefasciatus* was demonstrated.[10] High mortality was observed with dichloromethane, ethyl acetate and acetone extract of *Rocella montagani*. The dichloromethane extract of *Rocella montagani* was reported to have significant LC_{50} value of 126.16 µg/mL and one hundred percent mortality at 100 µg/mL was recorded.[11] The larvicidal activity of *Ramalina usnea* was reported by exhibiting LC_{50} value 4.85 and 4.48 µg/mL corresponding to 4-hydroxy-4-methoxy -6-propyl methoxy benzoate and usnic acid respectively.[12]

In this present study, lichen secondary compounds have been explored for larvicidal activity against *Aedes aegypti*. Further, antioxidant and anti-inflammatory activities of the lichen extract was also explored to confirm their biomedical potential. The study also evidenced the possible repellent formulations that have been prepared from the lichen extract which could be the effective alternative for the commercial Mosquito repellents.

MATERIALS AND METHODS

Sample collection

The mosquito eggs of *Aedes agepyti* have been collected from ICMR- vector control research centre field unit, Madurai. The eggs were maintained at room temperature.

Lichen identification

Lichen sample was identified based on the chemical and morphological appearance. Lichen substances present in the tissue of lichen thallus produce a colour change on the surface of the thallus while reacts with certain chemicals.[13] The chemicals routinely used for the spot test are as follows,

K Test: 10-25% of potassium hydroxide solution

C Test: A freshly prepared solution of calcium hypochlorite.

KC Test: Application of potassium hydroxide immediately followed by calcium hypochlorite.

P Test: 1-5% para-phenylene diamine.

Extraction

The lichen thallus was cleaned with distilled water after being washed with in running tap water. Then they were subjected to shade dry and ground to powder. Then, extract was prepared using methanol by cold maceration method. Approximately 4 g of lichen material was made to soak in 20 mL methanol and kept for 24 hr at ambient condition. After incubation it was filtered with Whatman No. 1 filter paper.[14]

Phytochemical screening of lichen extracts

The extract obtained was qualitatively tested for the occurrence of various phytochemical compounds including alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and glycosides etc., by the prescribed methods.[15]

Bioactivity test

Antioxidant activity

Antioxidant potential of lichen was assessed by DPPH and FRAP methods.

DPPH Assay

About 0.025 g of DPPH was made to dissolve in 100 mL of 80% methanol. Lichen extracts of different concentrations including 20 µL, 40 µL, 60 µL, 80 µL, 100 µL have been tested. About 200 µL of methanol with 5 µL DPPH was served as blank. A standard was used as Ascorbic acid. The various concentration of lichen extract (20 µL, 40 µL, 60 µL, 80 µL, 100 µL) was made up to 200 µL using methanol and 3 µL of methanolic DPPH was (0.1 mM) added and stored at dark condition for the period of 30 min. The aabsorbance was read at 517 nm.[16]

$$\text{DPPH Radical scavenging activity (\%)} = \frac{A0 - A1}{A0} \times 100$$

Where, A_0-Absorbance reading of the control solution, A_1-Absorbance reading of the test sample.

Frap Assay

About 2.5 mL of lichen extract was added to a solution of 2.5 mL phosphate buffer (0.2 Molar and pH 6.6) then 2.5 mL of potassium ferri-cyanide (1%) was added in a test tube and kept for incubation in a water bath for the period of 20 min at a

temperature of 50°C further cooled rapidly and then mixed with 2.5 mL of 10% trichloro acetic acid solution. The contents were spin at the speed of 3000 rpm for about 10 min. Then 2.5 mL of the top layer obtained was pipetted out and mixed with 2.5 mL of distilled water along with 0.05 mL of 0.1% of ferric chloride and kept for the period of 10 min. The absorbance was read at 700 nm.[17]

Anti-inflammatory activity

Anti-inflammatory potential of lichen extracts was assessed by albumin-denaturation and Protease inhibition methods.

