



PHYSICOCHEMICAL CHARACTERIZATION, BIOACTIVITY, AND ACARICIDAL POTENTIAL OF ESSENTIAL OILS FROM *Cymbopogon citratus* AND *Citrus sinensis* PEEL AGAINST ADULT *Amblyomma variegatum*

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ABSTRACT

The increasing resistance of ticks to synthetic acaricides has driven interest in plant-derived alternatives. This study evaluated the extraction yield, physicochemical properties, chemical composition and acaricidal efficacy of essential oils from lemongrass (*Cymbopogon citratus*) and orange peel (*Citrus sinensis*) against adult *Amblyomma variegatum*. Essential oils were obtained by hydro distillation and yields were calculated. Physicochemical properties were determined using standard Association of Official Analytical Chemists methods, while chemical composition was analyzed using gas chromatography–mass spectrometry. Acaricidal activity was assessed using the Adult Immersion Test at concentrations of 10%, 20%, and 30%, with mortality recorded at 24, 48, and 72 hours post-treatment. Amitraz (0.1%) served as the positive control, while extra-virgin olive oil was used as diluent and negative control. Lemongrass oil yielded 0.70% (2.0 g), whereas orange peel oil yielded 4.70% (14.0 g). GC-MS analysis identified citral (71.0%) as the major component in lemongrass oil and limonene (36.0%) in orange peel oil. Both oils exhibited concentration- and time-dependent acaricidal activity. At 30% concentration after 72 hours, mortality rates of 73.33% and 60.00% were recorded for lemongrass and orange peel oils, respectively. Although neither treatment achieved complete mortality comparable to amitraz, lemongrass oil consistently demonstrated greater efficacy. These findings indicate that both essential oils possess significant acaricidal potential with lemongrass oil showing superior activity. Their effectiveness at higher concentrations supports their potential as environmentally friendly alternatives for integration into tick management strategies.

Keywords: AIT (Adult Immersion Test), *Amblyomma variegatum*, GC-MS, Hydro-distillation, Lemon grass essential oil, orange peel essential oil

INTRODUCTION

Essential oils are concentrated volatile aromatic oils with strong aroma found in many plant families of Mediterranean and tropical country origin (Franz and Novak, 2020). They can be synthesized by all plant organs such as leaves, flower, stems, seeds, fruits, bark, buds, roots and twigs, they are lipid soluble and soluble in other organic solvents with a lower density than that of water. The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes. Their chemical composition and biological activities can vary with plant age, plant tissues, geographical origin, the plant organ used in the distillation process, type of distillation and the specie and age of the targeted parasite (Sharmeen *et al.*, 2021).

Essential oils are known for their medicinal and antiseptic properties, and their acaricidal, insecticidal and repellent activities against a wide range of pests are widely reported (Bakkali *et al.*, 2008; Isman, 2020). The efficacy of essential oil is usually due to their various components which may work in synergy (Sharma *et al.*, 2020). Essential oils interfere with physiologic, behavioral, metabolic and biochemical functions of insects. They have the ability to reduce arthropod/insect fecundity as a result of the volatile oils which may diffuse into eggs affecting vital processes associated with embryonic development (Lemay and Scott-Dupree, 2022). They induce premature oviposition, deter feeding, have larvicidal effect, delay development and suppress emergence of adults (Khater, 2012; Kaur *et al.*, 2023).

Essential oils are known to aid cellular accumulation and absorption of their components which are toxic to insect's nervous system (Chaudhari *et al.*, 2021). Terpinen-4-ol which is found in tea tree oil is known to inhibit arthropod

acetylcholinesterase which disrupts insect nervous system (López and Pascual-Villalobos, 2010; de Sena Filho *et al.*, 2023). Essential oils are known for their ability to disrupt the function of Octopamine (a biogenic amine which act as a neurotransmitter, neurohormone and neuromodulator) in insects which results in the breakdown of their nervous system (Martins Da Silva *et al.*, 2023). In addition to octopamine receptors, gamma amino butyric acid (GABA) receptors are target sites for compounds like thymol which disrupts the functioning of GABA synapses (Devnija *et al.*, 2022). The activities of acetylcholinesterase enzyme in insects is inhibited by monoterpenoids (Liu *et al.*, 2022). The hydrophobic nature of essential oils affects the cuticular waxes of insects by desiccation and blocks their spiracles leading to death (Agwunobi *et al.*, 2020)

Cymbopogon citratus belongs to the family Poaceae which grows in tropical and subtropical regions of the world (Oniha *et al.*, 2023). *Cymbopogon* species are perennial herbs with long thin leaves and are known for their strong lemony flavor due to high content of aldehyde citral which has two geometric isomers; geranial (citral a) and neral (citral b). One isomer does not occur without the other (Irfan *et al.*, 2022). A number of studies (Sharma *et al.*, 2021; Naser and Abdul-Ameer, 2023; Ashaq *et al.*, 2024) have been carried out to show the anti-microbial, anti-fungal and anti-oxidant activities of lemon grass.

Citrus sinensis belong to the family Rutaceae, they grow worldwide and they are the most popular fruit in Nigeria. The fruit skin (rind/peel) contains numerous small oil glands from which essential oils are derived which may also be called orange oil. Composition of the oil varies as a result of regional, seasonal and the method used for extraction. Several

compounds have been identified most of which belong to the terpene groups with limonene being the most abundant. Pinene, limonene and linalool in citrus peels are known to act as an insecticide (Visakh *et al.*, 2022). Limonene from citrus oils of oranges and other citrus fruit peels is used as a contact insecticide against ants, cockroaches, fleas and ticks. Limonene has low oral and dermal toxicity to mammals. Most essential oils used as pesticides works by disrupting the insect neurotransmission which is not present in humans and other vertebrates (Souto *et al.*, 2021).

