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RESEARCH ARTICLE

THE ESSENTIAL OIL OF JACARANDA MIMOSIFOLIA AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Essential oils are substances of plant origin and lipid nature. They are present in leaves, stems, flowers, and fruit rinds. Various methods are known for their extraction. In this study, the essential oil of *J. mimosifolia* was extracted using distillation dragging water vapor, and the antifungal properties of the extracted essential oil were tested.

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INTRODUCTION

As is well known, antimicrobial resistance is increasing, but the development of new drugs with antimicrobial activity is not increasing at the same rate. Antimicrobial resistance refers to the ability of microorganisms, including bacteria, viruses, fungi, and parasites, to survive and multiply despite drug treatment (Ventola, 2015). The current antibiotic discovery model is not delivering new agents at a rate that is sufficient to combat present levels of antibiotic resistance. This has led to fears of the arrival of a 'post-antibiotic era'. Scientific difficulties, an unfavourable regulatory climate, multiple company mergers and the low financial returns associated with antibiotic drug development have led to the withdrawal of many pharmaceutical companies from the field (Jackson *et al*., 2018). Therefore, it is necessary to search for new substances with antimicrobial properties. Various plant-derived substances with antibacterial and antifungal properties have been reported. Research shows that plants contain bioactive compounds such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, lectins, polypeptides, polyacetylenes, essential oils, which serve as foundations for antibiotic development (Angelini, 2024; Edeoga *et al.,* 2005; Rahman and Anwar, 2007). Essential oils are rich in beneficial chemical elements and have a wide range of applications in agricultura, food, cosmetics, medicine. They are derived from a variety of sources (including spices, herbs, fruits, and flowers) and contain a wide range of constituents, with hydrocarbon monoterpenes being particularly prominent. Many essential oils are constitutively expressed by plants or can be synthesized as self-defense mechanisms in response to pathogens. It has been reported that essential oils have antimicrobial properties that are dependent on their chemical composition and the number of single components (Angane *et al*., 2022; Hintz *et al*., 2015; Mehidi *et al*., 2024; Nazzaro *et al*., 2013). Various essential oils with antimicrobial properties have been reported. Therefore, this work shows the novel antifungal activity of *J. mimosifolia* essential oil.

MATERIAL AND METHODS

Source of material: In this study, the essential oil of *J. mimosifolia* was distilled from partially crushed flowers. The flowers were collected from a leafy jacaranda tree in the city of Puebla, México. The essential oil was prepared by distillation dragging water vapor from jacaranda flowers.

Biological material: The *Saccharomyces cerevisiae* strain was used. The strain of *S. cerevisiae* used was the yeast marketed for making bread. Yeast was stored in cryovials at -40°C in yeast peptone dextrose (YPD) broth with 20% glycerol until analysis.

Distillation dragging water vapor: The *J. mimosifolia* essential oil was obtained according to methodology previously described by Flores-Encarnación *et al*., (2025). For this, 20 grams of fresh flowers were partially crushed with a sterile mortar and pestle. The flowers of jacaranda were placed in a 500 mL round glass flask. Using 5 mm glass tubing, the water vapor generated in another 500 mL round flask (containing 200 ml of distilled water in boiling) was passed to 500 mL round flask containing 20 grams of ground jararanda flowers. This last flask was connected to a condenser to recover the *J. mimosifolia* essential oil entrained by the water vapor. The condensate was recovered in a 250 mL Erlenmeyer flask. After 3 hours, about 100 mL of distillate was recovered and protected from light using aluminum foil. Then, the distillate obtained was placed in a 500 mL glass separation funnel and 15 mL of chloroform was added, stirring vigorously for 30 min (releasing excess gas periodically). This process was carried out at room temperature in a gas extraction system. Phase separation between chloroform (below) and water (above) was immediately observed. To recover a larger amount of *J. mimosifolia* essential oil, the separating funnel was left to stand for 24-48 hours at room temperature in low light. The chloroform phase (below, containing the essential oil) was recovered in an amber glass bottle. Chloroform was removed from the *J. mimosifolia* essential oil using a continuous low flow of air passed over the surface of the chloroform phase for 3 hours at room temperature within a gas extraction system. The obtained *J. mimosifolia* essential oil was stored in a sterile 1.5 mL centrifuge tube and protected from light.

