Original Research Article

UNRAVELLING INSECT REPELLENT POWER OF LEMONGRASS

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Abstract:
This review article explores the potent insect-repelling attributes of Cymbopogon citratus, commonly known as Lemongrass. Beginning with an introduction to the significance of plant-based insect repellents, the focus narrows down to Lemongrass as a standout candidate. The article proceeds by detailing the extraction of Lemongrass essential oil, followed by its comprehensive GC–MS analysis, offering insights into the chemical composition of the oil. Citral is Known for its antimicrobial properties, Citronella Used as a natural insect repellent in various products like candles and sprays, Neral Contributes to the lemony aroma and possesses antimicrobial properties in perfumes, soaps, and cosmetics, Limonene Provides a citrusy aroma. The formulation of the oil and its varying effects, spanning from lethal to sublethal on insects, are also investigated, emphasizing its potential as an efficient insect deterrent. Central to the article is an examination of the bioactive compounds inherent to Lemongrass and its oil. Moreover, the review critically evaluates methodologies for repellent testing, ensuring a robust comprehension of experimental protocols. The article concludes by shedding light on emerging developments in plant-based repellents, underscoring the ongoing evolution of insect control techniques rooted in botanical resources. Through its comprehensive exploration of Lemongrass's insect-repelling capabilities, this review presents a holistic perspective, showcasing its potential to revolutionize insect management strategies.

Key Words: Lemongrass, Cymbopogon citratus, plant based Repellent

1. INTRODUCTION :

The majority of plants contain substances aimed at preventing attacks from plant-eating insects, falling into various classes like repellents, feeding deterrents, toxins, and growth regulators. These chemicals can be mainly categorized into five groups: (1) nitrogen compounds, mainly alkaloids, (2) terpenoids, (3) phenolics, (4) proteinase inhibitors, and (5) growth regulators. While their primary role is to defend against insects, many of these compounds also work effectively against mosquitoes and other biting insects like Diptera. This is particularly true for the volatile components that are released when the plant is being consumed by herbivores.[(1)]. Many of these compounds likely possess repellent characteristics due to their evolutionary origins from ancestors that consumed plants. A substantial portion of these substances initially evolved as natural deterrents against insects that fed on plants[2] The ability to react repellently to potentially harmful compounds has been preserved across the evolutionary lineage of Diptera (True Flies). Insects possess the capacity to detect scents by means of volatile odors binding to odorant receptor (OR) proteins found on hair-like structures called ciliated dendrites. These specialized odor receptor neurons (ORNs) are exposed to the external environment and are typically located on the insect's antennae and maxillary palps. Among these ORNs is OR83b, which plays a crucial role in the sense of smell and is inhibited by the widely recognized synthetic repellent DEET (N, N-diethyl-3-methylbenzamide).[3] are highly conserved across insect species[4] Plants commonly produce volatile “green leaf volatiles” when leaves are damaged in order to deter herbivores[5]. Moreover, various researchers have demonstrated significant reactions of odor receptors in mosquitoes to this category of airborne compounds, which includes substances like geranyl acetate and citronellal. [6] 6-methyl-5- hepten-2-one and geranylacetone [7]. Interestingly, the same odour receptors that respond to DEET also respond to thujone eucalyptol and linalool in Culex quinquefasciatus [2]. In Anopheles gambiae mosquitoes, the DEET receptor OR83b responds to citronellal's stimulation, but its activity is also influenced by the TRPA1 cation channel.[8]. However, it's quite likely that many volatile compounds originating from plants work as deterrents or repellents due to their significant vapor toxicity to insects (9,10). In the context of insect repellents formulated for personal use, like DEET, they have been linked to inducing adverse skin reactions, thus limiting the feasible utilization of the active ingredient across different products.[47] they are known to contain harmful and poisonous compounds which could pose threats to human health and toxicity to non-target organisms[48] The repellent efficacy of the chemical N,N-diethyl-3-methylbenzamide (DEET) over other products made it the compound of choice in pesticides[49]. However, concerns of its safety have not ceased to be considered.[50]