Inhibition of albumin denaturation method

About 5 mL of reaction mixture was taken at different concentrations (20 µL, 40 µL, 60 µL, 80 µL, 100 µL) added with 1 mL (0.1%) of bovine serum albumin, 1 mL Tris-HCl buffer of 7.8 pH and 1 mL of test solution. The aspirin and buffer solution were used as the control solution. The mixture was kept for incubation at 37°C for 20 min followed by heating in the water bath for 2-4 min at 72°C. After subsequent cooling, absorbance was recorded at 660 nm.[18]

$$\% \text{ Inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where, A_0-Absorbance of the control, A_1-Absorbance in the presences of sample.

Protease inhibition assay

About 2 mL of reaction mixture contains 0.06 mL of trypsin, one mL of 20 mM Tris HCL buffer of 7.4 pH and 1 mL of test extract at various concentrations (20,40,60, 80 and100 µL) have been kept at ambient condition for 20 min. Then 1mL of casein solution was added. After incubation 2 mL of 2 M per chloric acid was used to control the reaction. The sample was spin for the period of 15 min. Absorbance reading for the supernatant was read at 280 nm.[19]

$$\% \text{ Inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where, A_0-Absorbance of the control solution, A_1-Absorbance of the sample.

Formulation of repellent products

Incense product

The methanol extract of lichen was allowed to evaporate and residue was collected and used for the preparation of incense log. The product was subjected to evaluate the smoke toxicity. The mosquito repellent incense log was prepared by standard protocol: 10 g of kungiliyam flour, 3 g of gigat powder, 4 g of white wood powder, and appropriate quantity of lichen extract was added. All the ingredients were added with distilled water to obtain a semisolid consistency. Incense log (2.4±0.2 g weight approximately) was obtained by moulding technique and dried at shade conditions. The control incense log was prepared without the lichen extract. The incense stick was prepared using bamboo stick and the control incense stick was also prepared.

Cream

The crude extract of lichen was subjected to evaporate at ambient temperature and the residue was obtained and used for the preparation of cream. Pure petroleum jelly was weighed about

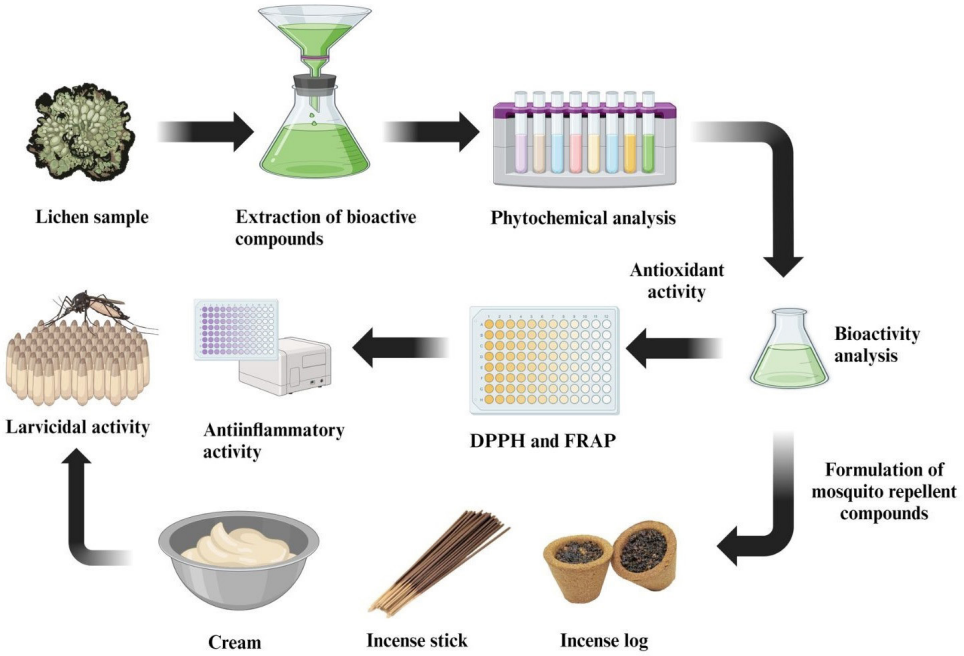

Figure 1: Overall Process flow diagram.

