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Antifungal activity of *Pelargonium graveolens* essential oil and its use in preserving fresh orange juice during short-term storage

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Abstract

The present study focused on the extraction and quality analysis of *Pelargonium graveolens* oil, as well as its use in preserving fresh orange juice during short-term storage as a natural alternative to synthetic compounds. The yield of extraction and physicochemical properties of the essential oil were evaluated, and subsequently tested for antimicrobial activity using the agar diffusion method. Freshly squeezed orange juice was transferred into three sterile 100 mL bottles. *P. graveolens* essential oil was sterilized by filtration through a 0.22 µm membrane under aseptic conditions and added at 0 µL (control), 50 µL, and 100 µL doses. Samples were stored at room temperature for microbial analysis. Gas chromatography–mass spectrometry (GC-MS) analysis of oil identified citronellol as the major constituent, followed by geraniol. The antifungal and antimicrobial activity of the essential oil was very significant against *Candida albicans*, but weak against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Interestingly, complete inhibition was observed in orange juice stored at room temperature with the highest essential oil concentration. Bacterial growth was also not observed in juice samples, regardless of whether they were treated with or without essential oil. The results indicate that the essential oil of *P. graveolens* exhibits significant antifungal activity and, therefore, may be used as a natural preservative in fresh juices to improve microbiological stability during short-term storage.

Key words: *Pelargonium graveolens*, essential oil, antibacterial activity, preservative.

Introduction

Medicinal and aromatic plants have garnered considerable interest in recent years due to the presence of natural products with antioxidant and antimicrobial properties. Most of these plants are safer than synthetic chemicals, which may present health and environmental hazards (Ben El Hadj *et al.*, 2020). The Geraniaceae contains more than 830 species in five genera (Bremer, 1992). One of these genera, *Pelargonium,* has about 283 species (Röschenbleck, 2014). Although *Pelargonium* was once more widely cultivated due to its rapid growth and usefulness, it is now grown mostly on a small scale and sometimes faces neglect (Boukhatem *et al.*, 2011).

For a long time, medicinal plants have been used to treat illnesses, partly due to their diverse chemical compounds and the way these compounds interact with one another. Essential oils from plants are increasingly used, either alone or in combination with other substances, to preserve food, as consumers prefer natural ingredients (Tongnuanchan and Benjakul, 2014). The essential oil from *Pelargonium graveolens* is considered safe for use in food by the U.S. FDA. It is known for its several health benefits, including anti-inflammatory and healing effects (Lis-Balchin, 2004; Moro Buronzo, 2008; Omar *et al.*, 2012; Kumar *et al.*, 2021). However, less is known about its ability to fight bacteria, especially in Algeria. There is limited research on this plant regarding its antimicrobial activity.

The ever-increasing prevalence of infections caused by opportunistic pathogens, such as *Candida albicans, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, presents a considerable challenge to both healthcare and food safety, particularly in environments like the processing and storage of citrus juices. *Candida albicans* is a dominant fungal pathogen implicated in both superficial and systemic candidiasis, frequently impacting immunocompromised individuals and serving as a model for studies on fungal biofilms and drug resistance (Lima *et al.*, 2024). *Pseudomonas aeruginosa*, a Gram-negative bacterium, is notorious for causing severe and persistent infections, particularly in immunocompromised patients and those exposed to contaminated foods or medical devices, aided by its strong resistance to conventional antibiotics and innate ability to form biofilms in diverse environments, including beverages like citrus juice (Srivastava *et al.*, 2022).

With the growing identification of multidrug-resistant strains, there is an urgent need for new antimicrobial strategies. Among natural alternatives, the essential oil of geranium (*Pelargonium graveolens)* has demonstrated broad-spectrum antimicrobial properties. Research has shown that geranium essential oil is highly effective in reducing the viability of *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, often at low minimum inhibitory concentrations (de Lima *et al.*, 2024; Bigos *et al.*, 2012; Srivastava *et al.*, 2022). Notably, studies indicate that geranium oil can be used alone or in combination

with other agents and may be explored for use in food matrices, such as citrus juice, showing promise in controlling microbial contamination in both clinical and food systems.