For millennia, humans have made use of the inherent repellent properties found in plant materials. This has often been accomplished by simply hanging crushed plants indoors, a practice that remains prevalent in developing nations and has endured across generations. Additionally, the repellent characteristics of plants have been employed for centuries through methods such as burning plants to create basic fumigants that deter troublesome mosquitoes. Over time, these repellent qualities were incorporated into oil-based formulations designed for application on the skin or clothing. This historical practice has been documented by scholars from ancient Greek, Roman, and Indian civilizations. In rural tropical communities where resources are limited, plant-based repellents continue to play a crucial role in guarding against mosquito bites. For many of these financially disadvantaged communities, traditional plant-based repellents stand as the only accessible means of protection against diseases carried by mosquitoes. [2] as in the Europe and North America [11]. Preferential inclination towards repellents emitting a "natural" fragrance stems from the belief that plants provide a reliable and trustworthy approach to ward off mosquito bites. The process of discovering new repellents derived from plants is significantly dependent on insights from ethnobotany.[12] Many plant oils, including citronella, clove, geranium, mint, nutmeg, pennyroyal, and soybean, exhibit the capacity to temporarily repel insects. Nevertheless, their rapid evaporation significantly limits the duration of their efficacy when employed in candles or applied to the skin.[2,47] Lemongrass, scientifically identified as Cymbopogon citratus, is a lofty and persistent grass esteemed for its fragrant characteristics. Among the scented grasses within the Cymbopogon genus, this specific plant stands out due to its essential oils that emit a delightful lemony fragrance.

Across the Asian region, Lemongrass holds a significant position as a fundamental element in enhancing health and well-being. Particularly in India, it is employed as a calming agent for the central nervous system.[45] they contain an essential oil having citral as main constituent, same as what is present in lemon peel. Leading phytochemical compounds in lemongrass leaf are terpineol, Dipentene, Limonene, α-terpineol, citronellol, methyl heptenone, dipentene, geraniol, limonene, nerol, farnesol, mainly (triterpenoids).[46]

2. METHODS :

2.1. Extraction of C. citratus Essential Oil :

Fresh leaves of Cymbopogon citratus were sourced from farms. The leaves underwent a meticulous cleaning process, including thorough rinsing with running tap water to eliminate any dirt or contaminants. After this initial step, the leaves were finely chopped into small fragments using excelsior. Employing a hydro-distillation technique with a Clevenger-type apparatus, the valuable essential oil (EO) derived from C. citratus was successfully obtained. This systematic procedure ensured the effective extraction and collection of the precious essential oil from the plant material.[13]

2.2. GC–MS Analysis of the Essential Oil :

The assessment of Lemongrass essential oil (EO) composition was conducted using a Trace GC Ultra-ISQ mass spectrometer from Thermo Scientific, located in Austin, TX, USA. The instrument was equipped with a TG–5MS capillary column (30 m × 0.25 mm × 0.25 µm). Before introducing the sample into the GC–MS system, the EO underwent a dilution process with n-hexane solvent (3 parts n-hexane to 1 part EO). The carrier gas employed was Helium (He) with a flow rate of 1 mL/min. The GC Autosampler AS1310 was integrated, enabling automated splitless injection of the diluted sample (2 µL).

The temperature programming for the column oven commenced with an initial temperature of 50 °C, which experienced gradual increments at 5 °C/min until reaching 250 °C. This temperature was then sustained for 2 minutes. Following this, the temperature was further raised to 310 °C at a rate of 25 °C/min and maintained for 2 minutes. The injector and MS transfer line were kept at consistent temperatures of 270 °C and 260 °C, respectively. The ionization voltage for electron ionization (EI) mass spectra was set at 70 eV, covering a mass-to-charge ratio (m/z) range of 50–650 through full-scan mode. Additionally, the ion source temperature was established at 250 °C.

For the purpose of identifying chemical constituents, the study utilized the Xcalibur 3.0 data system developed by Thermo Fisher Scientific Inc. in Austin, TX, USA, in 2014. This system employed calculations based on Standard Index and Reverse Standard Index measurements, aligning with existing GC–MS literature. To confirm the presence of a compound, a Match factor value equal to or greater than 650 was considered significant. This comprehensive approach greatly facilitated the identification of various components present within the sample.[2,13,14,15,16].

