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Essential Oil of Eucalyptus: a natural solution for treating pediculosis

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ABSTRACT

Objective: Pediculosis is a condition caused by the infestation of *Pediculus Humanus Capitis*. The pesticides used in current formulations exhibit toxicity and carcinogenic effects on consumers. This study aimed to investigate the pediculicidal activity of the essential oil from *Eucalyptus globulus* leaves, with the intention of adding it as an active ingredient in pediculicidal formulations to replace harmful chemicals.

Design/methodology/approach: *In vivo* tests were conducted to assess the repellency and mortality of the essential oil obtained through hydrodistillation. The major components were determined through gas chromatography-mass spectrometry analysis. Additionally, proximate, and chemical composition analyses were performed on the eucalyptus leaves using ASTM E871, ASTM E872, ASTM D1104, TAPPI T264, TAPPI T207, ASTM D1106, and ASTM D1104 methods.

Results: A repellency of 66.66% and 100% mortality within 2.26 minutes were obtained in the *in vivo* tests. The yield of hydrodistilled essential oil was 4 mL/kg, primarily composed of 71.04% 1,8-cineole, 18.94% 4-Ethyl-m-xylene, 2.72% γ-Terpinene, and 1.23% L-α-Pinene. Furthermore, the composition of eucalyptus leaves was determined as 61.25% moisture, 30.32% volatile matter, 6% ash, 2.40% fixed carbon, 11.22% acetone extractives, 33.03% water extractives, 31.49% lignin, 69.33% holocellulose, 62.09% cellulose, and 7.24% hemicellulose.

Limitations on study/implications: The pediculicidal activity study was conducted solely on the essential oil, and further testing on the formulation of the finished product is necessary.

Findings/conclusions: The pediculicidal activity study was conducted solely on the essential oil, and further testing on the formulation of the finished product is necessary.

Keywords: Essential oil, *Eucalyptus globulus*, pediculosis, physicochemical characterization.

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INTRODUCTION

Infestation of *Pediculus humanus capitis*, or head lice, is a chronic condition worldwide. In Mexicali, Baja California, Mexico, lice infestation is abundant due to the climatic conditions. Lice are six-legged arthropods that invade the host's hair in search of blood for feeding. The life cycle of head lice consists of three stages: eggs, colloquially known as nits, measuring 1 mm, are white and attached to the hair by a non-polar substance called cement; the second stage is nymphs, classified as such at birth, measuring less than 1 mm and being whitish; finally, the adult stage, where lice mature 2 to 3 weeks after birth,

measuring between 3 and 4 mm, are brown in color, and live for 3 to 4 weeks, constantly feeding (Herranz & Abad, 2008). Primary sources of contagion are primary education schools. The trend indicates an increase in lice infestations in summer due to children's exposure to high temperatures, creating conditions for lice to reproduce rapidly. Various studies have shown that lice have developed resistance and immunity to conventional treatments for their extermination, such as pyrethrins, pyrethroids, malathion, carbaryl, and benzyl benzoate, in addition to confirming their toxicity in humans and potential carcinogenic effects (Burkhart & Burkhart, 2000; McCage, Ward, & Paling, 2002; Herranz *et al.*, 2008; Veracx & Raoult, 2012). Currently, organic alternatives are being studied to treat pediculosis without affecting patients' health. Experimentation with the effectiveness of essential oils as pediculicides and ovicides has been chosen, yielding favorable results in the total or partial elimination of head lice. Essential oils have the ability to deplete Adenosine Triphosphate (ATP) levels in lice, leading to asphyxiation and/or dehydration (McCage *et al.*, 2002; Pearlman, 2004).

Eucalyptus globulus essential oil is one of the most commonly used as an insecticide, supported by studies generated through gas chromatography, allowing for the qualification and quantification of the chemical composition of essential oils. *In vivo* tests on lice endorse the attributions of the pesticide effects to 1,8-Cineole, which causes inhibition of acetylcholinesterase, contact toxicity, reproductive function impairment, and fumigant action against *Pediculus humanus capitis* and other species. α-Pinene and β-Pinene also possess fumigant activity (Choi *et al.*, 2006).

MATERIALS AND METHODS

Physicochemical Characterization

Table 1 presents the procedures used for proximate and chemical composition analysis. Each method was performed in triplicate.