MATERIALS AND METHODS

Plant Material Collection and Preparation

Fresh mature lemongrass (*Cymbopogon citratus*) was harvested from its natural habitat in and around Zaria metropolis (11°4'N, 7°42'E). Fruits of *Citrus sinensis* (orange) were obtained from a local market. The plant materials were authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. The orange fruits were manually peeled, and the peels were cut into small pieces. They were thoroughly washed with distilled water to remove surface impurities, and air-dried at ambient temperature. All samples were processed immediately after preparation to minimize degradation of volatile constituents.

Extraction of Essential Oils

Lemongrass Essential Oil Extraction

Approximately 300 g of fresh *Cymbopogon citratus* leaves was immersed in 500 mL of distilled water in a 1 L round-bottom flask and subjected to hydro distillation using a Clevenger-type apparatus, following the method described by Vikrant *et al.* (2023). Distillation was carried out for 3 hours to ensure exhaustive recovery of essential oils. The extraction was repeated as required to obtain sufficient yield. The oil was collected, volumetrically measured, and stored in amber glass vials at 4 °C until further analysis.

Orange Peel Essential Oil Extraction

Essential oil from *Citrus sinensis* peel was extracted by hydro distillation using a modified Clevenger-type apparatus, following Dangol *et al.* (2023) with slight modifications. Approximately 300 g of fresh peel was transferred into a 1 L flat-bottom flask containing 650 mL of distilled water. The mixture was subjected to hydro distillation for 180 minutes, with boiling chips added to ensure smooth boiling. Heating was applied gradually to maintain steady distillation, and distilled water was replenished as required to maintain the extraction volume. The distillate was collected and extracted with 20 mL of diethyl ether using a separatory funnel. The aqueous phase was discarded, and the organic phase was retained and dried over anhydrous magnesium sulfate. The solvent was allowed to evaporate under a fume hood at ambient temperature for 12 h to obtain the essential oil. The extraction was repeated as necessary to obtain sufficient yield. The recovered oil was measured and stored in amber glass vials at 4 °C until further analysis.

Determination of Extraction Yield

The percentage yield of essential oil was calculated based on the weight of oil obtained relative to the initial weight of plant material used, expressed as a percentage.

$$\frac{\text{Volume of Essential Oil Extracted} \times 100}{\text{Weight of Plant Materials}} \quad (1)$$

Physicochemical Analysis of the Extracted Essential Oils

The physicochemical properties of the extracted essential oils, including color, refractive index, specific gravity, acid value,

and peroxide value were determined using standard AOAC, 2019 procedures. Refractive index was determined at room temperature using an Abbe refractometer, while specific gravity was measured using the density bottle method. Acid value was determined by titration of the oil, dissolved in neutralized ethanol, with standardized potassium hydroxide solution. Peroxide value was assessed by iodometric titration to evaluate the extent of lipid peroxidation. All analyses were performed in triplicate, and results were expressed as mean \pm standard deviation.

Chemical Analysis of Essential Oil using Gas Chromatography–Mass Spectroscopy (GC –MS) Technique

The chemical composition of the essential oils of lemongrass (*Cymbopogon citratus*) and orange peel (*Citrus sinensis*) was analyzed using Gas Chromatography–Mass Spectrometry (GC–MS). The analysis was performed on a gas chromatograph coupled to a mass spectrometer (HP 5972 MSD, Hewlett-Packard, Palo Alto, CA, USA) equipped with a ZB-5MS Zebron capillary column (30 m \times 0.25 mm i.d, 0.25 μ m film thickness; Agilent Technologies). Helium was used as the carrier gas at a linear velocity of 39 cm s⁻¹. The oven temperature was initially held at 45°C for 2 min, then increased from 45°C to 165°C at a rate of 4°C min⁻¹, and finally raised to 280°C at 15°C min⁻¹. The injector temperature was set at 250°C. Samples (1 μ L) were injected in split mode with a split ratio of 50:1. Retention indices (RI) for all constituents were determined relative to a homologous series of n-alkanes (C₈–C₃₂) analyzed under the same chromatographic conditions. The components of the oils were identified by comparison of their retention indices with those reported in the literature. Further identification was achieved by comparison of their mass spectra with those stored in the NIST mass spectral library and published literature (Adams, 2017).

Ethical Clearance

Ethical approval for the use of animal for this study was obtained from Ahmadu Bello University, Committee on Animal Use and Care (ABUCAUC/2024/032), ABU, Zaria.

Tick Collection and Laboratory Propagation

Engorged adult female *Amblyomma variegatum* were manually collected from naturally infested cattle within the animal pen of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Collected ticks were placed in aerated plastic containers covered with muslin cloth and transported to the Veterinary Entomology Diagnostic Laboratory, Department of Veterinary Parasitology and Entomology, for identification and culture. Morphological identification was performed using a stereomicroscope by comparison with standard taxonomic descriptions and reference specimens. Only fully engorged, undamaged and motile females were selected for oviposition. Selected ticks were incubated at 28 °C and 80 % relative humidity (RH) in labeled Petri dishes until oviposition. Eggs laid were further incubated under the same conditions until hatching into larvae. Hatched larvae were fed on laboratory rabbits using ear-pouch infestation and allowed to engorge before detachment. Detached larvae were incubated to molt into nymphs, which were subsequently fed on goats using flank-pouch infestation until engorgement. Engorged nymphs were incubated to molt into adults, completing the laboratory life cycle required for bioassays.

