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Exploring larvicidal activity of *Prosopis juliflora* seedpods for Mosquito repellent formulations

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Abstract. Mosquitoes are one of the dangerous insects that transmit dreadful diseases such as Malaria, Chikungunya, Yellow fever, Dengue fever, Filariasis, Zika etc..The chemically derived insecticides cause allergic reaction to human and cause several environmental deteriorations. The present study aims to formulate Mosquito repellents from essential oil of *Prosopis juliflora*. The essential oil from *Prosopis juliflora* seedpods were extracted by hydro-distillation method with the yield was 8.5 ml/g. Phytochemical analysis was conducted to identify the presence of major bioactive compounds of the essential oil such as alkaloids, flavonoids, terpenoids, steroids and phenolic compounds. Bioactivity evaluation such as antioxidant, anti-inflammatory and larvicidal activity was performed. The maximum inhibitory concentration of essential oil was 75µg/ml with IC50 of 16.72 µg/ml and 26.18 µg/ml respectively for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay. Around 90% of larval mortality rate was observed at 100 µg/ml of essential oil. Mosquito repellent activity was evaluated for the formulation which demonstrated 76.6% protection against *Aedes agepyti*.

1 Introduction

Synthetic mosquito repellents are prepared from non-biodegradable chemicals such as N, N-diethyl-3-methylbenzamide (DEET), dimethylphthalate (DMP) and allethrin which cause severe environmental degradation and cause allergic reactions to humans. *Prosopis juliflora* is considered as a cheapest source exhibiting many pharmacological and therapeutic properties. *Prosopis juliflora* offers various pharmacological activities such as antioxidant, amoebicidal, antipustule, antipyretic, cytotoxic, anti-antigiardial, anthelmintic, antimalarial activity and larvicidal activity [1]. *Prosopis juliflora* produce large quantity of seedpods. The most significant bioactive constituents of this plant are terpenes, alkaloids, flavonoids and phenolic compounds. Some of these compounds may exhibit therapeutic activities such as antibacterial activity [2]. The presence of saponins show that *Prosopis juliflora* has antimicrobial and insecticidal activities. The presence of high fibre content indicates high nutritional value [3]. Biological properties of various extracts of the leaves, pods, and bark of *Prosopis juliflora* have been reported. Hydrodistillation is one of the conventional methods employed for essential oil extraction of *Prosopis juliflora* seeds. Soxhlet

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extraction for *Prosopis juliflora* seeds was carried out using hexane [4]. This particular method is difficult in achieving purity of the essential oil, because it retains solvent residues and some volatile compounds may degrade while extraction with solvents and heat. *Prosopis juliflora* leaves can be extracted by cold extraction method but it is a slow process and not suitable for essential oil extraction [5]. The present study highlights the extraction of essential from *Prosopis juliflora* seedpods and evaluating the larvicidal property against *Aedes agepyti*.

2 Materials and Methods

2.1 Extraction of essential oil by hydrodistillation

Prosopis juliflora seedpods were obtained from Marappampalayam, Namakkal, Tamil Nadu. The collected seedpods were dried in direct sunlight until the moisture content was removed and powdered using mixer blender. The powdered samples were stored at room temperature for further use. Ades ageypti mosquito egg was collected from ICMR- Vector Control Research Centre Field Unit, Chinna Chokikulam, Madurai, Tamil Nadu. The eggs were stored at room temperature.
Hydrodistillation is the conventional extraction method followed for oil extraction. The powdered materials were placed in round bottom flask containing distilled water [6]. The flask was attached to the condenser and heated in a heating mantle. The distillation process was continued for 5 hr. The organic phase was collected separately and dried using anhydrous sodium sulphate and filtered. The filtrate was kept under 4°C and the yields was calculated [7].

$$Yield\ (\%) = \frac{W1}{W2} \times 100$$

Where W1 = initial sample weight, W2 = weight of essential oil

2.2 Phytochemical screening

Phytochemical screening for the essential oil was conducted using standard procedure for the quantitative estimation of alkaloid, flavonoids, saponins, tannin, terpenoids, glycosides, steroids, phenolic compounds.