Culture: *S. cerevisiae* strain were cultivated on yeast peptone dextrose broth containing amoxicillin (16µg/mL) and gentamicin (40 µg/mL) and the following components of medium (g/L): 10 yeast extract, 20 peptone and 20 dextrose. The stationary cultures were grown at 30°C for 24 hours in glass tubes containing 5 mL of yeast peptone dextrose broth and were used as precultures. The yeast peptone dextrose agar plates containing 20 mL of medium were prepared. Sterile Petri dishes (150 mm) were used. Plates were inoculated by crossstriation with a stationary 24-hour preculture of *S. cerevisiae* in yeast peptone dextrose broth (Ab_{560nm}= 5).

Antifungal activity of essential oil: The antifungal activity of *J. mimosifolia* essential oil was determined using the technique of diffusion in agar using paper discs. For it, yeast peptone dextrose agar plates (containing 20 mL of medium) were prepared. Sterile Petri dishes (150 mm) were used. Plates were inoculated by crossstriation with a stationary 24-hour preculture of *S. cerevisiae* in yeast peptone dextrose broth (Ab_{560nm}= 5). Then, sterile filter paper disks (5 mm diameter) were placed on the surface of yeast peptone dextrose agar plates. Different amounts of essential oil were used: 1.05, 2.1, 4.2, 6.3, and 10.5 mg. The agar plates were incubated at 30°C for 24 h. The inhibition zones formed were observed. The analyses were conducted in triplicate.

Cell viability assay: The cell viability assay was performed using *S. cerevisiae* cells and the trypan blue dye according to modified methodology described by Castillo *et al*., (2009). For that, 1 mL of an active culture of *S. cerevisiae* (18-24 hours of culture, Ab_{560nm}= 5) was centrifuged at 3,000 r.p.m. for 10 min. The supernatant was removed and 200 µL of fresh yeast peptone dextrose broth were added (cell suspension). The cell viability assay was determined by mixing 10 µL of cell suspension and 10 µL of 0.1% trypan blue dye, and then placing 10 µL of the mix on a slide observing at **40X** power. Dead cells were observed in a deep blue color. All determinations were made in triplicate. For negative control, non-viable cells of *S. cerevisiae* were used. This cells were obtained by heating at 100°C for 10 minutes.

Effect of *T. vulgaris* essential oil on cell viability: The effect of *J. mimosifolia* essential oil on viability of *S. cerevisiae* cells was determined as follows. The cell suspension was prepared and mixed with the trypan blue dye as described before. Then, 1.05 mg of *J. mimosifolia* essential oil was added; this mixture was incubated at room temperature at 15 min. The preparations were observed at ***4*0X** power. All determinations were made in triplicate.

RESULTS

In this study, the *J. mimosifolia* essential oil was obtained and also the antifungal activity of the essential oil was determined. So, *J. mimosifolia* essential oil was prepared by distillation dragging water vapor from 20 grams of fresh flowers partially crushed. As described in Materials and Methods, fresh flowers partially crushed of jacaranda were placed in a 500 mL round glass flask and water vapor was passed through a glass tube from another round flask that generated the water vapor. The condensate was recovered in a 250 mL Erlenmeyer flask and the extracted essential oil was recovered using chloroform as described in Materials and Methods. Then the chloroform was removed from *J. mimosifolia* essential oil using a continuous low flow of air passed over the surface of the chloroform phase for 3 hours at room temperature within a gas extraction system. The obtained *J. mimosifolia* essential oil was stored in a sterile 1.5 mL centrifuge tube and protected from light (Fig. 1).