2.3. Formulation of Oil :

The ingredients in the formula are investigated at different levels and select the appropriate content to add to the product. The result is a diffuse product with main ingredients: Coconut oil (1%), Lemongrass essential oil (4%), Ethanol (5%), Tween 80 (8%) as emulsifier and BHT antioxidant

(0.1%). The resulted lemongrass essential oil requires room temperature and minimal temperature change to maintain its diffusion quality.[17]

3. Bioactive Compounds Present in Lemon Grass and its Oil:

Today, a wide range of ethno pharmacological applications of lemongrass are available. Its potential for promoting wellness can be attributed to the diverse secondary metabolites it generates. Examination of the grass verified the existence of fats, proteins, carbohydrates, fiber, minerals, and various bioactive compounds. These can be categorized into specific classes such as alkaloids, terpenoids, flavonoids, phenols, saponins, and tannins. Studies have also indicated the presence of anthraquinones, steroids, phlobotannins, and cardiac glycosides within lemongrass.[19]

Citral:-

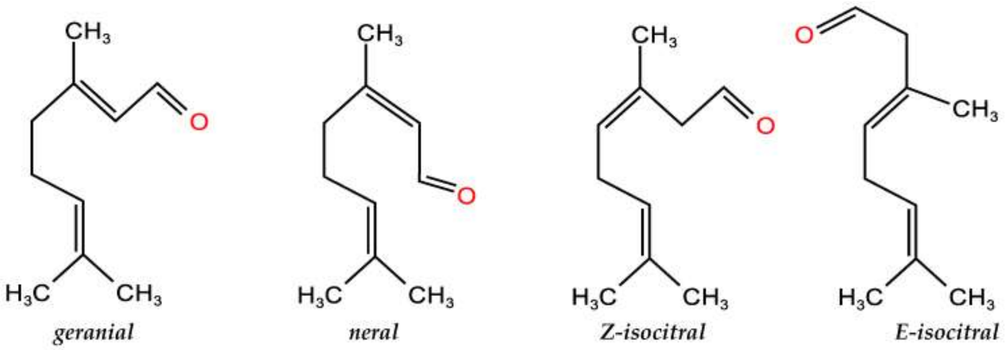


Figure 1. Chemical structures of the main compounds of the essential oils from citral chemotype C. camphora and C. bodinieri.[51]

Formula: C10H16O

Boiling point: 225 °C

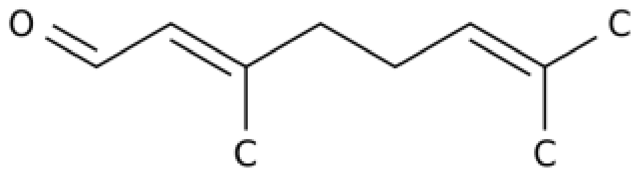
Molar mass: 152.24 g/moll

Companies treated with citral exhibited a dose-dependent reduction in body weight gain. They also demonstrated lower fasting glucose levels, improved glucose tolerance, decreased fasting plasma glucose, enhanced metabolic rate, and smaller adipocytes following the administration of the compound.

Purpose: Citral finds utility in the synthesis of vitamins A, lycopene, ionone, and methyl ionone. It is also utilized to mask the smell of smoke. The plant Cymbopogon citratus has displayed promising insecticidal and antifungal activity against pests commonly found in storage.

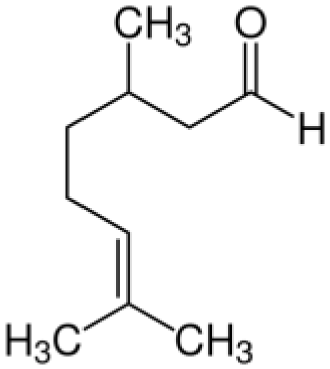
Toxicity: Citral undergoes rapid metabolism and is primarily excreted as metabolites, mainly through urine. The primary route of elimination is through urine. This chemical exhibits low acute toxicity in rodents, as indicated by oral or dermal LD50 values exceeding 1000 mg/kg. It poses skin irritation potential and does not cause eye irritation in rabbits [20].

Neral:-



Formula: $C_{10}H_{16}O$
Boiling point: 225 °C
Molar mass: 152.24 g/mol
Neral is an enal this is 3,7-dimethyloctanal with unsaturation at positions C-2 and C-6. It has been remoted shape the important oils of plant species like lemon. It has a position as an apoptosis inducer and a plant metabolite. It is an Enal and a monoterpenoid. Neral have a lemon heady fragrance, however, neral has a milder, and sweeter lemon odour. These compounds are used for my part or collectively relying upon the favoured heady fragrance or taste due to the fact that they're utilized in perfumes, sweet or even gentle drinks. Neral are examples of cis/trans isomeric alcohol molecules located in important oils(21).

