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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM SOME AROMATIC PLANTS AGAINST GASTROINTESTINAL PATHOGENIC BACTERIA

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ABSTRACT

Traditional medicinal practices in Mexico often involve the use of aromatic and medicinal plants to treat gastrointestinal diseases, as they have shown significant antibacterial activity against various clinically relevant bacterial strains. This study aimed to determine the antibacterial activity of essential oils extracted from the leaves of *Chenopodium ambrosiodes, Psidium guajaba, Citrus lemon, Lippia graveolens, Thymus vulgaris, Mentha spicata*, and *Ocimun basilicum*. **Materials and Methods:** The essential oils' chemical composition was analyzed using gas chromatography-mass spectrometry, and their antibacterial activity was evaluated using the Kirby-Bauer method. Additionally, antioxidant activity was determined. **Results:** The phytochemical screening revealed the presence of secondary metabolites such as tannins, flavonoids, and saponins. Notably, *Lippia graveolens* exhibited remarkable efficacy against most bacterial strains, except for *B. subtillis*. In **conclusion**, *the essential oils of Lippia graveolens, Citrus lemon, Thymus vulgaris, and Mentha spicata demonstrated the highest inhibition activity against Bacillus subtilis, Escherichia coli, Salmonella enteritidis, and Shigella dysenteriae.*

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INTRODUCTION

Traditional medicinal practice in Mexico incorporates the use of aromatic and medicinal plants to treat gastrointestinal diseases due to their demonstrated high antibacterial activity against various clinically relevant bacterial strains (Juarez Rosete et al., 2013). Mexico boasts one of the greatest floral biodiversity in the world, making it a valuable source of natural resources that can be utilized for human benefit, particularly in terms of healthcare (Biodiversidad Mexicana). However, the use of these plant species is primarily based on empirical knowledge, lacking scientific support and understanding of their potential beneficial effects. Additionally, these plants may contain metabolites that can be harmful to humans. Therefore, it is crucial to conduct phytochemical characterization and evaluate their biological activity against relevant pathogens. This is essential for gaining insights into the properties of the product, such as essential oils, to ensure their appropriate and safe use when interacting with biological systems.

Plants possess a diverse range of substances that are produced as part of their metabolic processes, including certain compounds developed as defense mechanisms against pathogenic microorganisms (Lujan, et al, 2010). Among these metabolites are essential oils, which are oily, aromatic, and volatile liquids. These oils are typically synthesized in specialized cells and are often concentrated in specific plant parts such as leaves, bark, or fruit. They consist of complex mixtures of hydrocarbons, terpenes, alcohols, carbonyl compounds, aromatic aldehydes, or phenols (Gutierrez, et al., 2008). Plant secondary metabolites have pivotal roles in plant-pathogen interactions,(Kumar et al. 2023), in addition, essential oils find application in medicine, fragrance production, flavor enhancement, and insecticides (Duarte et al., 2012). Many essential oils have demonstrated biocidal effects against bacteria, fungi, viruses, protozoa, insects, and plants (Di Vaio, et al., 2010). Gastrointestinal diseases are recognized as a global public health concern and represent a significant issue in Mexico.

They affect individuals of all ages, genders, and social statuses, primarily transmitted through contaminated water, food, and the fecal-oral route (Cecilia et al 2011). The aim of this study was to determine antibacterial activity of the following essential oils: *Chenopodium ambrosiodes, Psidium guajaba, Citrus lemon, Lippia graveolens, Thymus vulgaris, Mentha spicata* and *Ocimun basilicum* leaves

MATERIALS AND METHODS

Plant Material: *Chenopodium ambrosioides, Psidium guajaba, Citrus lemon, Lippia graveolens, Thymus vulgaris, Ocinum basilicum and Mentha spicata* leaves, collected from the municipality of Amacuzac, Morelos Mexico.

Essential oil extraction: The extraction process was conducted using steam distillation. A total of 500g of fresh plant material was crushed and placed in a stainless steel container. To facilitate extraction, 8 liters of water were added. Each sample underwent a 4-hour extraction period.