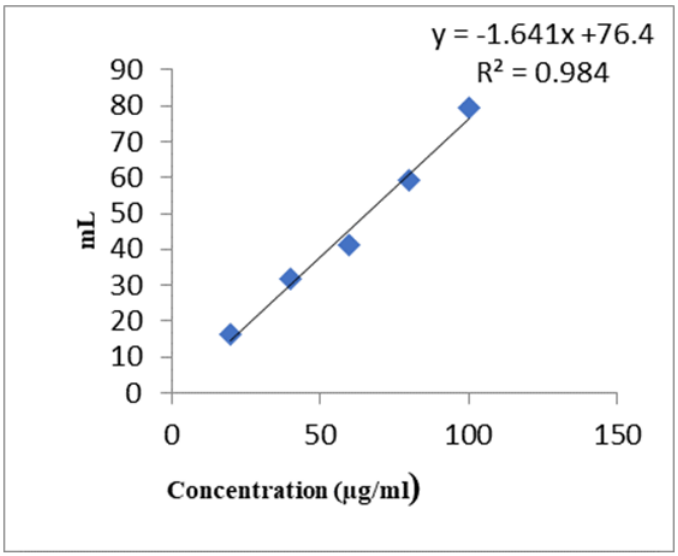

Figure 2: Antioxidant activity of lichen extract by DPPH assay.

22.73 g in 50 mL of beaker and heated in water bath at 50°C. At this molten stage, 1.73 g of lichen material was added and mixed properly[20] The prepared cream was evaluated in terms of pH, appearance, spreadability, irritancy and thermal stability.

Characterization of cream

Preliminary evaluation of formulated cream was carried in terms of different parameters including pH, spreadability and irritancy.[21]

pH

About 5 g of cream was weighted in a 100 mL beaker and dispersed in 45 mL of distilled H_2O. The pH of cream was measured at room temperature.

preadability

The spreadability was expressed in seconds. Glass slides of standard dimension was taken and the formulation of cream was placed between the two slides and pressed uniformly to spread. Better the spreadability was experienced for the lesser time of separation of slides.[22] The weight was removed and the upper slide was made to slip off freely by the application of force. The time taken was noted.

Homogeneity: The test was performed by physical touch.

Appearance: The appearance of the cream was identified in terms of colour, texture, roughness and odour.

Test for larvicidal activity

Aedes aegypti type larvae have been examined to evaluate the larvicidal property of lichen extract. The IV instar larvae of *Aedes aegypti* were kept in a beaker of 50 mL capacity comprising 40 mL water as a control. The stock solution was made by dissolving 0.04 g of extract in 2 mL methanol. From the stock solution various concentration of the test sample was formulated in the range of 20, 40, 60, 80 and 100 µg/mL. Four larvae for each concentration was set up and triplicate was done. One control group (without sample) was maintained. The mortality rate was documented for the exposure 24 hr. The dead larvae have been identified by their movement. The mortality rate was calculated by Abbott's formula,[23]

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

Repellent activity of cream

Repellent activity of the formulated cream was tested against selected human volunteers. Percent protection method was followed. Blood starved female adult mosquitoes of three to four days have been kept in a Barraud cage. The arm was cleaned using ethanol after allowing the arms to air dry, the dorsal side of the skin was made to expose and alcohol was used as the control. The cream formulated from lichen extract was applied on volunteer arm. The control and the cream treated arms have been introduced into the cage simultaneously. The numbers of bites were observed for 5 min. The triplicate was conducted. The protection percentage was obtained using the following formula.[24]

$$\text{\% Protection} = \left(\frac{\textit{No.of bites received by control arm} - \textit{No.of bites received by treated arm}}{\textit{No.of bites received by control arm}}\right) \times 100$$

Deterrent activity

Patch test

About 3 g of cream was kept on a small piece of fabric and applied to the sensitive area of the skin (behind ears)[25] The control (of similar cream of known brand) was applied. The skin behind ears was inspected after 24 hr. The test was repeated for three times.