Citronellal:-



Formula: $C_{10}H_{18}O$
Molar mass: 154.25 g/mol
Boiling point: 208 °C
Chemical properties: Citronellal has insect repellent properties, and research shows high repellent effectiveness against mosquitoes.
Odour: Citronellal is a monoterpenoid, the principle thing of citronella oil which offers it its unique lemon aroma. Citronellal ($C_{10}H_{18}O$) is a mono terpenoid aldehyde, the principle thing with inside the aggregate of terpenoid chemical substances that provide citronella oil its unique lemon scent. It has a function as a metabolite and an antifungal agent. It is a monoterpenoid and an aldehyde(22).

Limonene:-
Formula: $C_{10}H_{16}$
Boiling point: 176 °C
Molar Mass: 136.24 g/mol
Odour Like citrus fruits and commonly used in food products Limonene is a colourless liquid aliphatic hydrocarbon classified as a cyclic monoterpene and is the major component of citrus peel oils. The isomer, commonly found in nature as the flavour of oranges, is used as a flavouring agent in the manufacture of food products (23).

Table 1. Phytochemical screening of essential oil from lemongrass leaves by GC–MS analysis RT: Retention time (min).

RT	Area%	Compound Name	Match Factor (MF)
7.97	0.83	Isoneral	850
8.37	1.49	Isogeranial	900
8.60	0.71	Dihydronopol	750
9.77	35.00	Neral or β-citral (Citral B)	955
10.47	35.91	Geranial or α-citral (Citral A)	850
10.61	3.58	*trans*-Verbenol	750
10.71	0.91	Epoxy-linalooloxide	800
10.97	1.45	Geranyl vinyl ether	955
11.26	7.84	Nerylacetal	929
11.76	9.08	5-Octyldihydro-2(3H)-furanone	930
12.69	1.24	Geraniol acetate	950
13.74	1.24	(Z,E)-α-farnesene	939
16.77	0.72	β-Caryophyllene epoxide	850

4. Mechanism for Insecticidal Activity:

Lemongrass essential oil, notably containing citral, can influence cell proliferation by interacting with intracellular oxygen radicals and inducing oxidative stress. Furthermore, citral, along with other essential oil components, can affect neuroreceptors, disrupt signal transduction, lead to hormonal imbalances, cause membrane damage, and induce cytotoxicity in host organisms. In addition to citral, various other components within essential oils can target multiple sites in insects, potentially creating a synergistic effect that enhances their insecticidal properties. Insects have defense and detoxification mechanisms to mitigate the toxic effects of major components, but lesser-known minor components can evade these detoxification systems and induce cytotoxicity in insect cell lines, including ovarian cell lines.

Essential oils may also target essential metabolic enzymes, especially acidic phenolic components like geranial and neral, which can react with enzyme active sites and potentially lead to their inactivation. Phenoloxidase, an important marker for the insect defense system, is one such enzyme that can be affected by essential oils, potentially weakening the insect's immune system. Essential oils may interfere with the activation cascade of phenoloxidase, reducing its activity. Additionally, essential oils can enhance lactate dehydrogenase activity, a marker for evaluating cellular damage, and modulate the expression of genes associated with the biosynthesis of these enzymes.

Essential oils from various Cymbopogon species have been found to negatively impact haemocyte viability in insect larvae populations. Haemocytes play a significant role in maintaining the cellular and humoral immune system in insects, and their viability can be modulated by targeting insect immune system constituents or regulating immune responses. Such alterations can have detrimental effects on the hematopoietic system and endocrine gland activity, ultimately impeding overall insect growth and development.

Components of essential oils can vary in their effectiveness against different insects and the systems they target. These variations can be attributed to plant-dependent factors, such as the concentration of essential oil constituents, plant developmental stage, phenological conditions, and environmental factors, as well as insect-dependent factors, including the level of essential oil absorption by the insect, its developmental stage, cuticle thickness, and weight. Essential oils, being complex mixtures of components, can thus impact multiple systems in insects, including the nervous system, respiratory system, reproductive system, and defense mechanisms. Recent reports have indicated that essential oils may employ neurological mechanisms to inflict significant damage in insects.[18]

5. Considerations for repellent testing:

findings were identified within the past five years. These research works can be categorized as follows:

Ethnobotanical studies that assess plants traditionally used for repelling mosquitoes[24,25,26]. Laboratory investigations involving standard dose-response evaluations of plant solvent extractions, excluding DEET positive controls[2]. Laboratory evaluations employing standard dose-response assessments of plant solvent extractions or essential oils, accompanied by DEET positive controls [2]. These studies were complemented by GC-MS (gas chromatography-mass spectrometry) analysis[28]. Some studies also incorporated GC-MS analysis to identify oil constituents [29].