Organic extraction: After obtaining the water and oil emulsion, it was separated using a 1:1 ratio of hexane. The separation was carried out using a separation funnel, and the top layer, which contains the oil, was collected. This process was repeated three times. To further purify the collected oil, 3 grams of anhydrous sodium sulfate were added. The oil was then subjected to further extraction using rotary steam until it became solvent-free. Finally, the oil was stored at a temperature of 4°C.

Phytochemical characterization of essential oil: The chemical composition analysis of the essential oils was conducted using gas chromatography-mass spectrometry (Agilent Technologies 6850) with a capillary column measuring 30 meters (Sefadex B45). Helium was utilized as the carrier gas. For the analysis, 1 µl of the essential oil was diluted with 100 µL of dichloromethane before measurement. The identification of the chemical constituents was effected by means of a comparative analysis of the mass spectrum of the substances with those of the database of the system GC-MS (Nist 62.lib) , the literature, and retention index.

Antimicrobial susceptibility testing: The antibacterial activity of the essential oils was tested by the Kirby-Bauer disk diffusion susceptibility test at a concentration of 15 µL, the essential oils were tested against *Bacillus subtilis* and Gram negative bacteria: *Escherichia coli, Salmonella enteritiditis and Shiguella dysenteriae,* were used as a positive control sulfamethoxazole.

Antioxidant activity: For each aromatic plant, three tubes were prepared. In each tube, 5 µl of essential oil was added along with 1980 µl of DPPH. The mixture was allowed to sit for 2 hours in a dark place to ensure reaction completion. Subsequently, the samples were read using a spectrophotometer at a wavelength of 517nm.

RESULTS

The phytochemical screening (Table 1) indicates the presence of secondary metabolites such as Tannins, Flavonoids, Saponins.

It was observed that the volume of oil that produces a greater inhibition halo is 15 µL which indicates that the percentage of inhibition depends on the amount of oil. *Lippia graveolens* exhibited significant antibacterial activity against nearly all bacterial strains, except for *B. subtilis*. In contrast, the essential oil of *Mentha* demonstrated substantial antibacterial activity, as indicated in Table 3. The antioxidant activity of the essential oils, as presented in Table 4, indicated that all oils, except for *Psidium guajaba*, displayed significant antioxidant properties. However, the specific amount of essential oil required to inhibit 50% of the radicals is still under investigation.

DISCUSSION

Preliminary tests were conducted to identify the various types of compounds present in aromatic plants. These compounds can be analyzed using gas chromatography-mass spectrometry (GC-MS). Additionally, a thorough literature review was conducted to determine the inhibitory activity of these compounds. The results obtained show us that the essential oils of the leaves of *Psidium guajaba, Citrus lemon, Lippia graveolens, Thymus vulgaris, Mentha spicata and Ocimun basilicum* had inhibitory activity against *Bacillus subtilis, Escherichia coli, Salmonella enteritiditis and Shigella dysenteriae*, however *chenopodium ambrosides*extracts had no inhibitory activity, we consider that *chenopodium ambrosides* could have it for its activity against helminths. Johnson and Croteu (1987) suggest that this occurrence could be attributed to the extraction of essential oil from unripe plant material, resulting in a higher concentration of α-terpinene and a lower amount of ascaridol. On the other hand, *Lippia graveolens* has demonstrated exceptional efficacy against almost all bacterial strains. According to statistical analysis, the essential oil of *Lippia graveolens* exhibited the highest inhibitory effect against *S. enteritiditis*. Furthermore, it displayed an inhibition halo diameter of 16mm for *E.coli*, consistent with the findings reported by Albado Plaus (Albado et al., 2001). Citrus lemon and Mentha spicata essential oils also exhibited notable inhibitory effects against *B. subtillis*, with mean inhibition halo diameters of 2.10.

