HERBAL MOSQUITO REPELLENT SPRAY FROM CITRONELLA (CYMBOPOGON) OIL

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• ABSTRACT

The present study was conducted to evaluate the mosquito repellent activity of selected plant materials to develop safe and effective herbal mosquito repellent formulations by combining these materials. Seeds of Azadirachta indica were soaked in hexane overnight, and the extract was filtered and concentrated using a rotary evaporator.[1)] The same procedure was followed for leaves of Vitex negundo using both hexane and ethanol as solvents. Hydro-distillation was carried out using a Clevenger apparatus to obtain essential oils from the leaves of Ocimum sanctum, the rhizomes of Curcuma longa, and the peels of Citrus sinensis. Essential oils of Cymbopogon nardus leaves, Eucalyptus globulus leaves, and Syzygium aromaticum buds were procured from a trusted source. Ethanol solutions containing 10% (v/v) of each extract or essential oil were prepared and tested for mosquito repellent activity using the arm-in-cage method. In this method, a volunteer's forearm treated with 1 ml of the test solution was exposed to a cage containing 20 blood-seeking mosquitoes, and the number of mosquitoes that landed or attempted to bite the arm was recorded each minute for five minutes. Three replicates were conducted for each sample. After analyzing the repellency of each individual extract.

KEY WORDS : Cymbopogon nardus , Ocimum sanctum , Syzygium aromaticum , Curcuma Longa.

• INTRODUCTION

Insect bites, particularly from mosquitoes, can transmit serious diseases such as dengue, malaria, and yellow fever, which may become life-threatening if not treated promptly. These disease-carrying insects are commonly found in many Asian countries. To address this issue, both urban and rural communities must work together to implement effective mosquito control measures.[10)]Efforts should focus on identifying safe and non-toxic insecticidal methods to prevent the spread and reproduction of these disease vectors.

A wide range of mosquito repellent products is available in the market today. However, many of these commercial formulations have certain drawbacks. In particular, young children may experience adverse reactions such as skin irritation, rashes, swelling, or even eye infections due to the potential toxicity of these chemical-based repellents, which can also affect the skin and nervous system. This growing concern has encouraged scientists and researchers to develop repellents derived from natural and bio-based materials instead of synthetic chemicals. Many plants possess inherent mosquito-repelling properties that can be harnessed

for the production of safer and environmentally friendly repellents. In several regions, the use of chemical pesticides has already been restricted or banned because of issues such as insect resistance and adverse environmental impacts.

Mosquitoes are among the most troublesome blood-feeding insects that affect humans. Several species from the genera Anopheles, Culex, and Aedes act as vectors for numerous diseases such as dengue fever, malaria, yellow fever, Japanese encephalitis, and other viral or parasitic infections. Globally, mosquito-borne diseases impact more than 700 million people each year, resulting in over one million deaths annually. Malaria, caused by Plasmodium parasites and transmitted through the bites of infected female Anopheles mosquitoes, remains a major health threat, particularly to infants and young children in endemic areas. In 2012 alone, approximately 207 million malaria cases were reported worldwide, with an estimated 627,000 deaths. Historically, malaria has been endemic in Sri Lanka for centuries, with several severe outbreaks recorded over time.

One of the most severe malaria outbreaks occurred in 1934–1935, during which nearly 1.5 million people were infected and more than 80,000 lost their lives. In the years that followed, Sri Lanka made remarkable progress in controlling the disease, reducing malaria cases from over 400,000 in 1991 to zero by November 2012.

- **CHEMICALS**

Previous research has shown that the essential oils extracted from the leaves of several plants exhibit strong mosquito repellent properties. These include Cymbopogon nardus (citronella), Cymbopogon citratus (lemongrass), Cymbopogon winterianus (citronella), Ocimum basilicum (sweet basil), Ocimum sanctum (tulsi), Ocimum americanum (hairy basil), Eucalyptus citriodora and Eucalyptus globulus (eucalyptus), Rosmarinus officinalis (rosemary),Rose water, Melissa officinalis (lemon balm), Curcuma longa (turmeric) rhizomes, Citrus sinensis (sweet orange) peels, Citrus hystrix (kaffir lime) peels, Citrus limonum (lemon) peels, Syzygium aromaticum (clove) buds, and Pinus roxburghii resins. Additionally, extracts from Azadirachta indica (neem) seeds, Alpinia galangal (greater galangal) leaves, Vitex negundo, and Tribulus terrestris have also been investigated for their potential as natural mosquito repellents. The selection of these plant species was based on factors such as their availability, traditional usage, and prior scientific validation of their repellent effects.