2.3 Antioxidant activity

2.3.1 DPPH Radical Scavenging Assay

The free radical scavenging potential of the essential oil was determined by DPPH method. About 0.007 grams of DPPH was dissolved in 50ml of methanol. L-Ascorbic acid was the standard. Different concentrations (25µl, 50µl, 75µl, 100µl) of sample were made up to 1000µl with methanolic DPPH and stored in dark room for 30 minutes [5]. The absorbance was measured at 517nm.

$$\text{Inhibition (\%)} = \frac{A0 - A1}{A0} X100$$

Where, A0 = Absorbance of the control, A1 =Absorbance of the sample

2.3.2 Ferric Reducing Antioxidant Power (FRAP)

Reducing power of the essential oil was determined by FRAP assay [8]. About 2.5ml of phosphate buffer and 1% potassium ferrocyanate was added to different concentrations (25µl, 50µl, 75µl, 100µl) and incubated in water bath at 50ºC for 20 minutes. Then, 2.5 ml of tri-chloro acetic acid (TCA) was added followed by 0.5ml of ferric chloride and centrifuged for 3 min. The absorbance was read at 700 nm.

2.4 Anti-inflammatory activity

2.4.1 Inhibition of protein denaturation method

Inhibition of protein denaturation was conducted using Bovine Serum Albumin (BSA). Different concentrations (25µl, 50µl, 75µl, 100µl) of the test solution were taken. About 1 ml of 0.1% bovine serum albumin was mixed with 1ml Tris-HCl buffer (pH 7.8). The mixture was kept at 37ºC for 20 min followed by heating at 72ºC for 2-4 minutes in the water bath for denaturation and allowed to cool at room temperature. The absorbance was recorded at 660 nm (Modi et al., 2019).

$$\% \text{ Inhibition} = \frac{Ac - At}{Ac} X100$$

Where, Ac = Absorbance of control, At = Absorbance of the test sample

2.4.2 Protease inhibition assay

Protease inhibition assay was performed using trypsin as an enzyme source. Different concentrations (25µl, 50µl, 75µl, 100µl) of the sample were taken. About 0.06 ml of trypsin was added to all the test tubes followed by 1ml of 20mM Tris-HCL buffer (pH 7.4). The mixture was kept at 37°C for 10 minutes. Then, 1ml of casein solution was added. The reaction contents were re-incubated for 20 minutes. After incubation 2ml of 2M perchloric acid was added to arrest the reaction. The sample was spin at 15 min. The absorbance of the supernatant was measured at 280nm [9].

$$\% \text{ Inhibition} = \frac{Ac-At}{Ac} \times 100$$

Where, Ac = Absorbance of control, At = Absorbance of the test

2.5 Formulation of repellent compounds

2.5.1 Incense log and cream

The essential oil was used for the formulation of incense log. The incense log was prepared with kungiliyam flour, gigat powder, wood saw dust and plant extract. All the ingredients were mixed to form a semisolid paste using distilled water. Incense log was made by moulding method and shade dried. The control was made without essential oil. The cream formulation was prepared using pure petroleum jelly. About 22.73 g pure petroleum jelly was weighed in 50ml of beaker and it was heated in water bath at 50°C. At this molten stage, 1.73g of*Prosopis juliflora* essential oil was added and mixed properly [10]. The prepared cream was tested for pH, appearance, spreadability, irritancy and thermal stability.

2.5.2 Larvicidalbioassay

Aedes agepyti larvae were used to evaluate the larvicidal activity of essential oil. The early third instar larvae of Aedes agepyti were tested for 10, 20, 50, 100 and 200 ppm essential oil. The mortality rate was recorded at 24 hrs and 48 hrs of exposure [11].

$$\text{Mortality rate \%} = (1 - \frac{NO.of\ dead\ larve\ after\ treated}{No.of\ larve\ in\ control\ after\ treated}) \times 100)$$

Repellent activity of the formulated cream was evaluated in human volunteers. Blood starved female adult mosquitoes of three to four days old were kept in a Barraud cage. The hand of the human volunteer was cleaned using ethanol and dorsal side was exposed. The control and the exposed hand was introduced into the cage [12]. The number of bites were counted and triplicate was done. The protection was evaluated by the following formula

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

3 Results and Discussion

3.1 Extraction of essential oil

The yield of extracted essential from *Prosopis juliflora* was 8.5 ml/g. The overall process flow diagram is given in figure 1.