Preparation of Essential-Oil Treatments

Essential oils of lemongrass (*Cymbopogon citratus*) and citrus peel (*Citrus sinensis*) and varying concentrations (10, 20 and 30% respectively) was prepared using cold-pressed extra-virgin olive oil as diluent as well as negative control while 0.1% amitraz served as positive control.

Adult Immersion Test (AIT)

The acaricidal efficacy of the essential oils against adult *Amblyomma variegatum* was evaluated using the Adult Immersion Test described by Drummond *et al.*, (1973), with minor modifications following Tesfaye and Abate (2025). A total of 480 adult ticks of comparable weight were randomly assigned to four treatment groups (20 ticks per replicate, in triplicate): lemongrass essential oil, citrus peel essential oil, negative control (extra-virgin olive oil), and positive control (0.1% amitraz). Ticks in the essential-oil groups were further exposed to 10%, 20%, and 30% (v/v) concentrations. Each group of ticks were immersed in 5 mL of the respective treatment solution for 1 min with gentle agitation. Following immersion, ticks were removed, blotted dry on absorbent paper and transferred into labeled Petri dishes. All experimental groups were incubated at 28 ± 1 °C and 80 ± 5% relative humidity, mortality was recorded at 24 hours interval for up to 72 hours. Ticks that showed no movement and no response to mechanical stimulation were considered dead. Each treatment was conducted in triplicate and mortality rates were statistically compared among groups, where p < 0.05 was considered to be of significant difference.

Data Analysis

All experimental data were analyzed using IBM SPSS Statistics software (version 25.0; IBM Corp., Armonk, NY, USA). Percentage mortality of adult ticks was calculated for each treatment and concentration. Differences between treatments and concentrations were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc multiple comparison test to separate group means. For time-dependent mortality observations, two-way ANOVA was performed to examine the effects of treatment concentration and exposure time, including their interaction. All results were expressed as mean ± standard deviation (SD) from triplicate experiments. Statistical significance was accepted at p < 0.05.

RESULTS AND DISCUSSION

Essential Oil Yields of Lemon Grass and Orange Peel

The Table 1 presents the yield of essential oils extracted from lemon grass and orange peel under specific conditions. Both samples weighed 300 grams, and 500 ml of distilled water was used as the solvent. The oils were extracted through hydro-distillation using a Clevenger-type apparatus. Lemon grass yielded 2.0 grams of essential oil, which corresponds to a 0.70% yield. In comparison, orange peel produced 14.0 grams of essential oil, resulting in a 4.70% yield. orange peel’s yield was significantly higher - approximately 6.7 times greater than that of lemon grass. The longer extraction time for orange peel, combined with its naturally higher oil content, likely contributed to this difference.

Table 1: Yield of Essential Oil from Lemon Grass and Orange Peel

Samples	Sample Quantity (g)	Solvent (ml)	Extraction Process and Duration	Yield	
				(g)	(%)
Lemon grass	300	Distilled (500)	water Hydro distillation for 120 minutes	2.0	0.70
Orange peel	300	Distilled (500)	water Hydro distillation for 180 minutes	14.0	4.70

Physicochemical Properties of Extracted Essential Oils of Lemon Grass and Orange Peel

The results of the physicochemical properties of essential oils extracted from lemon grass and orange peel are shown in Table 2. The two oils share some similarities in appearance and solubility, with lemon grass oil having pale yellow color and orange peel oil ranging from pale yellow to orange. Both oils are soluble in ethanol, methanol, diethyl ether, and chloroform. Their odour, however, are distinct, with lemon

grass having a tangy, pungent scent and orange peel emitting a strong citrus aroma. Lemon grass oil has a higher boiling point (200°C) compared to orange peel oil (160°C), which is due to the differences in their volatile compounds. In terms of density, Lemon grass oil (0.85 g/mL) is slightly denser than orange peel oil (0.83 g/mL). Lemon grass oil is also marginally more viscous (3.0 mm²/s) than orange peel oil (2.8 mm²/s).

Table 2: Physicochemical Properties of Essential Oils of Lemon Grass and Orange Peel

Physicochemical Properties (Units)		Lemon Grass Oil	Orange Peel Oil
Physical properties	Colour	Pale yellow	Pale yellow to orange
	Odour	Tangy pungent	Strong Citrus
	Boiling point	200 °C	160 °C
	Refractive index	1.43	1.40
	Density (g/mL)	0.85	0.83
	Viscosity (mm ² /s)	3.0	2.8
	Solubility	Soluble in ethanol, methanol diethyl ether Chloroform	Soluble in ethanol, methanol diethyl ether Chloroform
Chemical properties	pH	6.0	6.5
	Acid value (mgKOH/g)	4.4	3.0
	Ester value (mgKOH/g)	16.5	12.5
	Saponification value (mgKOH/g)	11.5	15.0
	Water content (%)	1.0	1.5
Iodine value (gl/100g)	12.0	10.0	
Peroxide value (meq/ kg)	15.0	12.0	

With respect to their chemical properties, orange peel oil has a slightly higher pH (6.5) compared to lemon grass oil (6.0). Lemon grass oil exhibits higher acid (4.4 mg KOH/g) and ester values (16.5 mg KOH/g) than orange peel oil, which has acid and ester values of 3.0 mg KOH/g and 12.5 mg KOH/g, respectively. Conversely, orange peel oil has a higher saponification value (15.0 mg KOH/g) compared to lemon grass oil (11.5 mg KOH/g). Conversely, the saponification value (15.0 mg KOH/g) and water content (1.5%) of orange peel oil are higher when compared to that of lemon grass oil (11.5 mg KOH/g and 1.0% respectively). Lemon grass oil exhibits higher iodine and peroxide values (12.0 gI/100g and 15.0 meq/kg respectively) than orange peel oil (10.0 gI/100g and 12.0 meq/kg).