- Cymbopogon nardus (Citronella) Kingdom :

  Plantae Order : Poales

  Family : Poaceae

  Genus : Cymbopogon

  Species : C. nardus

Cymbopogon nardus, commonly known as citronella grass, is a coarse, tufted tropical plant indigenous to Sri Lanka. This perennial grass typically attains a height of about 1.5 to 1.8 meters, with stems resembling canes. The essential oil of citronella is obtained through the steam distillation of its leaves. Widely recognized for its insect-repelling properties, citronella oil is a key ingredient in many commercial pest control products, including sprays, lotions, and candles.

- **Extraction Process of Citronella Essential Oil**

Citronella essential oil is obtained from the leaves and stems of the *Cymbopogon nardus* plant through a systematic extraction process. Harvesting typically takes place four to five months after planting when the plants reach maturity. The best time for collection is early in the morning, as the essential oil content in the leaves is highest at that time.

After harvesting, the leaves and stems are separated from the remaining plant material, and any debris or impurities are removed. The plant parts are thoroughly cleaned to eliminate dust and contaminants, then dried in a well-ventilated, shaded area or using a low-temperature dehydrator to prevent the loss of volatile compounds. Once completely dried, the material is chopped or coarsely ground to increase surface area and enhance oil recovery during extraction.

The final citronella essential oil is stored in dark, airtight glass containers to protect it from light and oxidation, preserving its natural properties and potency. It is kept in a cool, dry environment until use. Before distribution, the oil is subjected to quality control tests to assess purity

• MALARIA

- Malaria is a life-threatening infectious disease transmitted by arthropod vectors, specifically caused by *Plasmodium* parasites spread through the bites of infected female *Anopheles* mosquitoes. Apart from mosquito bites, transmission may also occur through blood transfusions or the use of contaminated needles. Early signs of malaria often resemble mild febrile illnesses, making it difficult to identify in the initial stages. If left untreated, *Plasmodium falciparum* infection can progress rapidly and may result in death.[12]

- The primary vector species responsible for malaria transmission include *Anopheles stephensi* and *Anopheles culicifacies*. The main causative agents are:

- *Plasmodium vivax*
- *Plasmodium falciparum*
- *Plasmodium malariae*
- *Plasmodium ovale*

- *Plasmodium knowlesi*
- The incubation period ranges from 7 to 30 days. Common symptoms include high fever, chills, headache, nausea, vomiting, sweating, and body aches. Without timely treatment, malaria can progress to severe forms such as cerebral malaria (characterized by convulsions and coma), anemia, renal failure, hypoglycemia, and pulmonary edema. Symptoms typically appear 10 to 15 days after receiving an infectious mosquito bite.
- Certain types of malaria can cause serious illness and even death. Infants, children below five years, pregnant women, travelers, and individuals with compromised immunity, such as those living with HIV/AIDS, are at greater risk. Immediate medical care is essential for individuals showing severe symptoms, as early treatment of mild malaria can prevent life-threatening complications. Malaria during pregnancy may lead to premature birth or low birth weight in infants.
- Diagnostic methods include:
- Peripheral blood smear examination (considered the gold standard)
- Rapid diagnostic tests (RDTs)
- Polymerase chain reaction (PCR) for species identification
- Prevention and control strategies focus on early diagnosis, effective treatment, mosquito vector control, and the use of personal protective measures to reduce human-vector contact.

• DENGUE

Dengue, also known as breakbone fever, is a viral disease caused by the dengue virus (DENV), a member of the *Flavivirus* genus. The virus is transmitted to humans through the bite of infected *Aedes* mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus*. There are four distinct serotypes of the dengue virus—DEN-1, DEN-2, DEN-3, and DEN-4. The disease is prevalent in tropical and subtropical regions around the world, particularly in urban and semi-urban areas.