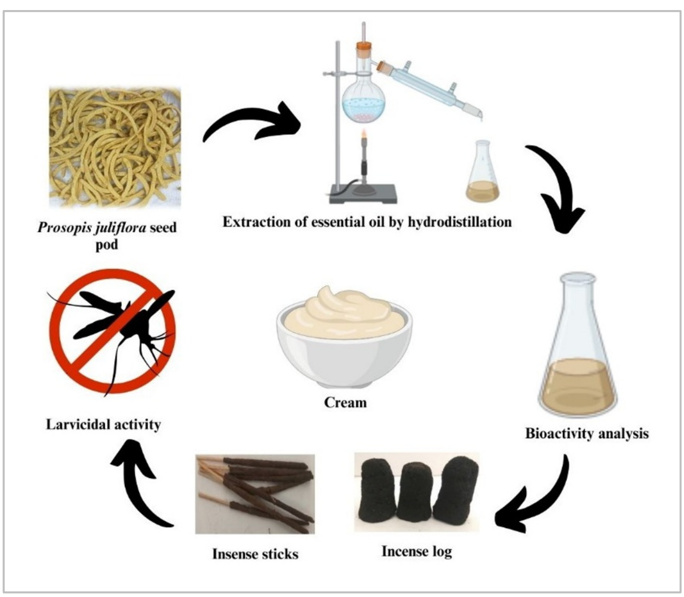

Fig. 1. Process flow diagram

3.2 Phytochemical screening of essential oil

The phytochemical screening of essential oil extracted from *Prosopis juliflora* seedpods evidenced the presence of important bioactive compounds including alkaloids, flavonoids, terpenes followed by steroids, phenolic compounds and saponins. Glycosides and tannins are not present.

3.3 Antioxidant activity

DPPH and FRAP assay were carried out for the essential oil obtained by hydro-distillation method. Figure 2(a) shows that for DPPH assay, the half maximal inhibitory concentration was obtained at 75µg/ml of 50.32 % inhibition. The minimal inhibitory concentration (IC50) at 50% inhibition was found as 16.72µg/ml. The essential oil demonstrated antioxidant property due to the occurrence of bioactive compounds including flavonoids and phenols. For FRAP assay, the minimal inhibitory concentration IC50 value was found as 26.18µg/ml (Figure 2b). The half maximal inhibitory concentration was at 75µg/ml with the inhibition of 49.14%. The free radical scavenging and reducing capability of the compound is considered as the significant indicator for potential antioxidant activity. The decrease in inhibition after 75µg/ml is observed due to the toxicity of the extract at higher concentrations.

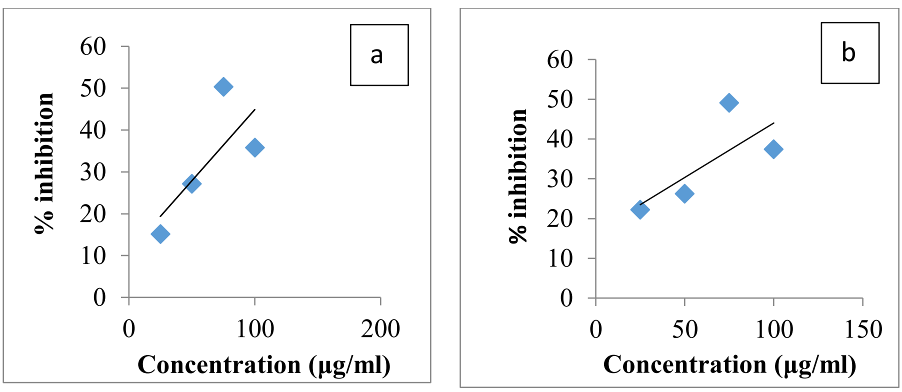

Fig. 2. IC50 value of essential Oil by a) DPPH and b) FRAP assay

3.4 Anti-inflammatory activity

The albumin denaturation and Protease inhibition assay were conducted for the essential oil obtained by hydro-distillation, figure 3 (a) shows the minimal inhibitory concentration (IC50) which was 22.10µg/ml. The half of maximal inhibitory concentration which was 100µg/ml with % inhibition of 46.28%. Presence of bioactive compounds responsible for the anti-inflammatory activity.

Figure 3 (b) shows the protease inhibition assay for the essential oil obtained by hydro-distillation method. The result shows the presence of anti-inflammatory property of essential oil due to the occurrence of phenols. The minimal inhibitory concentration (IC50) was 25.28µg/ml. The half of the maximal inhibitory concentration at 100µg/ml was 48.54%.