Chemical Components of the Essential oils of Lemon Grass and Orange Peel using Gas Chromatography – Mass Spectroscopy (GC –MS)

The chemical components of essential oils extracted from lemon grass and orange peel, analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS), as presented

in Table 3, highlights the diverse range of compounds present in both oils and their respective percentage compositions.

In lemon grass, the major component is Citral, which makes up 71.0% of the oil while in orange peel, Limonene is the predominant compound, accounting for 36.0% of the composition, followed by Beta-pinene (19.0%) and gamma-terpinene (10.0%). Both oils contain linalool and geraniol, although in varying proportions. lemon grass oil contains slightly higher amounts of these compounds (4.0 and 6.0%, respectively) compared to orange peel oil (3.5% each). Cineole is present in both oils, making up 1.0% in lemon grass and 2.0% in orange peel. Lemon grass oil features compounds such as Beta-Caryophyllene (3.0%), Camphene (2.2%), and Geranyl Acetate (1.8%) that are absent in orange peel oil. Orange peel oil contains a broader range of unique components, including Terpinen-4-ol (2.5%), D-nerolidol, Eucalyptol (both 1.0%), and smaller quantities of compounds like Methyl linoleate (0.3%) and Alpha-cadinene (0.1%). Lemon grass oil shows trace amounts of Gamma-murolene (2.0%) and Alpha-pinene (0.5%), while orange peel includes compounds like Delta-cadinene (0.2%) and Beta-eudesmol (0.05%) in trace amounts.

Table 3: GC-MS Identified Constituents and Relative Abundance of *Cymbopogon Citratus* and *Citrus Sinensis* Peel Essential Oils

Compound	Relative Composition (%) *	
	Lemongrass Oil	Orange Peel Oil
Limonene	2.5	36.0
Citral (Geranial and Neral)	71.0	-
Gamma – terpinene	-	10.0
Alpha – pinene	0.5	7.0
Citral	-	6.0
Linalool	4.0	3.5
Geraniol	6.0	3.5
Alpha – terpineol	0.3	3.0
Terpinen-4-ol	-	2.5
Cineole	1.0	2.0
Citronellal	0.5	1.5
D – nerolidol	-	1.0
Eucalyptol	-	1.0
Elemol	-	0.5
α – trans – Bergamotol	-	0.5
Methyl palmitate	-	0.4
Methyl linoleate	-	0.3
Beta – pinene	-	19.0
Delta – cardinene	-	0.2
Alpha – cardinene	-	0.1
Beta – eudesmol	-	0.05
D – Carvone	-	0.05
Neral (Z – Citral)	30.0	-
Beta – Caryophyllene	3.0	-
Camphene	2.2	-
Gamma – muurolene	2.0	0.3
Geranyl acetate	1.8	-
Terpinolene	1.0	-
Geranial	0.8	-
Neryl acetate	0.7	-
Beta – terpineol	0.2	-
Gamma – terpineol	0.1	-
Alpha - cadinene	0.05	-

*Values represent relative percentage composition based on GC-MS peak area normalization

Effectiveness of Essential Oil Treatments on Mortality of Adult *Amblyomma Variegatum*

The Table 4 is the result presenting the effectiveness of essential oils from Lemongrass and citrus peel on the

mortality of adult *Amblyomma variegatum* ticks over time (24, 48, and 72 hours).

Lemongrass and citrus peel oils at 10 % concentration, showed effectiveness, with mortality rates increasing gradually over time. After 72 hours, Lemongrass and citrus

peel oils resulted in 51.67 and 40.00 % mortalities respectively. At 20 % concentration, Lemongrass and citrus peel oils exhibited improved effectiveness compared to the 10 % treatments. Lemongrass oil reached 56.67 % mortality, while citrus peel oil achieved 48.33 % mortality after 72 hours. At 30 % concentration, the treatments were more

potent, with Lemongrass oil causing 73.33 % mortality and citrus peel oil reaching 60.00 % mortality after 72 hours. The positive control (0.1 % Acaricide) consistently caused 100 % mortality across all time points. In contrast, the negative control (100 % olive oil) had no effect, with 0.00 % mortality recorded throughout the experiment.

Table 4: Effectiveness of Essential Oil Treatment on Mortality of Adult *Amblyomma Variegatum*

Concentration	Treatment	24h Mortality	48h Mortality	72h Mortality
10%	Lemongrass	23.33 ± 2.89 ^a	36.67 ± 5.77 ^a	51.67 ± 2.89 ^a
	Citrus peel	23.33 ± 2.89 ^a	33.33 ± 7.64 ^a	40.00 ± 0.00 ^b
	Olive oil	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
	0.1% Amitraz	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d
20%	Lemongrass	40.00 ± 5.00 ^a	50.00 ± 0.00 ^a	56.67 ± 2.89 ^a
	Citrus peel	31.67 ± 2.89 ^b	40.00 ± 5.00 ^b	48.33 ± 7.64 ^b
	Olive oil	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
	0.1% Amitraz	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d
30%	Lemongrass	58.33 ± 7.64 ^a	68.33 ± 2.89 ^a	73.33 ± 2.89 ^a
	Citrus peel	51.67 ± 7.64 ^b	56.67 ± 2.89 ^b	60.00 ± 5.00 ^b
	Olive oil	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
	0.1% Amitraz	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d	100.00 ± 0.00 ^d