Many dengue infections remain asymptomatic or result in only mild illness, though in some cases, the infection can develop into a severe or even life-threatening condition. Common symptoms include high fever, intense headache, muscle and joint pain, nausea, vomiting, rashes, and pain behind the eyes. In severe cases, the disease may cause dangerously low platelet counts, internal bleeding, or shock.

Symptoms usually appear between 4 to 10 days after being bitten by an infected mosquito and typically last for about 2 to 7 days. The major mosquito vectors responsible for transmission are *Aedes aegypti* and *Aedes albopictus*.Diagnostic testing plays an important role in effective management and control of the disease. Laboratory-based and point-of-care diagnostic tools

include nucleic acid amplification tests (NAATs), enzyme-linked immunosorbent assays (ELISA), and rapid diagnostic tests (RDTs), all of which help confirm infection and guide timely medical intervention.

• LIFE CYCLE OF MOSQUITO

Understanding the various stages of a mosquito's life cycle helps in effectively preventing mosquito infestations both indoors and outdoors, as well as in selecting suitable repellents when necessary. Each mosquito species undergoes four distinct developmental stages as part of its life cycle.

- **Egg**: The mosquito life cycle begins when a female lays her eggs on or near stagnant water sources such as rainwater puddles, sewage, or plant bases where moisture collects. Some mosquito species deposit their eggs individually, while others lay them in clusters known as rafts. These eggs are remarkably resilient and can endure dry conditions for several months, remaining dormant until they come into contact with water. Once submerged, the eggs typically hatch within 24 to 48 hours, depending on environmental temperature.

- **Larva:** After hatching, the mosquito enters the larval stage. Mosquito larvae are aquatic, worm-like organisms that can be seen moving or wriggling at the water's surface. They feed on microorganisms and organic matter present in the water, contributing to their rapid growth. During this stage, larvae molt several times before progressing to the next phase. The duration of this stage varies from a few days to several weeks, influenced by temperature and nutrient availability.

- **Pupa:** After completing the larval stage, the mosquito develops into a pupa, commonly referred to as a "tumbler." Pupae remain aquatic but do not feed, as this stage serves primarily as a transitional or resting phase before adulthood. Despite being in a resting state, they remain active and can move or tumble in the water when disturbed. The pupal stage usually lasts for a few days to about a week, depending on environmental conditions.

- **Adult:** The final stage begins when the adult mosquito emerges from the pupal casing. After emergence, it rests briefly on the surface of the water while its wings and body harden. Once ready, the adult mosquito takes flight in search of food. It is only the female mosquito that feeds on blood, which is required for the development of her eggs. After obtaining a sufficient blood meal, the female finds a suitable place to lay eggs, thereby continuing the life cycle.

- Mechanism of Action of Citrus limon as a Mosquito Repellent

The mosquito repellent properties of *Citrus limon* are primarily attributed to the presence of bioactive compounds such as limonene, citral, β-pinene, and γ-terpinene. These constituents act through multiple mechanisms to repel or eliminate mosquitoes.[6]

- **Olfactory Disruption**
  - Mosquitoes locate their hosts by detecting carbon dioxide, lactic acid, and human skin odors through specialized odor receptors.
  - Compounds like limonene and citral interfere with these receptors, making it difficult for mosquitoes to identify and approach their hosts.
  - This masking of human scent effectively reduces mosquito attraction and biting activity.

- **Neurotoxic Action on Mosquitoes**
  - Limonene functions as a natural neurotoxin against insects.
  - It disrupts normal neurotransmission by interacting with gamma-aminobutyric acid (GABA) receptors, leading to paralysis and death.
  - It also inhibits the enzyme acetylcholinesterase (AChE), causing an accumulation of acetylcholine, which results in overstimulation of nerve cells and ultimately paralysis.

- **Cuticular Penetration**
  - Terpenes present in lemon essential oil can penetrate the mosquito's outer cuticular layer.
  - This penetration leads to dehydration and damage to the mosquito's cellular structure, resulting in death.

- **Antifeedant and Deterrent Effects**
  - Compounds such as citral and limonene produce a strong citrus odor that mosquitoes find unpleasant.
  - This scent acts as both a repellent and deterrent, discouraging mosquitoes from landing on or near treated surfaces.