Pilot-scale Hydrodistillation

The hydrodistillation extraction at a pilot scale began by loading 1 kg of previously cut eucalyptus leaves. It was brought to a boil in the presence of 7.19 L of water, creating a steam-vapor mixture (water and essential oil) (Peredo, Palou & López, 2009). The water vapor and essential oil mixture passed through a condenser, where it was cooled to low temperatures using cold water to reach room temperature. The mixture transformed into an unstable liquid emulsion composed of essential oil and hydrosol. Finally, the emulsion underwent decantation, and it was easily separated due to immiscibility caused by the difference in density. The result was the essential oil as the main product and aromatic water as a byproduct. The equipment used is shown in Figure 1.

Repellency and Mortality Tests

Figure 2 displays the methods used in the effectiveness tests of the essential oil from eucalyptus leaves. Repellency and mortality were studied in nymphs, young lice, and adult lice. Both tests were conducted in triplicate.

Empty Petri dishes were used as controls, and Petri dishes with purified water were used as placebos, following the methodology outlined for each test.

Table 1. Physicochemical characterization.

No.	Analysis	Standard method	Procedure
1	Moisture	ASTM E871, (1998)	Drying in oven at 65 °C for 48 h
2	Volatile Matter	ASTM E872, (2013)	Drying in oven at 950 °C for 7 min
3	Ashes	ASTM D1102, (2001)	Drying in oven at 580 °C for 4 h
4	Fixed Carbon	-	Percentage difference
5	Solvent Extractables	TAPPI T264, (2007)	Acetone extraction using a Soxhlet apparatus for 8 h, followed by drying in an oven at 105 °C for 4 h
6	Water Extractables	TAPPI T207, (1999)	Fractional distillation with water at 100 °C for 4 h, followed by drying in an oven at 105 °C for 4 h
7	Lignin	ASTM D1106, (2001)	Agitation at 200 rpm for 2 h with 72% sulfuric acid, followed by fractional distillation with 3% sulfuric acid for 4 h. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
8	Holocellulose	ASTM D1104, (1985)	Addition of acetic acid and sodium chlorite every 5 h with agitation, maintaining a temperature of 75 °C. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
9	Cellulose	Rowell, (2012)	Addition of 17.5% sodium hydroxide every 5 minutes for 20 min. Addition of water and allowing to rest for 1 h. Dilution with 8.3% sodium hydroxide and 10% acetic acid. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
10	Hemicellulose	-	Percentage difference

Figure 1. Pilot-scale hydrodistillation apparatus by Armenta *et al.*, 2023.

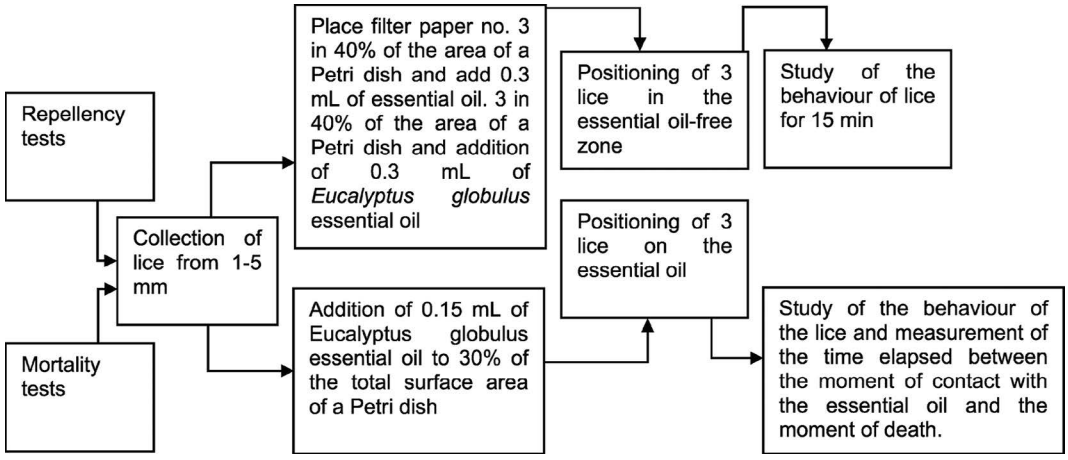

Figure 2. Methodology for determining pediculicidal activity.