Irritancy test

The cream was applied on the dorsal side of left-hand of 1sq.cm area and observed in the equal intervals upto 24 hr for irritancy and redness.

Smoke toxicity test

The repellent incense log of natural origin was formulated and used for smoke toxicity analysis. The experiment was performed by the arm-in-cage method. Hundreds of blood starved adult mosquitoes of three- or four-days old have been fed with sucrose and released into the cage and kept in tight shield. Triplicate was maintained. The data were compiled and mean value was subsequently used for the calculation. Two groups of controls were maintained. One group was treated with the incense product without the addition of active lichen extract as ingredient (Control II), the commercial mosquito coil was used as a positive control. After the trial, fed and unfed mosquitoes have been calculated. The effective protection offered by incense logs against

adult mosquitoes was recorded in terms of the percent unfed mosquitoes. The percent protection was estimated for the unfed mosquitoes using the given formul.[26]

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

RESULTS AND DISCUSSION

Aedes ageypti eggs collected from ICMR-Vector Control Research Centre Field Unit, Madurai were maintained at room temperature. Among the lichen samples identified, *Parmotrema* species was used for the study. The overall flow of the entire process is shown in Figure 1.

Phytochemical characterization of lichen sample

The phytochemical analysis of lichen extract evidenced the occurrence of bioactive chemical compounds such as phenolic compounds, flavonoid, terpenoids, saponins and tannins. From the result of phytochemical study, it was concluded that most of the phytochemical constituents have been extracted by methanol, hence it was used for further study. The presence of terpenoids confirmed that lichen extract has insecticidal activity and phenols are the important compounds of lichen that exhibit considerable antioxidant property. It was reported that almost all lichen extracts contain phytochemical compounds like proteins, tannins, carbohydrates and steroids etc.,[15]

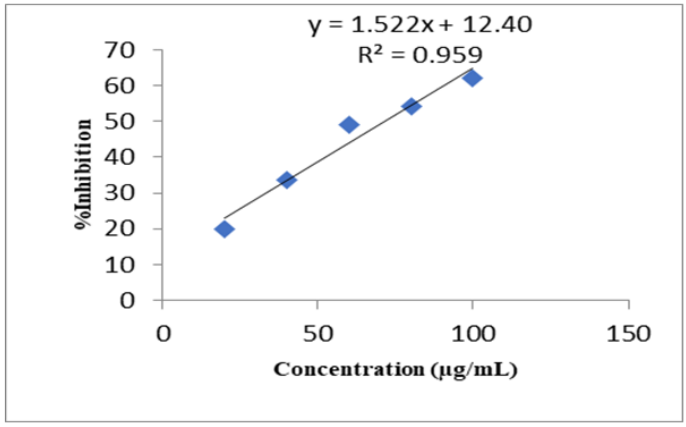

Figure 3: Antioxidant activity of lichen extract by FRAP assay.

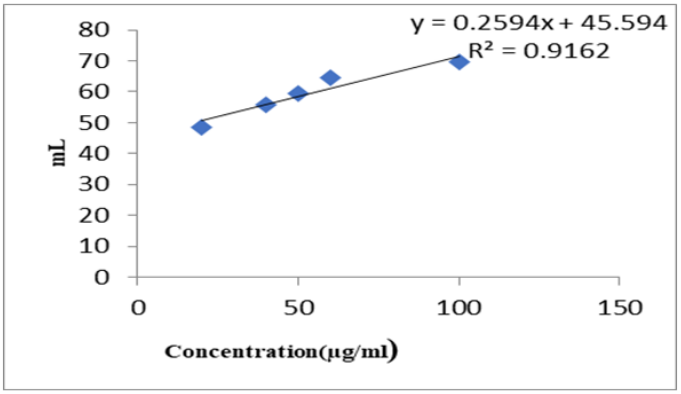

Figure 4: Anti-inflammatory activity of lichen extract by Albumin denaturation method.