Values are expressed as Mean ± Standard Deviation; Values with different superscript down the columns are significantly ($p < 0.05$) different. The three superscripts in the positive control are for the comparison at the 10, 20, and 30% essential oil concentrations respectively -ve: negative; +ve: positive. N is 60 adult ticks per each treatment group

Discussion

Essential oils from lemongrass (*Cymbopogon citratus*) and orange peel (*Citrus sinensis*) were successfully extracted using hydro-distillation, a technique widely recognized for its simplicity, affordability, and effectiveness in recovering volatile plant constituents (Perović *et al.*, 2024). The higher yield obtained from orange peel compared with lemongrass is consistent with previous reports demonstrating that essential-oil yield depends strongly on plant matrix, extraction method, and processing duration (Gupta *et al.*, 2023; Rahim *et al.*, 2023). Citrus peels contain large secretory cavities densely packed with oil, which readily rupture during distillation, whereas lemongrass leaves possess smaller oil cells distributed within fibrous tissues, resulting in comparatively lower oil recovery (Luthra *et al.*, 1991). Prolonged exposure of citrus peel to steam during extraction may further enhance oil release from glandular structures (Grover *et al.*, 2024).

The physicochemical properties of essential oils are critical determinants of their quality, stability, and potential applications (de Sousa *et al.*, 2023). The analysis of lemon grass and citrus peel oils reveals key differences in their physical and chemical characteristics, reflecting their distinct compositions. Both lemon grass and citrus peel oils are pale yellow, with citrus peel oil occasionally exhibiting a deeper orange hue, which is consistent with the presence of carotenoid compounds in citrus peels (Sharma *et al.*, 2023). The tangy pungent odor of lemon grass oil and the strong citrus fragrance of citrus peel oil highlight their respective high concentrations of citral and limonene, which are dominant aromatic components (Burt, 2004).

The boiling points of lemon grass oil (200°C) and citrus peel oil (160°C) suggest a difference in their volatile profiles. Lemon grass oil's higher boiling point reflects the presence of heavier molecules, such as citral, which is less volatile than limonene, the predominant compound in citrus peel oil (Azmir *et al.*, 2013). The refractive indices and densities for lemon grass and citrus peel are within the typical ranges for essential oils, indicating purity and consistency with standard extraction practices (Bustamante *et al.*, 2016). Both oils exhibit good solubility in organic solvents, making them versatile for industrial and pharmaceutical applications (Barros *et al.*, 2022).

The chemical analysis indicated a slightly acidic nature for both oils. Research has it that essential oils generally exhibit weak acidity due to the presence of organic acids and esters (Kumar *et al.*, 2020). The moderate acidic value of these oils can lower pH in localized environments and disrupt microbial membranes (Tatsadjieu *et al.*, 2009). The acid values of the two essential oils suggest the presence of free fatty acids that could influence their stability and susceptibility to oxidation (Machado *et al.*, 2023). The ester and saponification values of lemongrass and citrus peel oils are reflection of the esters which are biologically active compounds (geranyl acetate in lemongrass oil and linalyl acetate in citrus peel oil) These compounds are lipophilic in nature penetrating the exoskeleton of acarine and disrupting their physiology (Kim *et al.*, 2023b). Both lemongrass and orange peel oils exhibit good solubility in common organic solvents, showing their lipophilic nature (Barros *et al.*, 2022). This aligns with the general lipophilic and hydrophobic nature of essential oils which are rich in non – polar to moderately polar constituents (Burt, 2004).

The chemical composition of essential oils determines their aroma, therapeutic, acaricidal and repellent properties (Zhang *et al.*, 2024). Gas Chromatography–Mass Spectroscopy (GC-MS) analysis of Lemongrass and citrus peel oils reveals a wide range of bioactive compounds with distinct compositions for each oil. The predominant compound in lemongrass oil is citral. This is consistent with the characterization of *Cymbopogon* species, where citral (geranial and neral isomers) is often the primary constituent, accounting for 65–85 % of the total composition (Rassem *et al.*, 2016). Citral is known for its acaricidal, insecticidal, antimicrobial and anti-inflammatory properties, making them notably promising as a replacement to conventional acaricides (Gutiérrez-Pacheco *et al.*, 2023). Other notable compounds include linalool, geraniol and beta-caryophyllene which contribute to the oil's aromatic complexity and therapeutic effects. These components are also recognized for their antioxidant and antifungal properties (Ramadan *et al.*, 2015; Visakh *et al.*, 2022; Annou *et al.*, 2023). Citrus peel oil exhibits a significantly different profile, with limonene as the dominant compound (36 %). Limonene is the major monoterpene hydrocarbon in citrus oils and it is known for its

strong citrus aroma with acaricidal and insecticidal properties (Pagliaro *et al.*, 2023). Beta-pinene and gamma-terpinene are also other bio - actives that have been reported to contribute to the oil's antimicrobial and anti-inflammatory activities (de Sousa *et al.*, 2023; El Hachlafi *et al.*, 2024).

Lemongrass and citrus peel essential oils exhibited concentration- and time-dependent acaricidal activity against adult *Amblyomma variegatum*. Mortality increased progressively from 10% to 30% concentrations and with exposure time from 24 to 72 h, indicating cumulative toxic effects of the essential oil constituents. Similar dose-dependent acaricidal activity of plant essential oils against ixodid ticks has been reported by Chagas *et al.*, 2022 who observed increased tick mortality with higher concentrations of botanical extracts.