Given the widespread use and concerns associated with synthetic preservatives, this work studied *P. graveolens* essential oil as a natural alternative. The objective was to evaluate whether the oil can help prolong the shelf life of fresh fruit juices by inhibiting microbial spoilage during short-term storage.

Materials and methods

Plant material: The plant material of *Pelargonium graveolens* was harvested from Relizane city in February 2024 (0°33′21″ East, 35°44′14″ North and 98 m altitude).

Microbial strains: Three microbial strains (*Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 23235 and *Pseudomonas aerogenosa* ATCC 27853) were used to test the antimicrobial activity of the essential oil extracted from *Pelargonium graveolens.*

Biochemical characterization: The water content was measured as a percentage of the initial sample weight, following the method described by Chavagnat (1984). The ash content was determined and reported according to NF V05-113 (1972).

Extraction of essential oils by the hydrodistillation method: A twenty-gram sample of *P. graveolens* was used for oil extraction. Essential oils were extracted using a Clevenger hydrodistillation assembly and then brought to a boil for approximately 3 h at 150 °C. The oil was recovered and then dehydrated using a chemical desiccant, sodium sulfate, to eliminate all traces of water from the hydrodistillation process. The oil was then stored in a hermetically sealed, opaque bottle. The extraction yield was calculated according to:

Yield(%)= [(Weight of essential oil in grains/Weight of the fresh plant used in grams) x 100]

Determination of the chemical parameters of the oil: The chemical parameters of the oil, including the Acid Index (AI), Saponification Index (SI), and Peroxide Index (PI), were determined following standardized methods: AI was measured according to AFNOR-NFT-60-2000, SI according to NF ISO 365, and PI as described by Kouamé *et al.* (2015). These indices provide key information on the oil's quality, including its free fatty acid content, the average molecular weight of fatty acids, and the extent of oxidation. All analyses were performed in triplicate to ensure accuracy, and results were expressed as means with standard deviations.

Analysis of essential oils by CPG-MS: GC analysis was done by using a Gas Chromatograph coupled to a Mass Spectrometer (GC-MS) type Shimadzu QP2010. DB-5 ms was the type of the capillary column used (25 m × 0.25 mm × 0.25 µm). 1 µL of essential oil diluted to 10 % in cyclohexane was injected in splitless mode. Chromatogram acquisition was in SCAN mode. Then, Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The ion source and interface temperatures were set at 250 °C and 280 °C, respectively. The solvent delay time was 4 min. The detector was programmed to 250 °C. The mass spectra were recorded from 35 to 400 m/z at a scan rate of 0.3 s^{-1}. The compounds were identified by comparing their Kovats retention indices with those cited in the literature and provided by the spectral library banks (NIST) (Kaloustian and Hadji-Minagloyou, 2012).

Aromatogram: Approximately 15 mL of Mueller-Hinton (MH) medium was poured into each petri dish under the sterile zone of the Bunsen burner. After solidification, the microbial strains were inoculated by swabbing the surface with a turbidity of 0.5 MacFarland measured at 600 nm. Then, a disk of sterile Whatman paper was impregnated with 7 µL of essential oil. In parallel, sterile physiological water was used as a positive control. The microbial strains were tested in the same way against antibiotics. The sensitivity to different essential oils was organized according to the diameter of the inhibition zones, as proposed by Ponce *et al.* (2003).

Use of essential oil (EO) in the preservation of fresh orange juice: After obtaining one liter of squeezed orange juice, the addition of sterile essential oil was carried out. Sterilization of the essential oil was achieved by filtration through a 0.22 µm sterilizing filter membrane, which was performed under the sterile zone of the Bunsen burner. Three bottles, each with a volume of 100 mL of juice, were prepared. Each bottle was treated with 0 µL, 50 µL, and 100 µL of the EO. Fresh, unpasteurized juice was used in the study to preserve its native microbiological characteristics. The initial pH of the juice was 4.0. Filtration conditions applied for sample preparation were as described earlier in this section. Following preparation, the samples were stored under controlled conditions at 4 °C, protected from both air and light. The samples were subjected to natural microbial contamination for a period of 21 days.