- **Mechanism of Action of Eucalyptus as a Mosquito Repellent**

The mosquito repellent effects of *Eucalyptus* are mainly attributed to its essential oil, which is rich in active constituents such as 1,8-cineole (eucalyptol), α-pinene, limonene, and various other terpenes. These compounds work synergistically to interfere with the mosquito's olfactory system, masking human scent and hindering host detection. Additionally, certain components possess neurotoxic and irritant properties that can disrupt mosquito behavior, reducing their landing and biting activity.[21]

Material	Solvent Used	Process
Citronella Leaves	Hexane	Steam Distillation
Eucalyptus Leaves	Hexane	Clevenger Apparatus
Clove Bud	Hexane	Hydro-distillation
Sweet Orange Peel	Hexane	Cold Pressing

- **ROLE OF INGREDIENTS**

Sr. No.	Constituents	Role of Ingredients
1	Citronella essential oil (Cymbopogon nardus)	Strong scent and mosquito repellent
2	Eucalyptus essential oil (Eucalyptus globulus)	Masking the scent that attracts mosquito
3	Clove bud essential oil (Eugenia caryophyllus)	Strong scent and mosquito repellent
4	Sweet orange peel essential oil (Citrus sinensis)	The fragrance of the spray
5	Neem extract (Azadirachta indica)	Mosquito repellent
6	Hexane	Insect repellent
7	Ethanol	Solvent for active ingredient
8	Tween 80	Emulsifier
9	Lavender oil	Fragrance
10	Distilled water	Sovent

• EVALUATION OF HERBAL MOSQUITO REPELLENT SPRAY

- **pH Test:**

  The pH of the prepared spray was measured to be 6.4, indicating a slightly acidic nature. This ensures that the formulation is mild and safe for application on human skin.

- **Skin Irritancy Test:**

  A skin patch test was conducted to evaluate the potential for irritation. The spray was applied to a patch and placed on a volunteer's forearm for a duration of 48 hours. Observations taken after 24 hours showed no visible signs of redness, itching, or irritation on the skin surface, confirming the formulation's dermatological safety.[11]

- **Field Test:**

  Three different formulations of the herbal spray were subjected to field evaluation. The effectiveness of each was tested in areas with a high mosquito population. The formulation that provided the highest level of protection and repellency was identified as the most effective variant.

• Materials

- All ingredients used in the preparation of the mosquito repellent spray were obtained from verified suppliers to ensure consistency and quality. Each component was carefully chosen for its specific function in improving the product's effectiveness, stability, and safety.
- Lemon Essential Oil (5.00 mL): Extracted from fresh lemon peels, this essential oil served as the main active ingredient. It is recognized for its strong insect-repelling ability and pleasant citrus fragrance.
- Eucalyptus Essential Oil (5.00 mL): Derived from eucalyptus leaves, this oil provided additional mosquito-repelling properties and a fresh, cooling scent. It complemented the activity of lemon oil through synergistic effects.
- Ethanol (95–100%) (50.00 mL): Used as a solvent and natural preservative, ethanol helped dissolve the essential oils and contributed antibacterial properties, extending the spray's shelf life.
- Tween 80 (Polysorbate 80) (10.00 mL): A non-ionic emulsifying agent that facilitated the blending of hydrophobic essential oils with water, ensuring a uniform and stable final mixture.[4]

- Distilled Water (30.00 mL): Employed to dilute the formulation and adjust its viscosity, distilled water was chosen to prevent contamination from minerals or impurities present in regular water.

- All materials were prepared and handled under controlled laboratory conditions. Volume measurements were accurately carried out using calibrated glassware such as volumetric flasks and micropipettes. All apparatus were sterilized before use, and chemicals were stored properly according to supplier guidelines.

- Approximately 5 grams of each plant sample were accurately weighed and placed in an oven maintained at 105°C for about five hours until a constant weight was achieved. The final stable weight was recorded to determine the moisture content of the plant material.