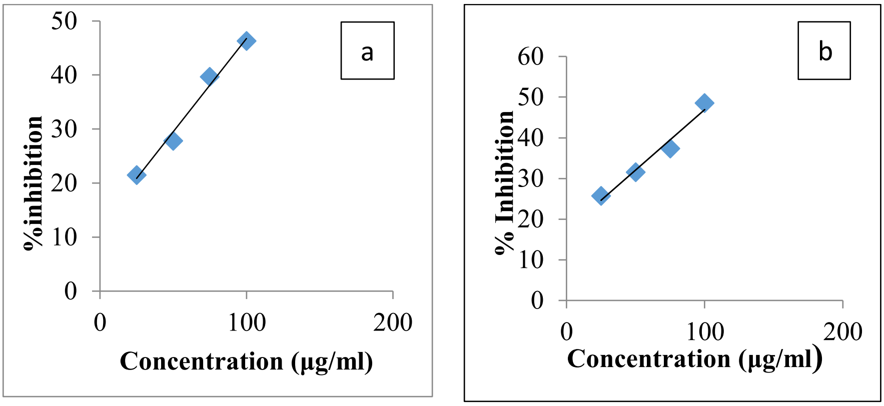

Fig. 3. IC50 value of essential Oil by a) Albumin denaturation and b) Protease inhibition assay

3.5 Larvicidal bioassay

The present study demonstrated the insecticidal property of essential oil extracted from *Prosopis juliflora* against Aedes aegypti larvae. Based on the experimentation, the maximum death rate was observed at the concentration of 100 ppm (Table 1). To increases the mortality rate of larvae, extended concentration and exposure time is needed.

Table 1. Larvicidal activity of Essential oil against 3rd instar stage Aedes ageypti larvae.

Concentration (ppm)	% of larval mortality rate at 24hrs	% of larval mortality rate at 48hrs
Control	0	0
25	0	0
50	10	50
75	20	60
100	40	90

3.6 Formulation of mosquito repellent

The incense log was prepared and shade dried for three days. The control incense log does not contain the active essential oil ingredient. The incense stick was also prepared using bamboo stick (Figure 4).

Fig. 4. Mosquito repellent Formulations

3.7 Repellent activity test

The repellent potential of the formulation (control I) tested exhibited 76.6 % protection against Aedes aegypti larvae which is comparable with the commercial product whereas the formulation without active essential oil (control II) demonstrated very lesser protection of 13.3%.

3.8 Discussion

Presence of alkaloids indicate the pharmacological significance of the essential oil (Ibrahim et al., 2013). Terpenes are major constituent for insecticidal property and antibacterial, antifungal, anthelmintic and antimalarial activities [13]. Insecticidal activity of *Argemone mexicane* was discussed [14], *Prosopis juliflora* and Tephrosia purpurea against Triboliumcastaneum (red flour beetle). Hexane extract of *Prosopis juliflora* seeds showed highest mortality rate against termite and cockroach and the LD50 values determined for each species was about 0.472% for termite and 1.07% for cockroach [04]. Larvicidal activity of *Vernonia cinerea*, *Cassia tora* and *Prosopis juliflora* against malaria vector *Anopheles stephensi* was demonstrated [15]. Among these acetone extract of *Prosopis juliflora* showed highest mortality rate. *Prosopis juliflora* exhibited highest mortality rate followed by *Vernonia cinerea* and *Cassia tora*. Larvicidal ability of *Prosopis juliflora* against *Anopheles subpictus*, *Culex quinqufasciatus* and *Aedes aegypti* was also reported [11]. *Prosopis juliflora* have number of secondary metabolites [16]. Mosquito repellent from *Curcuma longa* and *Embeliaribes* was formulated [17]. Mosquito repellent cream was formulated from essential oil [10]. Larvicidal, pupicidal and smoke toxicity effect of *Acanthospermumhispidum* leaf extract against *Anopheles stephensi*, *Culex quinqufasciatus* and *Aedes aegypti* was reported [18].

4 Conclusion

The present study investigated the insecticidal activity of essential oil extracted from *Prosopis juliflora* seedpods. The result showed that the oil has insecticidal activity and it worked against Aedes agepyti. The chemically derived insecticides are more harmful to human whereas plant derived products or metabolites serves as harmless agents. Thus, the presence of bioactive compounds in the *Prosopis juliflora* becomes a promising alternative. The presence of phytochemicals evidenced the biological potential of the essential oil which in turn signifies the pharmacological significance. Thus, the present investigation demonstrated that *Prosopis juliflora* is a best alternative to commercially available repellents and a cheap raw material as well.

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