Antioxidant activity

DPPH Assay

Free radical scavenging effect is recognized as one of the mechanisms involved in antioxidation.[27] This method is rapid, requiring affordable reagents.[28] The results of antioxidant activity by DPPH method is shown in Figure 2. Maximum percent inhibition was reported for the concentration at 100 µg/mL. Antioxidant activity of lichen was evidenced by the presence of phenols. The lesser the value of IC_{50} indicates that lichen extract exhibited higher free radical scavenging property. The percentage inhibition of the lichen sample confirmed the antioxidant potential of *Parmotrema* species.

The results of inhibitory concentration at 50% inhibition is 16.08 µg/mL which indicates that the methanol extract of lichen exhibited good radical scavenging property.[29] Total Phenol Content (TPC) assessed via the method of Folin-Ciocalteu for the methanolic lichen extracts of *Cetraria cucullata* and *Dactylina arctica* have been varied from 39.3 µg GA/mg to 113.5 µg GA/mg respectively.[30] Further, *Nephromopsis stracheyi* and *Asahinea scholanderi* exhibited higher total phenolic content of 84.2 µg GA/mg and 83.1 µg GA/mg respectively.[31] Aqueous extracts of lichens and organic extracts made up of acetone and methanol exhibited significant scavenging activity. Statistical analysis revealed that significant difference was recorded between the extracts and the control which was confirmed with $p<0.05$. The scavenging potential ranged from 29.99% to 90.93% for all lichen extracts. In particular, extracts from *Lasallia pustulata* demonstrated the highest DPPH radical scavenging effects compared to other lichen samples. The percent inhibition of solvent extracts such as acetone, methanol, and water of *Lasallia pustulata* have been observed as 90.93%, 69.87%, and 65.08%

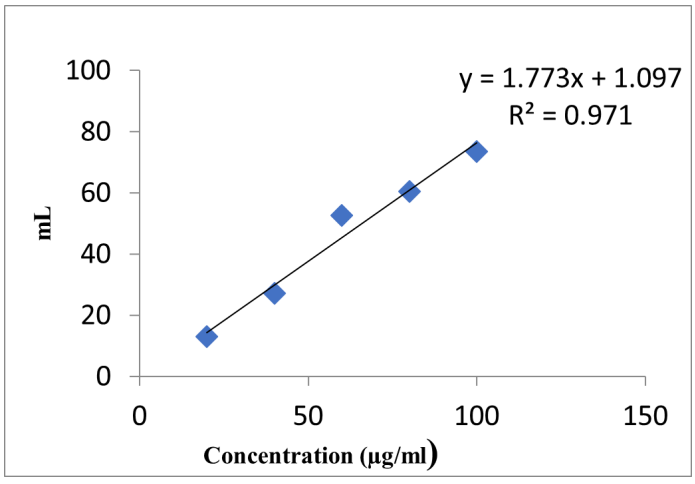

Figure 5: Antioxidant activity of lichen extract by protease inhibition method.

respectively.[32] Some lichen extracts demonstrated comparable or even superior antioxidant activity to standard antioxidants, indicating that lichen as a rich source of natural antioxidants.[33]

FRAP Assay

The FRAP assay is considered as one of the quick methods for the determination of antioxidant behaviour of the compound. The FRAP assay has shown significant result ensuring antioxidant potential which is ensured from the increasing reducing power. The results of FRAP assay stated that methanol extract of lichen exhibited significant reducing power. The Maximum percent inhibition was reported with the concentration of 100 µg/mL (Figure 3).