Gas Chromatography-Mass Spectrometry

To determine the major components of the essential oil from eucalyptus leaves, gas chromatography-mass spectrometry (GC-MS) was used. The essential oil was characterized using an Agilent Technologies 7890A Network GC-MS system. Mass spectra were obtained by electron impact ionization at 70 eV energy. The initial temperature was set at 50 °C for 2 minutes and then increased at a rate of 5 °C/min until reaching 250 °C. Helium was used as the carrier gas, with an inlet pressure of 12.667 psi. Samples were prepared by dissolving 20 microliters of the essential oil in 980 microliters of dichloromethane. From the resulting solution, 1 microliter was taken for injection (Jaramillo *et al.*, 2022).

RESULTS AND DISCUSSION

Physicochemical Characterization

Table 2 presents the results of the proximate analysis and chemical composition analysis performed on *Eucalyptus globulus* leaves in Mexicali, Baja California.

The results were compared with another species of *Eucalyptus globulus* located in Morelia, Michoacán. Additionally, comparisons were made with *Eucalyptus pellita* and *Eucalyptus citriodora* species located in Cuba, as well as *Gossypium hirsutum* L and *Triticum aestivum* L, two endemic species of Mexicali. The moisture content is considered high, the ash content

Figure 3 shows the design of each test.

Table 2. Physicochemical Characterization of *Eucalyptus globulus* leaves.

Analysis	Content (%)	Standard deviation
Moisture	61.2598	0.3039
Volatile Matte	30.3263	0.2242
Ashes	6.0040	0.2271
Fixed Carbon	2.4097	0.0881
Acetone Extractables	11.2288	0.6186
Water Extractables	33.0398	0.6659
Total Extractable	44.2687	0.9716
Lignin	30.6632	0.3992
Holocellulose	69.3367	0.7414
Cellulose	62.0946	0.6336
Hemicellulose	7.2420	0.1340

is within the average range, while the volatile and fixed carbon contents are low. The results of acetone extractables are low, whereas the water extractables are considerably higher. The lignin content is moderately high, the holocellulose and cellulose contents are high, and the hemicellulose content is low.

Essential oil extraction

Table 3 presents the results of the essential oil extraction in a pilot-scale system.

GC-MS

The essential oil of *Eucalyptus globulus* leaves is mainly composed of 71.04% of 1,8-cineol, 18.94% of 4-Ethyl-m-xylene, 2.72% of γ-Terpinene, and 1.23% of L-α-Pinene (Armenta *et al*., 2023).

Pediculicidal Activity

The results of the repellency tests showed an effectiveness of 66.66%, while the mortality tests exhibited a 100% effectiveness within an average time of 2.66 minutes. The essential oil of eucalyptus demonstrated outstanding mortality rates, eliminating lice in a shorter time compared to essential oils of *Citrus sinensis* and *Cymbopogon nardus*. In terms of repellency, all three essential oils showed similar results. During the tests conducted with the control group, the lice moved along the entire surface of the Petri dish for the duration of 15 minutes. In the placebo tests, for repellency, the lice explored the entire Petri dish, including the area with water, but found it difficult to move in that section. Once out of

Table 3. Results of eucalyptus essential oil extraction.

Plant material	m_{plant} (kg)	V_{water} (L)	V_{AE} (mL)	Yield ($V_{AE}/m_{vegetal}$)
Eucalyptus globulus	1	7.50	4.40	4.40
	1	6.58	4.60	4.60
	1	7.50	4.00	4.00
Mean	1	7.19	4.33	4.33

the water, they resumed normal activity. In the mortality tests, when the lice were placed directly on the water, they remained there for an average of 12 minutes. 83% of the lice managed to leave the water without apparent harm, while 17% remained in the water, showing signs of life throughout the entire test.

CONCLUSIONS

The physicochemical analyses conducted on *Eucalyptus globulus* indicate that it is a viable option for obtaining essential oils using physical conversion technologies such as steam distillation or hydrodistillation. The repellency and mortality tests confirmed the pediculicidal activity of the essential oil from *Eucalyptus globulus* leaves obtained through hydrodistillation. The essential oil of *Eucalyptus globulus* demonstrated favorable results in terms of its repellent and inhibitory properties within short time frames. These characteristics are mainly attributed to the high content of 1,8-cineol, followed by L-α-Pinene. It is recommended to develop a pediculicidal formulation based on this essential oil and conduct further tests to determine its lethal concentration once the formulation is completed.

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