- The solvent removal process was carried out using a rotary evaporator, as illustrated in Figure 10. This equipment operates by applying heat to a rotating flask under reduced pressure, facilitating efficient solvent evaporation. The rotation of the flask spreads the liquid across its inner surface, thereby increasing the surface area and enhancing evaporation. Heating is typically achieved by partially immersing the flask in a warm water bath. Since the apparatus functions under vacuum conditions, the solvent evaporates at a temperature lower than its normal boiling point, preventing the thermal degradation of dissolved compounds and ensuring the preservation of the sample's bioactive constituents.

- Preparation of Essential Oils from Plant Extracts :-

A. Citronella Oil -

Citronella essential oil is obtained from the leaves and stems of *Cymbopogon nardus* through a carefully controlled extraction process. Harvesting takes place four to five months after planting, typically in the morning when the oil concentration within the leaves is highest. After collection, the leaves and stems are separated, cleaned thoroughly, and dried either under shade in a well-ventilated setting or in a dehydrator at a low temperature to preserve the oil's natural properties.[17]

The dried material is then chopped or coarsely ground to increase the surface area for extraction. Among the available techniques—solvent extraction, cold pressing, and steam distillation—the latter is most commonly used. In steam distillation, steam produced from heated water is passed through the plant material, causing the volatile oil to evaporate. The vapor mixture is then condensed into liquid, separating the essential oil from water as it floats to the top. The collected oil can be purified if necessary, ensuring maximum retention of its natural aroma and active compounds. It is then stored in dark glass containers in a cool, dry place to prevent degradation by light or oxidation. Quality control tests are conducted to verify its purity and stability before packaging and labeling.

B. Sweet Orange Oil -

Sweet orange oil is primarily extracted through the cold-press technique, which preserves its characteristic citrus fragrance. Fresh orange peels are prepared and pressed manually using a firm surface and a natural sponge. The pressure applied helps release the essential oil from the peel glands into the sponge. The squeezed sponge then releases an oil emulsion into a collection vessel. Once allowed to settle, the pure essential oil separates naturally and is carefully collected. This method is highly valued for preserving the authentic aroma of orange peel oil compared to heat-based extraction methods. The obtained oil is stored in airtight containers to maintain its freshness and potency.[16]

C. Eucalyptus Oil -

Eucalyptus essential oil is extracted using a steam distillation technique involving a Clevenger apparatus. Fresh eucalyptus leaves are thoroughly washed to remove impurities, then chopped or crushed to increase the exposed surface area for extraction. The distillation apparatus is assembled with a round-bottom flask partially filled with distilled water. The prepared leaves are added to the flask, ensuring they are properly submerged. The mixture is gently heated using a hot plate to avoid rapid boiling.[18]

- As steam forms, it carries the volatile oil components through the condenser, where the vapors cool and condense into a liquid. This distillate collects in the separation chamber of the apparatus, where the lighter essential oil floats atop the water. The oil layer is separated, filtered through clean cheesecloth or filter paper to remove any plant residues, and transferred to dark glass bottles for storage. The oil is kept away from direct sunlight in a cool environment to preserve its quality. This procedure can be repeated to produce larger quantities using fresh leaves and distilled water.

• SIDE EFFECTS MOSQUITO REPELLENT SPRAY

- ApproximatelMosquito repellent creams and roll-ons are commonly used to protect against mosquito bites. However, frequent or improper use may lead to several skin and health concerns. While these products are effective, awareness of their possible side effects is important for safe use.
- Skin Irritation and Rashes: Many repellents contain active chemicals such as DEET, which can cause skin redness, itching, or mild rashes, particularly in individuals with sensitive skin.[15]
- Allergic Reactions: Some users may experience burning sensations, swelling, or irritation following application due to allergic sensitivities to specific components.