Microbiological analysis of juice: Total coliforms, fecal coliforms, *Salmonella* sp, yeast, and mold (OJAR, 2017) were sought and counted in the juice. The analysis was conducted on Days 0, 10, and 21. Decimal dilutions were prepared according to the protocol of Guiraud (2003). From decimal dilutions ranging from 10^{-3} to 10^{-1}. 1 mL was placed in a petri dish, followed by 15mL of VRBL agar. After solidification, a surface layer of the same medium was poured (about 4 mL). After a second solidification, the inverted petri dishes were incubated in an oven set at 44 °C for 24 h for fecal coliforms and at 37°C for total coliforms. The colonies obtained were purplish. The enumeration of coliforms was done according to the standard NF ISO 4832. A volume of 1 mL of each dilution was aseptically collected in a tube containing the selenite cysteine mixture, the whole was incubated at 37 °C for 24 h, then, a volume of 15 mL of the Hektoen medium was poured into petri dishes, after solidification, 100 µL of the previous preparation was placed on the surface and then spread. The dishes were then incubated at 37°C for 24 hours. The detection of *Salmonella* was done according to Gelde (1996). A volume of 100 µL of the 10^{-1} dilution was inoculated on the surface. Incubation was carried out at a temperature of 25°C for 3 to 5 days. Yeasts and molds varied in shape, color, appearance, and size. The enumeration of yeasts and molds was done by Guiraud (2003).

Statistical analysis: The sample was individually analyzed in triplicate for its chemical composition and antimicrobial activities and the data were reported as mean ± standard deviation. The difference between the control and samples was determined by

one-way analysis of variance followed by the Duncan's Multiple Range Test using SPSS statistical software.

Results

Physicochemical analysis of the plant: The proximate analysis of *P. graveolens* plant material revealed that it contained a high proportion of moisture, with a water content of 82.33 ± 1.53%, resulting in a dry matter content of 17.67 ± 1.53%. The mineral fraction, expressed as ash content, was found to be 11.33 ± 0.58%. These findings suggest that the fresh plant material is predominantly composed of water, with a moderate dry matter fraction and a notable level of mineral constituents.

Table 1. Proximate analysis of *P. graveolens*

Parameter	Value
Water content (%)	82.33 ± 1.53
Dry matter (%)	17.67 ± 1.53
Ash content (%)	11.33 ± 0.58

Physicochemical characteristics of essential oils: The physicochemical analysis of the essential oil (EO) extracted from *P. graveolens* is summarized in Table 2. The extraction yield was determined to be 0.10 ± 0.03%. The acidity index (AI) was measured at 13.09 ± 1.21, indicating a moderate acid content in the oil. The saponification index (SI) was 69.5 ± 2.35 mg KOH/g, indicating the degree of esterification. The peroxide index (PI) was relatively low, at 4 ± 0.50 meq O_2/kg, indicating good oxidative stability of the oil. The pH value of the essential oil was slightly acidic, with a recorded value of 4.8 ± 0.2. These values indicate that *P. graveolens* essential oil exhibits a modest extraction yield, moderate acidity and saponification values, a low peroxide content, and a mildly acidic character. The low variability in the values (as indicated by the standard deviations) suggests good consistency in oil characteristics across samples. *P. graveolens* a yellowish colour, a viscous appearance and a pronounced floral odour characteristic of this plant, characterized essential oil.

Analysis of essential oils by GC-MS: Based on the chromatogram analysis of the essential oil, ten chemical constituents were identified, comprising a total of 99.86 % of the oil's composition. The major component was citronellol, accounting for 46.23%, followed by geraniol at 22.11%. Chrysontenone was the least abundant constituent among those identified (Table 3, Fig. 1). This composition indicates a predominance of oxygenated monoterpenes, which are often responsible for the characteristic aroma and bioactivity of the essential oil. Citronellol is a major component of geranium essential oil and contributes significantly to its antimicrobial activity and is of industry importance (Kumar *et al.,* 2021).