However, it's noteworthy that a significant number of studies didn't adhere to the accepted standard methodology[30], warranting a cautious interpretation of their findings. Only a limited number of studies[31, 32] examined safety or adverse effects, and a solitary study incorporated randomization and blinding[33]. Furthermore, most repellent studies disregarded the necessity for an adequate number of human participants to minimize sampling error[2].

For the advancement of plant-based repellents in the future, it is imperative to adhere to the established WHO methodology[30]. This includes employing a DEET control for facilitating straightforward comparisons across multiple studies. Additionally, reporting standard errors is crucial for comprehending the reliability of the observed protective effects of the repellent compound. By following these guidelines, the development of plant-based repellents can be significantly enhanced.

6. Promising developments in plant based Repellents:

The development of plant-based repellents is advancing in response to consumer demands for safe, user-friendly, and environmentally sustainable methods of protecting against arthropod bites. A key focus in this endeavor is enhancing the durability of effective repellents with high volatility, such as citronella. Numerous research studies have explored the enhancement of plant oil formulations using nanoemulsions to extend their effectiveness over time.[34,35], improved formulations and fixatives[36,37]. Additionally, alternative approaches such as spatial activity [38,39] and excitorepellency [40] have been under investigation.

A notable development is the sole clinical study involving PMD, which has shown promise in reducing malaria incidence[2]. This discovery is particularly exciting because PMD can be derived from distilling leaves of E. citriodora or chemically modifying citronellal[41], which can be sourced from plants of the Cymbopogon genus. These plants are already cultivated for essential oil production and timber in malaria-endemic regions including South America (with around 6 million trees in Brazil), southern China, India, Sri Lanka, Congo (Zaire), Kenya, and various countries in southern Africa. The local production of insect repellent has the potential to alleviate the burden of importing such products in developing nations. Further advancements have been made in understanding the mechanisms of plant-based repellents in insects. Several studies have delved into the behavioral mode of action of repellents through structure-activity analyses of contact versus spatial repellency or olfactometry. For example, DEET has been shown to hinder mosquito response to human odor, while Ocimum forskolei repels insects without inhibiting their response to human odor[2]. Additionally, research has revealed that citronellal directly activates cation channels[42], akin to the excitorepellent effect of pyrethrin – another terpene derived from plants (2). This contrasts with DEET's inhibitory effect[43].

The realm of developing repellents from plants remains fertile due to the abundance of insecticidal compounds naturally present in plants as defenses against insects. While modern pyrethroids are the backbone of the ongoing malaria elimination initiative, these synthetic analogues are modeled after pyrethrins, initially discovered in the pyrethrum daisy, Tanacetum cinerariifolium, native to the Dalmatian region, and Tanacetum coccineum, originating from Persia. The insecticidal component, composed of six esters (pyrethrins), resides in small oil-containing glands on the seed case surface within the flower head. This natural defense shields the seed from insect attacks. Although pyrethrins are potent insecticides relatively benign to mammals, it's important to note that numerous other plants produce compounds that are highly toxic to mammals or skin irritants. Thus, the term "natural" does not automatically equate to "safe". Recent years have witnessed a breakthrough in the realm of plant-derived repellents, with PMD demonstrating efficacy and safety comparable to DEET in disease prevention. The World Health Organization (WHO) has recognized repellents as valuable tools for disease prevention alongside insecticide-based vector control strategies. The assessment and development of plant-based repellents have become more rigorous, and progress in dispensing methods for plant-based volatiles holds promise for extending the duration of repellency and subsequently enhancing efficacy [2].

7. Conclusion:

This study presents the repellent and insecticidal properties of Lemongrass oil. The study demonstrates that the essential oil of lemongrass can serve as an insect repellent, being both safe for humans and the environment. The article further provides informative insights into the potential future impacts of plant-based insect repellents.

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