Across all concentrations (10–30%), lemongrass essential oil consistently produced higher mortality than citrus peel oil. At 10%, moderate mortality was observed for both oils, with lemongrass showing greater residual activity at 72 h. Increasing the concentration to 20% resulted in enhanced acaricidal activity for both treatments, with lemongrass maintaining superior efficacy. At 30%, the highest mortality values were recorded, with lemongrass oil achieving 73.33% mortality at 72 h compared with 60.00% for citrus peel oil. These results indicate that the acaricidal effect of both oils is concentration dependent, with lemongrass exhibiting stronger toxicity against adult *A. variegatum*.

The higher efficacy of lemongrass essential oil may be attributed to the presence of oxygenated monoterpenes such as citral, neral, and geraniol, which are known to disrupt membrane integrity, interfere with respiration, and induce paralysis in arthropods. Similar acaricidal activity of *Cymbopogon citratus* essential oil against ticks has been reported by Oliveira *et al.*, 2020, who documented significant mortality following exposure to lemongrass oil. The comparatively lower activity of citrus peel oil may be due to the predominance of limonene, a highly volatile monoterpene that may reduce residual toxicity. Nevertheless, citrus essential oils have been reported to exhibit notable acaricidal activity against ticks through contact toxicity and fumigant action, as reported by Salman *et al.*, 2020

The progressive increase in mortality with exposure time observed at all concentrations suggests gradual penetration of essential oil constituents through the tick cuticle, resulting in cumulative toxicity. Similar observations have been reported by Benelli and Pavela (2018), who noted that plant essential oils require sufficient exposure time to exert maximum acaricidal effects. The absence of mortality in the olive oil control confirms that tick mortality resulted from the essential oil treatments, while the complete mortality recorded with amitraz indicates the higher efficacy of conventional synthetic acaricides.

CONCLUSION

The results indicate that both lemongrass and citrus peel essential oils possess appreciable acaricidal properties against adult *Amblyomma variegatum*, with efficacy increasing with concentration and exposure time. Lemongrass essential oil consistently exhibited greater activity than citrus peel oil, suggesting stronger acaricidal potential. Although neither treatment achieved complete mortality comparable to amitraz, the substantial mortality recorded at higher concentrations highlights their potential as environmentally friendly alternatives for tick control and their possible inclusion in integrated tick management strategies.

REFERENCES

- Adams, R. P. (2017). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. *Gruver, TX USA: Texensis Publishing.*
- Agwunobi, D. O., Pei, T., Wang, K., Yu, Z., and Liu, J. (2020). Effects of the essential oil from *Cymbopogon citratus* on mortality and morphology of the tick *Haemaphysalis longicornis* (Acari: Ixodidae). *Experimental and Applied Acarology*, 81, 37-50.
- Annou, G., Mosbah, S., Raache, I., Belhedre, Z., Derbal, B. D., and Khelil, A. (2023). Physicochemical Indexes and Evaluation of Antioxidant, Antihemolytic and Antibacterial Activities of *Citrus sinensis* and *Citrus limon* Peel Essential Oils. *Al-Qadisiyah Journal For Agriculture Sciences*, 13:(1), 7-17.
- Ashaq, B., Rasool, K., Habib, S., Bashir, I., Nisar, N., Mustafa, S., Ayaz, Q., Nayik, G. A., Uddin, J., and Ramniwas, S. (2024). Insights into chemistry, extraction and industrial application of lemon grass essential oil-A review of recent advances. *Food Chemistry: X*, 101521.
- Association of Official Analytical Chemists. (2019). *Official methods of analysis* (21st ed.). AOAC International.
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., and Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117:(4), 426-436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils—A review. *Food and Chemical Toxicology*, 46(2), 446–475. <https://doi.org/10.1016/j.fct.2007.09.106>
- Barros, M., Redondo, L., Rego, D., Serra, C., and Miloudi, K. (2022). Extraction of Essential Oils from Plants by Hydrodistillation with Pulsed Electric Fields (PEF) Pre-Treatment. *Applied Sciences*, 12:(16), 8107. <https://doi.org/10.3390/app12168107>
- Benelli, G., and Pavela, R. (2018). Repellence of essential oils and selected compounds against ticks—A systematic review. *Acta Tropica*, 179, 47–54.
- Bustamante, J., van Stempvoort, S., García-Gallarreta, M., Houghton, J. A., Briers, H. K., Budarin, V. L., Matharu, A. S., and Clark, J. H. (2016). Microwave assisted hydro-distillation of essential oils from wet citrus peel waste. *Journal of Cleaner Production*, 137, 598-605. <https://doi.org/10.1016/j.jclepro.2016.07.108>
- Burt, S.(2004).Essential oils: Their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology*, 94: (3), 223- 253
- Chagas, A. C. F., Domingues, L. F., Passos, W. M., Prates, H. T., Leite, R. C., and Furlong, J. (2022). Acaricidal activity of plant essential oils and their constituents against ticks: A review of current advances and limitations. *Veterinary Parasitology*, 305, 109690.