This finding is in line with the research conducted by Herrera Arias (2006), where *Thymus vulgaris* essential oil demonstrated inhibitory activity against *Salmonella spp*. and *E.coli*, resulting in an inhibition halo diameter of 15mm, which aligns with the results obtained in this study. This activity is due to phenolic compounds and other active ingredients such as aromatic amino acids and sulfur-containing compounds (Alu'datt et al., 2011). In addition, Porte and Godoy, (2008) as cited by Alu'datt, M. H., (2018),point out that thyme oil contains a bioactive component such as thymol and carvacrol at 20%-54%, (Matiz et al., 2015),also identified the presence of 1,8-cineole as a major component as well as B-pinene with, o-cymene (Coy A and Acosta G. 2013.), gamma-terpinene, limonene, borneol and linalool(Luengo, 2006) However, *Ocinum basilicum* and *Psidium guajaba* essential oils exhibited relatively lower activity, although there are studies that demonstrate the activity of Ocinum basilicum as Calderon Mestanza, J. (2013), It has been described that the activity may vary due to the percentage of metabolites present (Isoestragol, humulene, eucalyptol, B-linalool), this may be due to the procurement

Table 1. Phytochemical screening of the secondary metabolites (-) Negative test, + low concentration, ++ medium concentration, and +++ high concentration

Especie	Tannins	Flavonoids	Saponins
Psidium guajaba	+++	+	++
Thymus vulgaris	+++	+++	++
Citrus lemon	++	(-)	++
Chenopodium ambrosiodes	+	(-)	++
Lippia graveolens	+++	+	+
Ocinum basilicum	+	+	++
Mentha spicata leaves	+	+	++

Table 2. Preliminary analysis of the antibacterial activity of essential oils against *E. coli*

Inhibition halo (cm)					
Oil amount	*Mentha spicata* leaves	*Ocinum basilicum*	*Lippia graveolens*	Sulfamethoxazose	Bacitracim
5 µL	1.2	0.1	2.0	1.1	NP
10 µL	0.8	0.2	2.2	1.5	NP
15 µL	1.3	0.3	2.5	1.5	NP

Table 3. Inhibition halos of essential oils aromatic plants against different bacteria strains

Inhibition halo				
Specie	*E. coli*	*S. enteritiditis*	*B. subtillis*	*S. Dysenteria*
Ocinum basilicum	0.16^{d}	0.26^{d}	0.20^{e}	0.23^{d}
Thymus vulgaris	1.13^{c}	1.10^{c}	1.10^{c}	0.86^{b}
Citrus lemon	1.70^{b}	1.63^{b}	0.60^{d}	1.66^{a}
Psidium guajaba	0.10^{d}	0.10^{d}	0.10^{f}	0.10^{d}
Mentha spicata	1.16^{c}	1.26^{c}	2.10^{a}	0.63^{c}
Lippia graveolens	1.90^{a}	2.03^{a}	1.26^{b}	1.83^{a}
Chenopodium ambrosiodes	Whitout inhibition			

Different letters indicate statistically significant differences (α=0.05)

Table 4. The antioxidant activity from essential oils aromatic plants

Specie	Antioxidant potential (%)
Psidium guajaba	30.1 ± 10
Chenopodium ambrosiodes	100 ± 0
Citrus lemon	100 ± 0
Lippia graveolens	100 ± 0
Thymus vulgaris	100 ± 0
Ocinum basilicum	77.4 ± 0.7
Mentha spicata	100 ± 0

area, or Ocinum basilicum species, in addition, different genetic and environmental factors, plant nutritional status and other factors that may influence the composition of the oil must be considered, (Calderon Mestanza, J. 2013). Regarding *Psidium guajaba* leaves, Rodríguez Amado (2013) reported antidiarrheal activity, Bermúdez-Vásquez, M. J. et al ,(2019) reported activity against bacteria *Escherichia coli*(ATCC 25922), however, they also point out that although guajava leaf essential oil may be mainly composed of sesquiterpenes such as caryophyllene and selinene. These components will be affected depending on the place of procurement. (Montero-Recalde et al., 2018, Bermúdez-Vásquez et al., 2019).

CONCLUSION

It was determined that the essential oils extracted from *Lippia graveolens*, *Citrus lemon, Thymus vulgaris*, and *Mentha spicata* leaves exhibited significant inhibitory effects against gastrointestinal pathogenic bacteria, including *Bacillus subtilis, Escherichia coli, Salmonella enteritiditis*, and *Shigella dysenteriae*. On the other hand, *Ocinum basilicum* and *Psidium guajaba* demonstrated minimal inhibitory activity against these bacteria.

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