- Dryness and Skin Damage: Continuous application of repellent creams can reduce the skin's natural moisture, causing dryness, roughness, or peeling.
- Acne and Breakouts: Certain formulations have an oily base that may clog pores, resulting in pimples or acne flare-ups.
- Eye and Respiratory Discomfort: Unintentional contact with the eyes or inhalation of repellent vapors can lead to watering eyes, throat irritation, or breathing difficulties.
- Hormonal Imbalance: Some synthetic repellents contain compounds suspected of interfering with hormonal activity, posing potential risks for children and long-term users.(19)
- Toxicity from Prolonged Exposure: Repeated absorption of chemical ingredients through the skin over extended periods may affect vital organs such as the liver or the nervous system.
- Reactions on Children's Skin: Children's skin is more sensitive, and the use of strong chemical-based roll-ons or creams may lead to irritation, redness, or allergic reactions.
- Localized Irritation from Roll-Ons: Roll-on repellents, when applied directly to the skin, may cause localized burning, discoloration, or sensitivity depending on their concentration.
- Environmental and Health Hazards: Many repellents are formulated with synthetic substances that can negatively impact both environmental ecosystems and human health with continued exposure.
- y 5 grams of each plant sample were accurately weighed and placed in an oven maintained at 105°C for about five hours until a constant weight was achieved. The final stable weight was recorded to determine the moisture content of the plant material.
- The solvent removal process was carried out using a rotary evaporator, as illustrated in Figure 10. This equipment operates by applying heat to a rotating flask under reduced pressure, facilitating efficient solvent evaporation. The rotation of the flask spreads the liquid across its inner surface, thereby increasing the surface area and enhancing evaporation. Heating is typically achieved by partially immersing the flask in a warm water bath. Since the apparatus functions under vacuum conditions, the solvent evaporates at a temperature lower than its normal boiling point, preventing the thermal degradation of dissolved compounds and ensuring the preservation of the sample's bioactive constituents.

• Advantages

- Highly effective in repelling mosquitoes and other types of flying insects.(29)
- Completely natural and non-toxic, making it safe for human use and environmentally friendly.(27)
- Can be applied or used in multiple forms such as essential oils, sprays, and aromatic candles.(29)
- Possesses a pleasant and refreshing citrus scent that enhances its usability.(27)

- **Disadvantages**

- Generally less potent compared to chemical repellents containing DEET.[29]
- May cause mild skin irritation or allergic reactions in sensitive individuals.[27]
- Provides limited duration of protection, requiring more frequent application than synthetic alternatives.[29]

- **REFERENCES**

1. Krati and Dr. Jaya Martolia (2024). An extensive review of various in-vitro techniques used for evaluating antimicrobial activity. *IP International Journal of Comprehensive and Advanced Pharmacology*, 9(3).

2. Neeru, Shilpi Kashyap, Esha Vatsa, Jitendra Singh, and Ankush Sundriyal (2016). Analysis of total phenolic content, total flavonoid content, and overall antioxidant capacity in different extracts of *Roylea elegans* Wall. (aerial parts). *World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS)*, 5(6), 1884–1891.

3. Neeru, Esha Vatsa, Jitendra Singh, and Ankush Sundriyal (2016). Pharmacognostic standardization parameters of *Roylea elegans* Wall. (aerial parts). *International Journal for Pharmaceutical Research Scholars (IJPRS)*, 5(2), 133–140.

4. Kundan Singh Bora and Esha Vatsa (2016). Pharmacognostic evaluation of *Dendrobium macraei* Lindl. *Universities Journal of Phytochemistry and Ayurvedic Heights (UJPAH)*, 1(20), 29–36.

5. Amit Sharma, Bharat Parashar, Esha Vatsa, Shilpa Chandel, and Surbhi Sharma (2016). Phytochemical screening and anthelmintic activity of *Cedrus deodara* (Roxb.) leaves. *World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS)*, 5(8), 1618–1628.

6. Esha Vatsa, Samriti Faujdar, Nidhi Sharma, Shilpa Chandel, and Mehak Aggarwal (2022). *Dendrobium macraei Lindl.: A review of the medicinal significance of this orchid based on contemporary evidence. Chinese Journal of Medical Genetics*, 31(3), 560–571

7. Krati, Babita Rawat, Abhishek Bhardwaj, and Amandeep Singh (2025). *An analytical review of Indian barnyard millet (Echinochloa frumentacea). International Journal of Pharmaceutical Technology and Biotechnology*, 12(1), 1–7

8. Krati, Jaya Martolia, and collaborators (2024). *A detailed review of in-vitro methodologies used for assessing antimicrobial activity. Educational Administration: Theory and Practice*, 30(6), 2977–2984