The results of inhibitory concentration at 50% inhibition is 16.08 µg/mL which indicates that methanol extract of lichen reported radical scavenging activity. The reducing capability of a compound is the major indicator of its antioxidant property. The Ferric Reducing Ability (FRAP) was employed to assess the ability of lichen extracts to reduce ferric ions. Phenolic compounds are well-known antioxidants that can scavenge free radicals and prevent oxidative damage.[34] The rate of FRAP activity depends on the specific lichen samples and solvent used for the extraction of bioactive compounds. Antioxidants were extracted using n-hexane, methanol, and water and the results shown that all lichen extracts revealed noteworthy FRAP antioxidant capacity.[35] He study further revealed that the methanol extracts showed the highest FRAP activity whereas water extracts exhibited optimum activity, n-hexane extracts revealed very less activity.

Table 1: Characterization of mosquito repellent cream.

Sl. No.	Parameters	Cream
1	PH	7
2	Spreadability	5.1 g cm/sec
3	Irritancy	No irritant

Anti- inflammatory

Albumin denaturation method

Anti-inflammatory potential was assessed by method of inhibition of albumin denaturation. Maximum percent inhibition was reported with the 100 µg/mL concentration of lichen extract (Figure 4). The anti-inflammatory capability of lichen was evidenced by the presences of tannins. The lesser value of IC_{50} indicates a higher protein denaturation of the lichen sample.

The results of inhibitory concentration at 50% inhibition is 17.02 µg/mL which indicates that the methanol extract of lichen exhibited anti-inflammatory activity. The bioactive compounds of lichen help to treat or reduce the inflammation or swelling. The anti-inflammatory ability of the *P. hypotropa* extract was assessed by its BSA denaturation as prescribed by[36] by *in vitro* method. The tested lichen extract exhibited a dose-dependent activity against heat-induced denaturation of BSA. The results revealed that for the concentration of 1000 µg/mL, the lichen extract exhibited 38.39% inhibition. Protein denaturation is one of the mechanisms responsible to inflammatory actions through tissue degeneration, functionality losses, and releasing pro-inflammatory mediators.[37]

Protease inhibition assay

Anti-inflammatory activity was determined by protease inhibition assay which is shown in Figure 5. Maximum percent inhibition

Table 2: Comparison of mosquito repellent against number of bites received during repellent activity.

Repellent activity observation	No. of mosquitoes tested	No. of bites received		
		Mosquito repellent creams		
		Control I	Control II	Commercial product
12.00-12.15	30	1	10	-
12.15-12.30	30	4	7	1
12.30-12.45	30	2	16	2
12.45-1.00	30	3	22	3
1.00-1.15	30	4	18	3

Control I- Cream with active compound; Control II- Cream without active compound.

Table 3: Repellent Activity and Percent Protection of Formulated Cream.

Mosquito repellent product	Total No. of mosquitoes in cage	Total No. of bites received	% protection
Control I	30	4	86.6
Control II	30	22	26.6
Commercial product	30	3	90

Control I-Product with active compound; Control II-Product without active compound; Fed-active; Unfed- Dead.

Figure 6: Incense products and Repellent cream prepared by using *Parmotrema* lichen extract.

was reported with the 100 µg/mL concentration of lichen extract. The IC_{50} value is 27.5µg/mL which indicates that methanol extract of lichen revealed better anti-inflammatory activity. *Usnea barbata* exhibited the highest protease inhibition activity with a percentage inhibition of 72.5±3.2% corresponding to 100 µg/mL concentration. Other lichen species such as *Cladonia rangiferina* and *Parmelia sulcata* also demonstrated substantial protease inhibition, showing percentage inhibitions of 65.8±2.5% and 58.3±4.1% respectively, at the same concentration. The observed protease inhibition activity of lichen extracts confirms the presence of secondary substances such as depsides, depsidones, and usnic acid. Usnic acid, in particular, has been reported for its protease inhibitory properties.[38]

Larvicidal bioassay

The result of mortality rate of *Aedes aegypti* for different concentration of methanol extract indicated that 100 ppm solution exhibited 30% mortality rate and maximum death rate was observed at this maximum concentration. The mortality rate of larvae increases with concentration and also exposure time.