Table 2. Physicochemical characteristics of the essential oils of *P. graveolens*

Parameter	Value
Extraction yield (%)	0.10 ± 0.03
AI	13.09 ± 1.21
SI (mg KOH/g)	69.5 ± 2.35
PI (meq O2/kg)	4 ± 0.50
pH	4.8 ± 0.2

Table 3. Results of chromatographic analysis of essential oil

Kovats retention index	Compounds	Percentage (%)
1097	Linalool	5.87
1123	Chrysontenone[b]	0.36
1143	Camphor	2.09
1164	Isomenthone	8.80
1189	α-terpinéol	1.20
1128	Citronellol[a]	46.23
1255	Géraniol	22.11
1275	Citronellyl formiate	8.15
1300	Géranyl formiate	3.49
1586	Davanone	1.56
	Total	99.86

a: major compound, b: very small quantities

Aromatogramm: The results of the aromatogram are shown in Fig. 2. It was observed that *P. graveolens* EO appeared to be antifungal, following the diameter of the inhibition zone recorded against *Candida albicans* (30 ± 1.0 mm), which indicated that this strain is susceptible to this bioactive substance. Whereas, the two bacterial strains, *Staphylococcus aureus* and *Pseudomonas aeruginosa,* were found to be tolerant to the oils studied, with inhibition diameter zones of 7.66 ± 0.57 mm and 6.83 ± 0.76 mm, respectively.

Use of essential oil in the preservation of fresh orange juice: Based on the microbiological analysis over storage periods of 0 days, 10 days, and 21 days, the essential oil of *P. graveolens* demonstrated high antifungal activity against yeasts and moulds in juice samples (Table 3). Specifically, no fungal growth was detected in juice bottles treated with the 100 µL dose of the

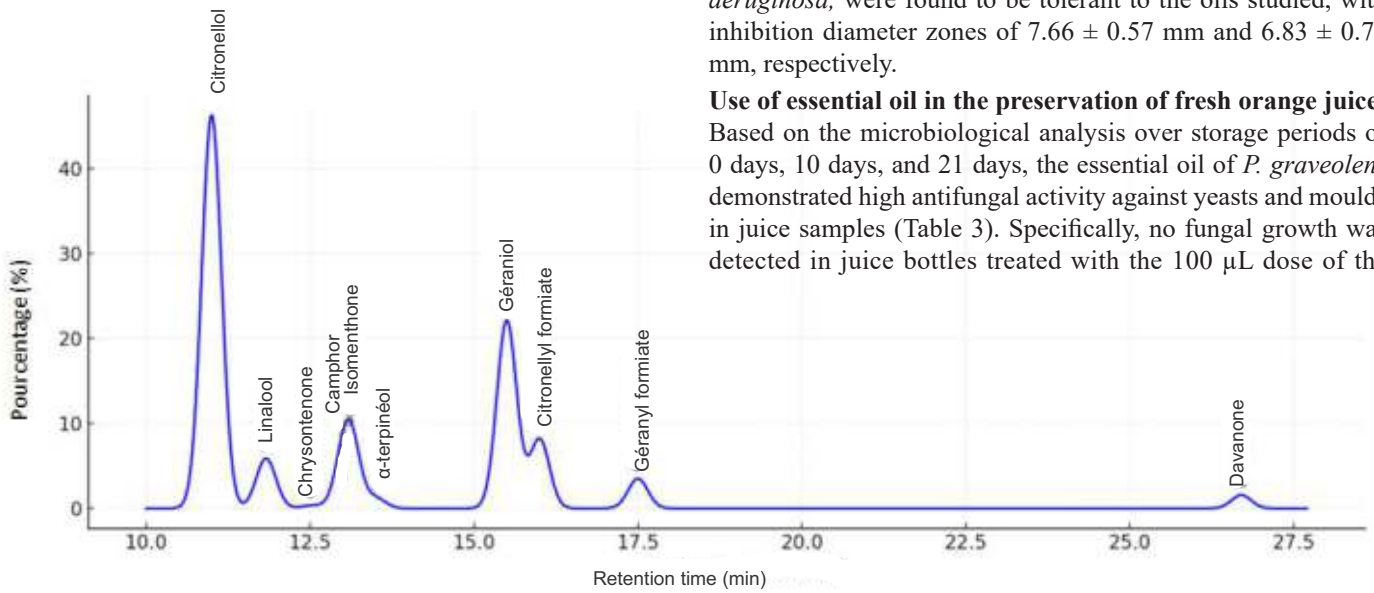

Fig. 1. GC-MS chromatogram of *P. graveolens* essential oil showing identified chemical