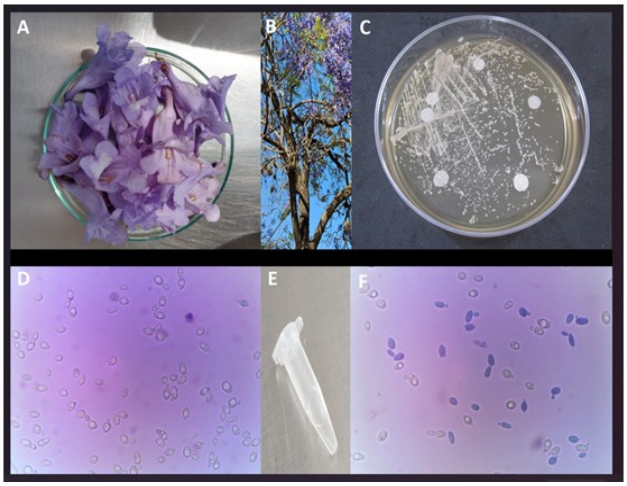

Fig. 1 The antifungal activity of *J. mimosifolia* essential oil. A. The flowers of *J. mimosifolia*. B. The *J. mimosifolia* tree. C. *J. mimosifolia* essential oil on *S. cerevisiae* growth. Essential oil increasing amounts (1.05, 2.1, 4.2, 6.3, and 10.5 mg) were placed in counterclockwise direction, starting with the top. D. Cells of *S. cerevisiae* mixed with 0.1% trypan blue dye. E. *J. mimosifolia* essential oil obtained. F. Cells of *S. cerevisiae* treated with *J. mimosifolia* essential oil for 15 min and stained with 0.1% trypan blue dye.

The antifungal activity of *J. mimosifolia* essential oil was determined. For this, the technique of disk by diffusion in agar on *S. cerevisiae* growth was used. So, Petri dishes containing yeast peptone dextrose agar were inoculated by crossstriation and then sterile filter paper discs were placed on the surface of

yeast peptone dextrose agar plates adding different amounts of essential oil: 1.05-10.5 mg. The agar plates were incubated at 30°C for 24 h. The results are shown in Fig. 1C. As shown in the figure, low amounts of *J. mimosifolia* essential oil did not inhibit the growth of *S. cerevisiae*. However, at the highest quantities of *J. mimosifolia* oil tested, it was possible to observe zones of growth inhibition of *S. cerevisiae*. With this, the antifungal effect of the essential oil of *J. mimosifolia* was verified. To determine the direct effect of the essential oil, *S. cerevisiae* cells were treated with *J. mimosifolia* during 15 min and cell viability was determinated using trypan blue dye as described in Materials and Methods. Dead cells were observed in a deep blue color. The results are shown in Fig. 1D and Fig. 1F. Fig. 1D shows *S. cerevisiae* cells stained with trypan blue dye and not treated with *J. mimosifolia* essential oil. As seen in this image, the cells of *S. cerevisiae* were not stained by trypan blue which indicated that the cells were intact. Fig. 1F shows the results obtained when the *S. cerevisiae* cells were incubated for 15 min with *J. mimosifolia* essential oil. *S. cerevisiae* cells were stained due to the action of *J. mimosifolia* essential oil. Dead cells were observed in a deep blue color. In this image it also can be seen that approximately 50% of *S. cerevisiae* cells were intracellularly permeated by the dye, meaning that *J. mimosifolia* essential oil had a lethal effect on *S. cerevisiae*. The cells maintained their characteristic morphology but not their viability.