Formulation of mosquito repellent compound

The herbal incense log was prepared in collaboration with Sree Nandini Sambrani Company, Dindigul District, Tamil Nadu. The incense log and stick were obtained as shown in Figure 5. The control Sambrani was prepared without the active lichen extract. The incense stick can also be prepared with bamboo stick.

The formulated cream as shown in Figure 6 has pH 7, spreadability 5.1 cm/sec and no irritancy was observed (Table 1).

Repellent activity test

Table 2 shows the repellent activity test for the sample (control I), cream without presence of sample (control II) and commercial cream against *Aedes agepyti*. The control I exhibited 86.6% protection against *Aedes aegypti*.

Smoke toxicity test

To assess the efficiency of incense product, smoke toxicity of incense product was performed. There are three types of repellent formulations used are commercial coil, incense product with sample (control I), (control II) incense log/stick prepared without the plant ingredient. There were 30 adult mosquitoes are used for testing smoke toxicity against *Aedes aegypti*. Protection of 26.6% was recorded.

The data presented in Table 3 reveals the significant insights of the repellent activity and percent protection of the formulated cream in comparison to controls and a commercial product against mosquitoes. Control I, representing the product containing the active compound, displayed a notably high level of protection with 86.6% efficacy. This suggests that the active compound effectively repels mosquitoes, resulting in a substantial reduction in the number of bites received. Conversely, Control II, lacking the active compound, exhibited significantly lower protection, with only 26.6% efficacy. The high efficacy observed in Control I and the commercial product indicates that the active compound utilized in the formulated cream worked better.

In the present study, the active lichen ingredient in the formulation providing repellent activity to the cream. Moreover, the formulated cream demonstrated comparable efficacy to the commercial product, achieving 90% protection against mosquito bites. This similarity in efficacy suggests that the formulated cream could serve as a promising alternative to established commercial repellents, potentially offering consumers similar levels of protection against mosquitoes. The implications of these findings extend beyond mere repellent efficacy. Given the global health risks associated with mosquito-borne diseases, effective mosquito control measures are essential. Furthermore, these results highlight the importance of ongoing research and development in mosquito repellents. Continued efforts to optimize formulations could lead to improvements in efficacy,

safety, and environmental sustainability. Future studies might focus on enhancing the longevity, user-friendliness, and skin compatibility of the repellent cream, thereby further increasing its appeal to consumers. In summary, active compound in conferring repellent activity to the formulated cream has comparable efficacy to a commercial product suggests its potential as an effective solution for mosquito protection, with broader implications for public health and mosquito control initiatives. Further research and development efforts could enhance the formulation and consumer acceptance of the repellent cream, ultimately contributing to more effective mosquito control strategies.

CONCLUSION

In order to overcome the negative effects of commercial mosquito repellent products, natural repellent is proposed using lichen extract of *Parmotrema* species. The result revealed that the lichen has larvicidal activity against *Aedes aegypti*. The study evidenced that lichen secondary compounds are the promising source of antioxidant, larvicidal and anti- inflammatory activity. The formulation of incense product and cream demonstrated significant repellent potential and results of smoke toxicity examination supported the percentage protection offered by the formulations. Incense products demonstrated better protection against *Aedes agepyti* and it was compared with commercial formulations. Smoke toxicity test exhibited significant protection against *Aedes agepyti*. Hence, the result of present study evidenced that lichen has potential larvicidal activity against *Aedes agepyti* and the formulated repellents are non-toxic and eco-friendly possess no harmful side effects to humankind thus exhibiting a potential alternative to the existing chemical-based repellents available in market. Thus, the study confirmed the biomedical potential of lichen secondary compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 2,2- Diphenyl-1-Picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **IC_{50}:** Half Maximal Inhibitory Concentration.

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