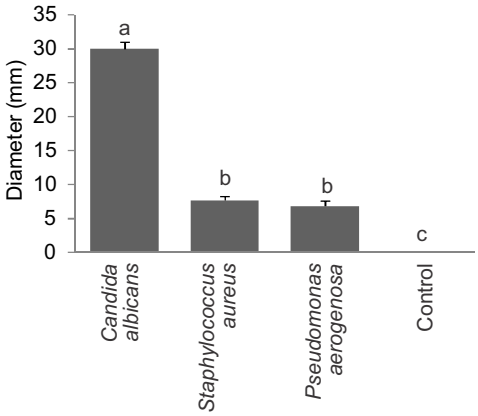

Fig. 2. Antimicrobial activity of *Pelargonium graveolens* essential oil against *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*

essential oil, even after 21 days. In contrast, the 50 µL dose allowed the presence of fungal colonies at a count of 8×10^2 CFU/mL after the same storage duration. The control juices without EO showed visible microbial mats, indicating significant fungal contamination (Fig. 3).

Notably, throughout the 21-day storage period, no bacterial contamination, including total coliforms, fecal coliforms, or Salmonella, was detected in the juices, regardless of the essential oil treatment. This suggests that the juice preparation and storage conditions maintained effective bacterial hygiene control, while the *P. graveolens* essential oil

Discussion

This study aims to enhance the value of *P. graveolens* essential oils as a biopreservative for foodstuffs. The moisture percentage of *P. graveolens* oil falls within the acceptable range (5% to 8%), indicating that the formulation can be stored for an extended period and is unlikely to be easily contaminated by bacteria (Boukhatem *et al.*, 2010). The variation in water content can be attributed to the varietal factor, the degree of maturation, pedoclimatic characteristics, and storage conditions. The organoleptic parameters of our EO were in accordance with those listed in AFNOR standards. The extraction yield obtained was lower than that recorded in the work of Boukhatem *et al.* (2010), where they found a value of 0.25% compared to a simple decantation (0.2%).

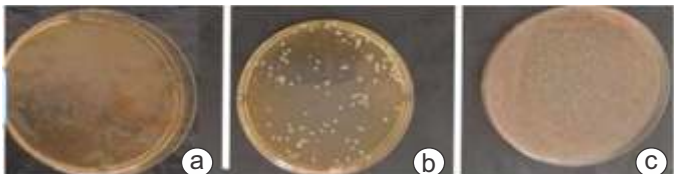

Fig. 3. Yeast colony on Petri dishes. a. Sample containing EO (1 µL/mL), b. Sample containing EO (0.5 µL/mL), c. Control (0 µL/mL)

Pelargonium essential oil from the Comoros was richer in citronellol (29.98%) than that from South Africa and Madagascar, whose concentrations were 20.24% and 28.51%, respectively (Omar *et al.*, 2012). Samples of *Geranium rosat* were collected in the region of Blida, in the commune of Chiffa, of which Citronellol appeared as the major constituent of the EO (19.22 %), followed by geraniol (14.03 %) (Atailia and Djahoudi, 2015). The results obtained were the same as those found in the same plant harvested in Taounate, Morocco, with a difference in percentage and the presence of other chemical constituents. Specifically, citronellol was found to be 26.98%, and geraniol was found to be 14.12% (Chraibi *et al.*, 2021). Referring to the results of the antibiogram, this oil demonstrated potent antifungal activity compared to the antibiotics tested.