- Chaudhari, A. K., Singh, V. K., Kedia, A., Das, S., and Dubey, N. K. (2021). Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. *Environmental Science and Pollution Research*, 28, 18918-18940.
- Dangol, S., Poudel, D. K., Ojha, P. K., Maharjan, S., Poudel, A., Gurung, R., and Bhattarai, S. (2023). Essential oil composition analysis of *Cymbopogon* species from Eastern Nepal by GC-MS and chiral GC-MS and antimicrobial activity of some major compounds. *Molecules*, 28:(2), 43. <https://doi.org/10.3390/molecules28020543>.
- de Sena Filho, J. G., de Almeida, A. S., Pinto-Zevallos, D., Barreto, I. C., de Holanda Cavalcanti, S. C., Nunes, R., Teodoro, A. V., Xavier, H. S., Barbosa Filho, J. M., and Guan, L. (2023). From plant scent defense to biopesticide discovery: Evaluation of toxicity and acetylcholinesterase docking properties for Lamiaceae monoterpenes. *Crop Protection*, 164, 106126.
- de Sousa, D. P., Damasceno, R. O. S., Amorati, R., Elshabrawy, H. A., de Castro, R. D., Bezerra, D. P., Nunes, V. R. V., Gomes, R. C., and Lima, T. C. (2023). Essential oils: Chemistry and pharmacological activities. *Biomolecules*, 13:(7), 1144.
- Devrnja, N., Milutinović, M., and Savić, J. (2022). When scent becomes a weapon—plant essential oils as potent bioinsecticides. *Sustainability*, 14:(11), 6847.
- Drummond, R. O., Ernst, S. E., Trevino, J. L., Gladney, W. J., and Graham, O. H. (1973). *Boophilus annulatus* and *Boophilus microplus*: Laboratory tests of insecticides. *Journal of Economic Entomology*, 66:(1), 130–133. <https://doi.org/10.1093/jee/66.1.130>
- El Hachlafi, N., Kandsi, F., Elbouzidi, A., Lafdil, F. Z., Nouioura, G., Abdallah, E. M., Abdnim, R., Bnouham, M., Al-Mijalli, S. H., and Naceiri Mrabti, H. (2024). Extraction of bioactive compound-rich essential oil from *Cistus ladanifer* L. By microwave-assisted hydrodistillation: GC-MS characterization, in vitro pharmacological activities, and molecular docking. *Separations*, 11:(7), 199.
- Franz, C., and Novak, J. (2020). Sources of essential oils. In *Handbook of essential oils* (pp. 41-83). CRC press.
- Grover, S., Aggarwal, P., Kumar, A., Kaur, S., Yadav, R., and Babbar, N. (2024). Utilizing citrus peel waste: a review of essential oil extraction, characterization, and food-industry potential. *Biomass Conversion and Biorefinery*, 1-22.
- Gupta, A., Ranjan, K. R., Yadav, N., Deeksha, and Mishra, V. (2023). Essential oils: Chemical composition and methods of extraction. *Essential Oils: Extraction Methods and Applications*, 871-889.
- Gutiérrez-Pacheco, M. M., Torres-Moreno, H., Flores-Lopez, M. L., Velázquez Guadarrama, N., Ayala-Zavala, J. F., Ortega-Ramírez, L. A., and López-Romero, J. C. (2023). Mechanisms and Applications of Citral's Antimicrobial Properties in Food Preservation and Pharmaceuticals Formulations. *Antibiotics*, 12:(11), 1608.
- Irfan, S., Zahra, S. M., Murtaza, M. A., Zainab, S., Shafique, B., Kanwal, R., Roobab, U., and Ranjha, M. M. A. N. (2022). Characterization of lemongrass oleoresins. In *Handbook of oleoresins* (pp. 261-283). CRC Press.
- Isman, M. B. (2020). Botanical insecticides in the twenty-first century: Fulfilling their promise? *Annual Review of Entomology*, 65, 233–249. <https://doi.org/10.1146/annurev-ento-011019-025010>
- Kaur, G., Kaur, R., and Kaur, S. (2023). Essential oil used as larvicides and ovicides. *Essential Oils: Extraction Methods and Applications*, 427-442.
- Khater. (2012). Prospects of botanical biopesticides in insect pest management. *Journal of Applied Pharmaceutical Science*. <https://doi.org/10.7324/japs.2012.2546>
- Kim, S., Cha, J., Choi, Y.-S., Yang, H.-W., Jang, H. W., and Kang, M.C. (2023). Anti-melanogenic effects of the essential oil from *Citrus reticulata* blossom on B16-F10 melanoma cells and in vivo zebrafish embryos. *Process Biochemistry*, 134, 1-8.
- Kumar, A., Singh, P. P., Gupta, V., and Prakash, B. (2020). Assessing the antifungal and aflatoxin B1 inhibitory efficacy of nanoencapsulated antifungal formulation based on combination of *Ocimum* spp. essential oils. *International Journal of Food Microbiology*, 330, 108766. <https://doi.org/10.1016/j.ijfoodmicro.2020.108766>
- Lemay, J., and Scott-Dupree, C. (2022). Management of insect pests on cannabis in controlled environment production. In *Handbook of Cannabis Production in Controlled Environments* (pp. 253-290). CRC Press.
- Liu, Z., Li, Q. X., and Song, B. (2022). Pesticidal activity and mode of action of monoterpenes. *Journal of Agricultural and Food Chemistry*, 70:(15), 4556-4571.
- López, M. D., and Pascual-Villalobos, M. J. (2010). Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Industrial Crops and Products*, 31:(2), 284-288. <https://doi.org/10.1016/j.indcrop.2009.11.005>
- Luthra, R., Srivastara, A.K., and Ganjewala, D.(2007). Sources of essential oils and their secretory structures in *Cymbopogon*. *Asian Journal of Plant Sciences* 6:(1), 194- 200
- Machado, M., Rodriguez-Alcalá, L. M., Gomes, A. M., and Pintado, M. (2023). Vegetable oils oxidation: mechanisms, consequences and protective strategies. *Food Reviews International*, 39 :(7), 4180-4197.
- Martins Da Silva, P. H., Prado, E. P., Ferreira-Filho, P. J., Francisco, J. P., Quiqui, E. M. D., Silva, C., and Guerreiro, J. C. (2023). Insecticidal and antifeedant bioactivities of *Melaleuca alternifolia* essential oil on *Ascia monuste orseis*. *Revista Colombiana de Entomología*, 49:(2).
- Naser, H. J., and Abdul-Ameer, F. M. H. (2023). Plants Extract Oils and Their Antimicrobial Activity in Treatment of Denture Stomatitis: Lemongrass Essential Oil (A review of literature). (*Humanities, social and applied sciences*) *Misan Journal of Academic Studies*, 22:(45), 360-371 E.
- Oliveira, J. N., Remédio, R. N., Santos, A. C. G., Campos, D. R., Fonseca, A. H., and Monteiro, C. M. O. (2020). Ultrastructural and morphological changes induced by