9. Esha Vatsa, Samriti Faujdar, Shilpa Chandel, Nidhi Chaudhary, Ashok Kumar, and Neeru (2023). *Evaluation of anti-inflammatory properties of the whole plant of Dendrobium macraei Lindl. European Chemical Bulletin*, 12(Special Issue 1), 657–664

10. Esha Vatsa, Samriti Faujdar, Nitin Kumar, Nidhi Chaudhary, Shilpa Chandel, Neeru, and Mehak Aggarwal (2023). *Recent investigations supporting the medicinal potential of the orchid Dendrobium macraei Lindl.*

11. Rani N., Wany A., Vidyarthi A. S., and Pandey D. M. (2013). *Investigation of citronella leaf-based herbal mosquito repellent formulations using natural binders. Current Research in Microbiology and Biotechnology*, 1(3), 98–103

12. Devi L. D. (2022). *Study on knowledge and awareness related to selected mosquito-borne diseases and their preventive measures. Asian Journal of Nursing Education and Research*

13. Salunke M. R., Bandal S. C., Choudhari D., Gaikwad T., and Dubey M. (2022). *A review of herbal mosquito repellents and their effectiveness. International Journal of Science and Development*, 7, 204–214.

14. Gupta D. K., and Gupta R. A. (2020). *Formulation and evaluation of polyherbal mosquito repellent preparations. Research Journal of Pharmacy and Technology*

15. Sharma M., Nagori K., Jain V., and Balan N. S. (2023). *Recent advances and opportunities in the development of safe and natural herbal mosquito repellents. Research Journal of Pharmacy and Technology*

16. Anil Kumar and Esha Vatsa (2025). *AI-powered embryo selection: A transformative review of advancements and innovations. South Eastern European Journal of Public Health*, 26(1), 6223–6230

17. Lohani V., A. R. A., Kundu S., Akhter M. Q., and Bag S. (2023). *Single-cell proteomics combined with spatial profiling: Tools, methodologies, and recent developments*

18. Amandeep Singh, Deepak Nanda, Ashok Kumar, and Abhishek Bhardwaj (2023). *In vitro assessment of anti-inflammatory properties of Ageratum conyzoides leaves using the human red blood cell membrane stabilization technique. International Journal of Research in Pharmaceutical*

19. Singh A., Nanda D., Bhardwaj A., and Kumar A. (2024). *Pharmacological evaluation of the anthelmintic potential of Callistemon citrinus (bottle-brush plant). IP International Journal of Comprehensive and Advanced Pharmacology*

20. Yogesh Tiwari, Amandeep Singh, Bhupendra Kumar, and Ashok Kumar (2017). *In vitro analysis of alpha-amylase inhibitory activity from bark extracts of Ficus auriculata. International Journal of Innovative Science and Research Technology*

21. Weng D. C., Latip J., Hasbullah S. A., and Sastrohamidjojo H. (2015). *Optimization and assessment of oil yield in citronella essential oil extracted from Cymbopogon nardus*

22. Tiwari P. and Sahu P. K. (2018). *Phytoconstituents and therapeutic potential of Eucalyptus tereticornis. Research Journal of Pharmacy and Technology*

23. Chang C. P. and Dobashi T. (2003). *Formulation of alginate complex capsules containing eucalyptus essential oil for controlled release applications.*

24. Misni N., Nor Z. M., and Ahmad R. (2016). *Identification of potential plant-based repellents effective against Aedes aegypti mosquitoes.*

25. Bhamare U. U., Mali Y. S., and Shaikh A. Z. (2020). *Neem: A versatile agent in natural medicine. Research Journal of Pharmacognosy and Phytochemistry*.

26. Kumar P., Singh S., and Sharma S. (2018). *Evaluation of mosquito repellent properties of essential oils derived from Indian medicinal plants. Journal of Essential Oil Research*

27. Jagessar R. C., Singh S., and Kumar P. (2019). *Assessment of mosquito repellent activity using various herbal extracts. Journal of Alternative and Complementary Medicine*

28. Singh S., Kumar P., and Sharma S. (2020). *Role of plant-derived alkaloids and terpenoids as natural mosquito repellents. Journal of Pharmaceutical Sciences*

29. Gupta S., Kumar P., and Singh S. (2020). *Development and evaluation of herbal-based mosquito repellent formulations.*