DISCUSSION

Medicinal and aromatic plants have been utilized as a natural source of remedies and healthcare for millennia (Ansari *et al*., 2023; Chaachouay *et al*., 2023; Chaachouay and Zidane, 2024; Okigbo *et al*., 2009). Multiple disciplines of study and diverse investigation methods have been included in drug discovery from medicinal plants (Chaachouay and Zidane, 2024). In this context, essential oils have been the subject of study due to their multiple functions. The essential oils are substances obtained from plant materials as leaves, fruits, branches, seeds, bark, flowers by different methods. The essential oils are secondary metabolites produced by plants in order to provide a defense function or attraction (Burt, 2004; Butkiené *et al*., 2015; Citarasu, 2010; Cowan, 1999; Flores-Encarnación *et al.*, 2016). In this study, the *J. mimosifolia* essential oil was obtained and its antifungal activity was determined. The essential oil was prepared by distillation dragging water vapor from fresh flowers partially crushed of jacaranda. This methodology was a simple strategy for extracting *J. mimosifolia* essential oil. It has several advantages, including its low cost, its relatively simple operation, and the production of an active biological product with antimicrobial properties. It has been reported that the most-used method for essential oil extraction is steam distillation due to its simplicity and low investment requirements. Due to the importance of this extractive method, technological updates represent an immense opportunity for improving this component of essential oil production (Machado *et al*., 2022). On the other hand, the essential oil constituents in six species of *Jacaranda* (*J. acutifolia*, *J. caucana*, *J. copaia*, *J. decurrens*, *J. filicifolia* and *J. mimosifolia*) have been reported. Mostafa *et al*., (2015) extracted the essential oil from flowers of *J. acutifolia* by hydrodistillation and reported the main components: *n*-dodecanoic acid (17.48%), *n*-tetradecanoic acid (15.59%), *n*-hexadecanoic acid (10.98%), hexahydrofarnesyl acetone (8.2%), *n*-decanoic acid (7.9%), and nonacosane (7.71%). In addition, these authors reported that the essential oil showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. In the present study, the antifungal activity of *J. mimosifolia* essential oil was determined using technique of disk by diffusion in agar on *S. cerevisiae* growth. Different amounts of the *J. mimosifolia* essential oil were tested: 1.05-10.5 mg. The results indicated that low amounts of *J. mimosifolia* essential oil did not inhibit the growth of *S. cerevisiae*. However, at the highest quantities of *J. mimosifolia* oil tested, it was possible to observe zones of growth inhibition of *S. cerevisiae*. To determine how quickly the essential oil acts on *S. cerevisiae* cells causing death, a direct test was performed. So, *S. cerevisiae* cells were incubated with *J. mimosifolia* essential oil during 15 min and cell viability was determinated. The results indicated that the essential oil had an antifungal effect, producing the entry of the trypan blue dye into approximately 50% of the cells observed. Dead cells were observed in a deep blue color. The antifungal activity of *J. mimosifolia* essential oil was greater than that recorded using amphotericin-B and fluconazole when performing direct tests (confronting *S. cerevisiae* cells against the antifungal) (data not shown). Yuana *et al.* (2018) extracted the essential oil from *J. cuspidifolia* Mart tree branches. They reported that the major constituents of the essential oil were palmitic acid (31.36%), (*Z*)- 9,17-octadecadienal (12.06%), ethyl palmitate (3.81%), perhydrofarnesyl acetone (2.07%), *γ*-maaliene (1.88%), cedro (1.42%) and 9,12-octadecadienoic acid ethyl ester (1.42%). In addition, *J. cuspidifolia* essential oil showed antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* with minimum inhibition concentration values of 17.3 mg/mL, 12.9 mg/mL and 16.0 mg/mL, respectively.

Finally, *J. mimosifolia* essential oil has been little studied, so the present study provides important information about its potential use as an antimicrobial agent. Furthermore, as mentioned before, the extraction of essential oil from *J. mimosifolia* is easy to perform and the production cost is low, which represents an important advantage for this natural resource to be considered as a possible alternative for the recovery of substances of plant origin with antimicrobial properties, in this case antifungal properties. Further studies are needed to understand its mechanism of action and other properties.

CONCLUSION

In this study, the *J. mimosifolia* essential oil was extracted. Using a simple methodology, the essential oil was obtained, and its antimicrobial properties were tested using *S. cerevisiae* as a biological model. The *J. mimosifolia* essential oil acted quickly, killing *S. cerevisiae* cells. Testing with bacteria and other pathogenic fungi is necessary to verify the spectrum of activity of the essential oil obtained.

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