On the other hand, the screening of the antimicrobial activity of the essential oil of *Geranium odorant* revealed that Gram-positive bacteria are more sensitive to the inhibitory action of this essential oil than Gram-negative bacteria. The latter have an intrinsic resistance to biocidal agents, which was related to the nature of their bacterial wall (Neidhardt, 1996). According to Marino *et al.* (1999), Gram (+) bacteria was generally more sensitive to essential oils than Gram (-) bacteria. *Pelargonium asperum* EO had a strong antibacterial activity against *S. aureus* (ATCC 43300) and *E. coli* of clinical origin. These results seemed contradictory to many studies that confirmed the effectiveness of this compound (essential oil of *P. graveolens*) against various bacteria, including *S. aureus*; however, it was more active against *C. albicans* (Atailia and Djahoudi, 2015). Hammer *et al.* (1999) conducted an exhaustive study on the antimicrobial activity of about ten essential oils and their major compounds. Their study revealed that geranium essential oil has a strong inhibitory power against *C. albicans*. The antifungal activity of essential oils has been the subject of numerous *in vitro* scientific studies for several years. However, the methods used to evaluate this activity were varied and can sometimes give different results depending on the experimental conditions specific to each manipulation. Moreover, the antibacterial activity of plant extracts depends on several factors, including the harvest period, climatic conditions, extraction method, chemical composition, type of microorganisms tested, and the conditions under which the tests are carried out (Al-Reza *et al.*, 2009). To achieve a significant antimicrobial effect in a food matrix, higher concentrations of

Table 3. Yeast and mold counts (CFU/mL) in fresh orange juice treated with *P. graveolens* essential oil over refrigerated storage at 0, 10, and 21 days

Storage day	Treatment	Dose (µL per bottle)	Bottle volume (mL)	Dose (µL/mL)	Yeasts CFU/mL	Molds CFU/mL	Total YM CFU/mL	LOD (CFU/mL)	Result vs LOD	Visible growth notes
0	Control	0	100	0	0 (ND)	0 (ND)	0 (ND)	10	< LOD	No colonies; no visible mats
0	EO	50	100	0.5	0 (ND)	0 (ND)	0 (ND)	10	< LOD	No colonies; no visible mats
0	EO	100	100	1	0 (ND)	0 (ND)	0 (ND)	10	< LOD	No colonies; no visible mats
10	Control	0	100	0	300	20	320	10	> LOQ	Visible microbial colonies
10	EO	50	100	0.5	50	10	60	10	> LOQ	Visible microbial colonies
10	EO	100	100	1	0 (ND)	0 (ND)	0 (ND)	10	< LOD	No colonies; no visible mats
21	Control	0	100	0	1000	100	1100	10	> LOQ	Visible microbial colonies
21	EO	50	100	0.5	800	30	830	10	> LOQ	Visible microbial colonies
21	EO	100	100	1	0 (ND)	0 (ND)	0 (ND)	10	< LOD	No colonies; no visible mats

LOD: lowest detectable level; LOQ: lowest quantifiable level. ND = not detected (< LOD). 'Detected, < LOQ' indicates presence without reliable quantification.

essential oils, typically 5 to 20 µL/g, were necessary. It is known that the "normal" microbial load of most foods ranges from 10^4 to 10^^{6} CFU/g. The massive presence of microorganisms in food products sometimes leads to very serious repercussions for the consumer's health. Citronellol is a monoterpene with anti-yeast and anti-fungal effects. The highest inhibition zone value was observed against *Microsporum canis* (64 mm), *Trichophyton mentagrophytes* (64 mm), and *Trichophyton rubrum* (39 mm) using 10 mL of the oil (Tolba *et al.*, 2015). Aliphatic alcohols (citronellol: 7.25 % and methyl eugenol: 8.21 %) and aldehyde (citronellal: 76.33 %) of the essential oil of *Eucalyptus citriodora* could be responsible for its antifungal activity (Traore *et al.*, 2013).

In conclusion, the antimicrobial activity of *P. graveolens* EO was tested on three microbial strains. The results obtained showed intense antifungal activity against Candida albicans but low antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa*. The EO preserved the natural orange juice in terms of yeast and mold growth. Even its color, odor, and appearance remained intact during storage at room temperature. This research represents a solution to the deterioration of fresh products, such as natural juices obtained from citrus fruits, which can be industrialized without compromising microbiological and organoleptic stability, while avoiding the use of chemical additives that appear to be harmful to human health.

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