- Cymbopogon citratus* essential oil in the tick *Haemaphysalis longicornis* (Acari: Ixodidae). *Experimental and Applied Acarology*, 81, 227–241.
- Oniha, M. I., Ahuekwe, E. F., and Akinpelu, S. O. (2023). Phytochemical Contents of Essential Oils from *Cymbopogon* Species: A Tropical Medicinal Plant. In *Tropical Plant Species and Technological Interventions for Improvement*. IntechOpen.
- Pagliaro, M., Fabiano-Tixier, A.-S., and Ciriminna, R. (2023). Limonene as a natural product extraction solvent. *Green Chemistry*, 25:(16), 6108–6119.
- Perović, A. B., Karabegović, I. T., Krstić, M. S., Veličković, A. V., Avramović, J. M., Danilović, B. R., and Veljković, V. B. (2024). Novel hydrodistillation and steam distillation methods of essential oil recovery from lavender: A comprehensive review. *Industrial Crops and Products*, 211, 118244.
- Rahim, M. A., Ayub, H., Sehrish, A., Ambreen, S., Khan, F. A., Itrat, N., Nazir, A., Shoukat, A., Shoukat, A., and Ejaz, A. (2023). Essential components from plant source oils: A review on extraction, detection, identification, and quantification. *Molecules*, 28:(19), 6881.
- Ramadan, M., Ali, M., Ghanem, K., and El-Ghorabe, A. (2015). Essential oils from Egyptian aromatic plants as antioxidant and novel anticancer agents in human cancer cell lines. *Grasas y Aceites*, 66:(2), e080-e080.
- Rassem, H. H. A., Nour, A. H., and Yunus, R. M. (2016). Techniques for extraction of essential oils from plants: A review. *Australian Journal of Basic and Applied Sciences*, 10:(16), 117–127.
- Salman, M., Abbas, R. Z., Israr, M., Abbas, A., Mehmood, K., and Khan, M. K. (2020). Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle. *Veterinary Parasitology*, 283, 109178.
- Sharma, A., Gumber, K., Gohain, A., Bhatia, T., Sohal, H. S., Mutreja, V., and Bhardwaj, G. (2023). Importance of essential oils and current trends in use of essential oils (aroma therapy, agrofood, and medicinal usage). In *Essential Oils* (pp. 53-83). Elsevier.
- Sharma, K., Guleria, S., Razdan, V. K., and Babu, V. (2020). Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. *Industrial Crops and Products*, 154, 112569.
- Sharma, S., Habib, S., Sahu, D., and Gupta, J. (2021). Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass. *Medicinal Chemistry*, 17:(1), 2-12.
- Sharmeen, J. B., Mahomoodally, F. M., Zengin, G., and Maggi, F. (2021). Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. *Molecules*, 26:(3), 666.
- Souto, A. L., Sylvestre, M., Tölke, E. D., Tavares, J. F., Barbosa-Filho, J. M., and Cebrián-Torrejón, G. (2021). Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. *Molecules*, 26:(6), 4835.
- Tatsadjieu, N. L., Jazet Dongmo, P. M., Ngassoum, M. B., Etoa, F.-X., and Mbofung, C. M. F. (2009). Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus* Link ex Fries. *Food Control*, 20:(2), 161–166. <https://doi.org/10.1016/j.foodcont.2008.03.008>
- Tesfaye, T. and Abate, A. (2025). *In vitro* and *in vivo* screening of commonly used acaricides against Ixodid ticks in South Omo pastoral areas, South-Western Ethiopia. *International Journal Agricultural Research Innovation Technology*, 15(1): 1-10. <https://doi.org/10.3329/ijarit.v15i1.82749>
- Vikrant P. Katekar, Anand B. Rao, and Vishal R. Sardeshpande (2023). A hydro-distillation-based essential oils extraction: A quest for the most effective and cleaner technology, *Sustainable Chemistry and Pharmacy*, Volume 36, 101270, ISSN 2352-5541, <https://doi.org/10.1016/j.scp.2023.101270>.
- Visakh, N. U., Pathrose, B., Chellappan, M., Ranjith, M., Sindhu, P., and Mathew, D. (2022). Chemical characterisation, insecticidal and antioxidant activities of essential oils from four *Citrus* spp. fruit peel waste. *Food Bioscience*, 50, 102163.
- Zhang, J., Zhang, M., Bhandari, B., and Wang, M. (2024). Basic sensory properties of essential oils from aromatic plants and their applications: A critical review. *Critical Reviews in Food Science and Nutrition*, 64(20), 6990-7003.

