EXTRACTION OF PHYTOCHEMICALS, FORMULATION, EVALUATION AND DEVELOPMENT OF HERBAL MOSQUITO INCENSE REPELLENT STICKS

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College: *Indian Academy Degree College (Autonomous), Bengaluru*
Branch: *Department Of Life Sciences-Biotechnology*
Guide(S): *Dr. Ramachandra Murthy Tts*
Dr. Erumalla Venkata Nagaraju
Student(S): *Ms. Vinitha Murugan*
Ms. Ramya K.S

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Introduction:

Mosquito-borne diseases such as malaria, dengue fever, and Zika virus pose significant public health threats globally. While synthetic mosquito repellents have been widely used, their adverse environmental and human health impacts have raised concerns. Natural mosquito repellents, derived from plants and essential oils, offer a safer and more sustainable alternative. This project aims to establish herbal mosquito repellents from *Ocimum Sanctum* (Tulsi), *Citrus Sinensis* (Sweet Orange), *Azadirachta Indica* (Neem). *Callicarpa Tomentosa* (Beautyberry Plant) as a viable alternative to synthetic products, fostering a transition toward sustainable and eco-friendly practices in public health and pest management. This aligns with global efforts toward reducing chemical pollution and enhancing the utilization of natural resources. Moreover, natural repellents are biodegradable, non-toxic, and environmentally friendly. They also offer additional benefits, such as antioxidant and anti-inflammatory properties.

Objectives:

- ✓ To extract and identify the essential oil constituents from selected plant species using hydro-distillation and gas chromatography-mass spectrometry (GC-MS) techniques.(Accomplished)

- ✓ To evaluate the antioxidant activity of the extracted essential oils using the DPPH radical scavenging assay
- ✓ The IC50 values will be calculated to determine the concentration of essential oils required to scavenge 50% of DPPH radicals.
- ✓ To assess the mosquito larvicidal and adulticidal activities of the extracted essential oils against selected mosquito species.
- ✓ To evaluate the ichthyotoxic activity of the extracted essential oils against non-target aquatic organisms.
- ✓ To formulate and test the efficacy of mosquito repellent sticks using the extracted essential oils.
- ✓ To evaluate the repellent activity of the formulated mosquito repellent sticks.
- ✓ Correlation analysis will be performed to determine the relationship between the essential oil constituents and their biological activities

Methodology:

1. **Collection and extraction of essential oil**

Fresh plant material of *Ocimum Sanctum* (Tulsi), *Citrus Sinensis* (Sweet Orange), *Azadirachta Indica* (Neem), *Callicarpa Tomentosa* (Beautyberry Plant) will be collected and washed with distilled/deionized water. The method that will be adopted for the extraction of volatile oil from leaves will be done by Hydro-distillation (Clevenger type apparatus). During this technique, 150g of raw feed material will be soaked in solvent. The solvent that will be used is water. Then, the solvent and raw feed material will be heated until they form vapours for 3h, then the vapours will be allowed to cool down and collect at the end in a receiving funnel. The oil sample that will be extracted in 10 mL of diethyl ether (Et2O) will be dehydrated over anhydrous sodium sulphate and will be kept in a refrigerator for further analysis. The percentage yield of oil will be calculated on the basis of fresh weight of the plant materials used for extraction.

2. **Identification of Essential Oil Constituents:**

a) **Phytochemical (qualitative test):** Various qualitative tests are performed to identify the classes of compounds present in the plant extract.

- ✓ **Test for Terpenoids:** Crude extract dissolved in 2ml of chloroform and was evaporated to dry. To this, 2ml of concentrated H2SO4 are added; a reddish-brown coloration at the interface indicates the presence of terpenoids.
- ✓ **Test for Alkaloids**: Crude extract will be mixed with 2ml of 1% HCl and heated gently. Mayer's or Wagner's reagents are then added to the mixture. Turbidity of the resulting precipitate will be taken as evidence for the presence of alkaloids.
- ✓ **Test for flavonoids (Shinoda test):** Crude extract mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour will appear after few minutes which indicated the presence of flavonoids.
- ✓ **Test for phenols and tannins:** Crude extract mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration will be indicating the presence of phenols and tannins.
- ✓ **Test for saponins:** Crude extract will be mixed with 5ml of distilled water in a test tube and it will be shaken vigorously. The formation of stable foam is taken as an indication for the presence of saponins.
- ✓ **Test for glycoside (Salkowski's test):** Crude extract mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 is added carefully and shaken gently. A reddish-brown colour will indicate the presence of steroidal ring, i.e., glycone portion of the glycoside

(b) Phytochemical (Quantitative Analysis)

Quantitative Analysis will be used to determine the concentration of essential oils. This step will usually involve instrumental techniques like mass spectroscopy or gas chromatography. The identification of essential oil constituents will be carried out with a Clarus 580 Gas Chromatograph (Perkin-Elmer, USA) equipped with a SQ8S MS detector. The analysis will be performed on an Elite-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a flow rate of 1.0 mL/min. The column temperature will be programmed at 60°C, then increased to 220°C at 3°C/min, and finally held at 220°C for 7 min. The injector and ion source temperature will be kept at 250°C. The essential oil constituents will be identified by matching the

acquired spectra against the built-in reference of the NIST spectral library and by comparing experimental retention indices (RI) obtained with the published bibliographic literature *(R. P. Adams et al.,*2007*).*

3. DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) Radical Scavenging Activity

The free radical scavenging activity of essential oils will be evaluated by adding 0.1 mL of essential oils (dissolved in dimethyl sulfoxide) to 3.9 mL of 0.2 Mm DPPH in methanol (MeOH). The mixture will be shaken vigorously and incubated in dark conditions at room temperature for 30 minutes. A decrease in the absorbance will be monitored at 515 nm (UV-Vis Spectrophotometer) against MeOH as the control and ascorbic acid as the standard. Radical scavenging activity will be measured as the percentage of DPPH decolorization by using the following equation.

$$\% \text{ Inhibition} = [\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}}] \times 100$$

The inhibitory concentration (IC_{50}) of the essential oil needed to scavenge 50% of DPPH is calculated from the plot of concentration versus percentage of inhibition.

4. Mosquito Culture

Larvae will be reared in large pans containing 1.5L of distilled water. The larvae will be fed ad libitum with Special Kitty cat food (Amazon). Each pan will receive approximately 4 pellets and the water will be changed every 3 days. This regimen will ensure that larvae are not crowded, as high larval densities can suppress insecticide resistance. When pupae start to appear, they will be removed daily and placed into small cups with water within BugDorm-1 Insect Rearing Cages (Amazon). Cages with the adults will be maintained in an insectary at 80% humidity and 27°C with a light/dark cycle of 14/10, respectively.

5. Mosquito Larvicidal Bioassay

The extracted oils will be used in five different concentrations (1%, 2%, 3%, 4%, and 5%) and their efficacy will be evaluated by the standard WHO method. Each replicate will contain 10ml of the oil solution, which will be placed in 100ml glass beakers. Batches of 10 late 3rd and early 4th instar larvae will be exposed in each beaker containing the crude oil solution. A total of three replicates will be conducted

for each concentration, and against each replicate, a control will be present. The numbers of dead larvae will be counted after 24 to 48-hours interval. The experiment will be conducted under lab conditions at 27°C with relative humidity.

6. **Preparation of Mosquito Repellent Stick**

The fresh *Azadirachta Indica* (Neem Leaves) and (neem bark), *Cinnamomum cassia* (Cinnamon bark), *Callicarpa Tomentosa* (Beautyberry Plant), will be dried until they get completely dry, which will take about 3 days. Then, the dried leaves will be transferred to a mortar and pestle and crushed into a fine powder. All the required ingredients will be taken along with white copal resin (binding agent) taken in a mortar, except oil and water, and mixed thoroughly. Into this mixture, a specified amount of essential oil (*Ocimum Sanctum* (Tulsi), *Citrus Sinensis* (Sweet Orange), *Azadirachta Indica* (Neem) and *Callicarpa Tomentosa* (Beautyberry Plant) will be added and blended evenly (3 different formulations F1, F2, F3). Finally, water will be added as per requirement for binding the sticks (bamboo incense sticks). Then, the prepared mixture will be filled into stick mould and kept for drying. The sticks will be removed from the mould and placed in a Hot Air Oven for 30 minutes to get dry. Once the sticks have dried, they will be stored securely to prevent moisture from being trapped inside and will be used for further studies.

7. **Mosquito Repellence Test**

Mosquito repellency tests will be conducted in mosquito-abundant regions such as home corners, bushes, tea stalls, drainage corners, and cafeterias. The garden area of the college premises, overseen by the Department of Life Sciences at Indian Academy Degree College Autonomous, Bangalore, will be tested in the evening and nighttime. Additionally, the study will be conducted for 30 days in the form of a questionnaire. The data collected will be studied statistically and represented.

Result and Conclusion:

1. **Collection and extraction of essential oil**

Fresh plant materials of Ocimum sanctum (Tulsi), Citrus sinensis (Sweet Orange), Azadirachta indica (Neem), and Callicarpa tomentosa (Beautyberry Plant) were collected and thoroughly washed with distilled/deionized water. The volatile oils from the leaves were extracted using the hydro-distillation method

with a Clevenger-type apparatus. Approximately 150g of each raw plant material was soaked in water and heated for 3 hours to produce vapors. These vapors were then condensed and collected in a receiving funnel. The extracted oil was dissolved in 10 mL of diethyl ether (Et2O), dehydrated using anhydrous sodium sulfate, and stored in a refrigerator for further analysis.

Figure 1: **collection of plant sources**

Figure 2: extraction of oil by hydro-distillation method

Figure 3: separation of volatile oil from aqueous phase

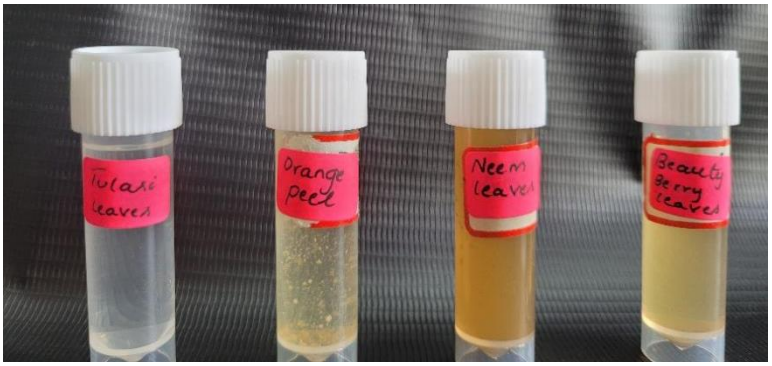

Figure 4: storage vials containing volatile oils

2. Identification of Essential Oil Constituents:

(a) Phytochemical (qualitative test)

Phytochemical screening of the extracted essential oils was carried out using various qualitative tests to identify the presence of different bioactive compounds. The presence of terpenoids was confirmed by a reddish-brown coloration at the interface after adding concentrated H2SO4 to the chloroform-dissolved extract. Alkaloids were detected by the formation of a turbid precipitate upon addition of Mayer's or Wagner's reagent. The Shinoda test indicated the presence of flavonoids by a pink scarlet coloration. Phenols and tannins were identified by a blue-green or black coloration with FeCl3. Saponins were confirmed by the formation of stable foam upon vigorous shaking with distilled water. Glycosides were

detected through the appearance of a reddish-brown color in the Salkowski's test, indicating the presence of a steroidal ring structure.

Figure 5: Phytochemical test for essential oils

8. Ingredients required for Preparation of Mosquito Repellent Stick

DISCUSSION:

The present study successfully demonstrated the extraction of essential oils from *Ocimum sanctum, Citrus sinensis, Azadirachta indica*, and *Callicarpa tomentosa* using the hydro-distillation method. The oil yields varied based on the plant species and were calculated relative to the fresh weight of the plant materials. These essential oils were then subjected to phytochemical screening, which revealed the presence of various bioactive compounds including terpenoids, alkaloids, flavonoids, phenols, tannins, saponins, and glycosides. The abundance of these phytochemicals is known to contribute to insecticidal, antioxidant, and anti-inflammatory properties, making them suitable candidates for mosquito repellent formulation.

The extracted oils were used to develop eco-friendly herbal mosquito repellent sticks. The formulation included natural binders, fillers (such as wood powder or charcoal), natural fixatives, and essential oils as the active ingredients. These ingredients not only ensured the proper shape, burning quality, and consistency of the sticks but also enhanced their mosquito repellent efficacy. The combination of plant-based essential oils and traditional stick-making components presents a sustainable alternative to synthetic chemical repellents, aligning with the growing need for environmentally responsible and health-conscious pest control methods.

Future Scope:

This project lays the foundation for the development of safe, effective, and eco-friendly herbal mosquito repellents. Building upon the current findings, future research can explore the following areas:

1. Large-scale Extraction and Commercialization: Scaling up the extraction process and optimizing yield and cost-effectiveness for commercial production of herbal mosquito repellent products.

2. Advanced Analytical Studies: Further identification and quantification of bioactive compounds using advanced techniques such as LC-MS/MS and NMR to understand the mechanisms of action at the molecular level.

3. Formulation Improvements: Developing different forms of mosquito repellents such as sprays, creams, gels, and diffusers using the extracted essential oils for wider consumer preferences and applications.

4. Long-term Toxicological Studies: Conducting extensive safety assessments including dermal toxicity, allergenicity, and long-term environmental impact to ensure product safety for humans and non-target organisms.

5. Field Trials and Efficacy Testing: Implementing large-scale field trials to evaluate real-world efficacy of the formulated products under different environmental conditions and against various mosquito species.

6. Integration into Public Health Programs: Collaborating with health departments and NGOs to promote the use of natural repellents in mosquito-prone areas, especially in regions with high disease burden and low access to synthetic products.