









Antimicrobial activity of *Cymbopogon schoenanthus* essential oil extracted by a solar distillation system

Actividad antimicrobiana del aceite esencial de *Cymbopogon schoenanthus* extraído mediante un sistema de destilación solar

Atividade antimicrobiana do óleo essencial de *Cymbopogon schoenanthus* extraído por um sistema de destilação solar

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Rev. Fac. Agron. (LUZ). 2025, 42(2): e254227

ISSN 2477-9407



DOI: [https://doi.org/10.47280/RevFacAgron\(LUZ\).v42.n2.XI](https://doi.org/10.47280/RevFacAgron(LUZ).v42.n2.XI)

Received: 25-02-2025

Accepted: 03-05-2025

Published: 28-05-2025

Crop production

Associate editor: Dra. Lilia Urdaneta  
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

Keywords:

Medicinal and aromatic plants
Camel grass
Dilutions
Essential oil
Inhibition zones

Abstract

Cymbopogon schoenanthus (L.) Spreng is an aromatic medicinal plant that grows in Algeria. It is commonly called camel grass and known by the local name “Lemmad.” Traditionally, it has been used to treat diverse diseases. Therefore, this study aimed to evaluate the antimicrobial activity of the essential oil of *Cymbopogon schoenanthus* (EOCS), grown in Timiaouine, Algeria. A solar distillation (SD) system was used to extract the oil. This extraction occurred in June 2023. Subsequently, the physical properties were determined. The antimicrobial activity against four bacteria and one yeast was evaluated using the agar diffusion test. Various dilutions were used, and the inhibition zones were measured to determine the extent of antimicrobial activity. In this study, the yield was estimated to be 0.97 %, with a density of 0.8490 and a refractive index of 1.4850. The antimicrobial properties of this essential oil (EO) showed efficacy against *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) at dilutions of 1/3 and 1/4, with inhibition diameters varying between 15 to 9 mm. On the other hand, other strains showed resistance throughout all dilutions. This research indicates that the EOCS extracted using the SD process has effective antibacterial properties against Gram- bacteria. Moreover, additional research is required to clarify and assess the effects of the chemical composition, thereby enhancing our understanding of the underlying mechanism.

Resumen

Cymbopogon schoenanthus (L.) Spreng es una planta medicinal aromática que crece en Argelia. Se llama comúnmente hierba de camello y se conoce con el nombre local de «Lemmad». Tradicionalmente, se ha usado para tratar diversas enfermedades. Por lo tanto, este estudio tuvo como objetivo evaluar la actividad antimicrobiana del aceite esencial de *Cymbopogon schoenanthus* (EOCS), cultivado en Timiaouine, Argelia. Para la extracción del aceite se empleó un sistema de destilación solar (SD). Esta extracción tuvo lugar en junio de 2023. Posteriormente, se realizó la determinación de las propiedades físicas. Mediante el test de difusión en hoyos en agar se evaluó la actividad antimicrobiana frente a cuatro bacterias y una levadura. Se utilizaron varias diluciones y se midieron las zonas de inhibición para determinar el alcance de la actividad antimicrobiana. En este estudio, el rendimiento se estimó en un 0,97 %, con una densidad de 0,8490 y un índice de refracción de 1,4850. Las propiedades antimicrobianas de este aceite esencial (AE) mostraron su eficacia contra *Escherichia coli* (EC) y *Klebsiella pneumoniae* (KP) en diluciones de 1/3 y 1/4, con diámetros de inhibición que variaban entre 15 y 9 mm. Por otra parte, otras cepas mostraron resistencia en todas las diluciones. Esta investigación indica que el EOCS extraído mediante el proceso SD tiene propiedades antibacterianas eficaces contra las bacterias Gram-. Además, se requieren investigaciones adicionales para aclarar y evaluar los efectos de la composición química, mejorando así nuestra comprensión del mecanismo subyacente.

Palabras clave: plantas medicinales y aromáticas, hierba de camello, aceite esencial, diluciones, zonas de inhibición.

Resumo

O *Cymbopogon schoenanthus* (L.) Spreng é uma planta medicinal aromática que cresce na Argélia. É vulgarmente chamada erva de camelo e conhecida pelo nome local “Lemmad”. Tradicionalmente, tem sido utilizada para tratar diversas doenças. Por conseguinte, este estudo teve como objetivo avaliar a atividade antimicrobiana do óleo essencial de *Cymbopogon schoenanthus* (EOCS), cultivado em Timiaouine, na Argélia. Foi utilizado um sistema de destilação solar (DS). Esta extração teve lugar em junho de 2023. Posteriormente, foram determinadas as propriedades físicas. A atividade antimicrobiana contra quatro bactérias e uma levedura foi avaliada utilizando o teste de difusão em ágar. Foram utilizadas várias diluições e as zonas de inibição foram medidas para determinar a extensão da atividade antimicrobiana. Neste estudo, o rendimento foi estimado em 0,97 %, com uma densidade de 0,8490 e um índice de refração de 1,4850. As propriedades antimicrobianas deste óleo essencial (OE) mostraram eficácia contra *Escherichia coli* (EC) e *Klebsiella pneumoniae* (KP) em diluições de 1/3 e 1/4, com diâmetros de inibição variando de 15 a 9 mm. Por outro lado, outras cepas apresentaram resistência em todas as diluições. Esta investigação indica que o EOCS extraído através do processo SD tem propriedades antibacterianas eficazes contra bactérias Gram-. Além disso, é necessária investigação adicional para clarificar e avaliar os efeitos da composição química, melhorando assim a nossa compreensão do mecanismo subjacente.

Palavras-chave: planta medicinal e aromática, erva-de-camelo, diluições, óleo essencial, zonas de inibição.

Introduction

Throughout history, the use of therapeutic plants has been ingrained in fundamental human understanding. In contemporary times, despite the incredible developments in scientific disciplines, a significant portion of the global populace, up to 80 % in developing countries, continues to rely on botanical resources for treating illnesses (Ekor, 2014).

In Algeria, medicinal plants typically grow spontaneously. The Timiaouine region is nationally recognized for its rich diversity of medicinal and aromatic plants, but they remain relatively unknown in the country. For these reasons, an in-depth study has been undertaken on one of these plants, *Cymbopogon schoenanthus* L. (CS).

This plant, called camel grass, is known in Algeria as ‘Lemmad’ or ‘Tiberimt’. It is a perennial species that belongs to the Poaceae family (Quézel and Santa, 1962). It has a natural abundance of essential oils (EOs) and a strong aromatic smell with important medicinal value (Heiba and Rizk, 1986). Furthermore, EOs exhibit excellent biological effects, including antimicrobial (Hashim *et al.*, 2017; Bellik *et al.*, 2019; Aous *et al.*, 2019; Sawadogo *et al.*, 2022), and antioxidants properties (Shaaban *et al.*, 2012; Naima *et al.*, 2016; Malti *et al.*, 2020), antiproliferative effects (Park *et al.*, 2014; Yagi *et al.*, 2016), as well as insecticidal activity (Aous *et al.*, 2019).

In short, the quality of EO is affected by diverse factors, such as geographical conditions, seasonal variations, types of plant species, harvest timing, as well as extraction methods (Dhifi *et al.*, 2016). Among all these factors, it is clear that the extraction technique significantly affects the quality of EO. This is achieved through conventional methods such as hydrodistillation, steam distillation, mechanical expression, and solvent extraction (Karakaya *et al.*, 2014), as well as solar distillation (SD), which is a novel alternative for extracting high-value EO compared to other methods. This preference can be attributed to its free energy source, economic benefits, and positive environmental impact (Lukose *et al.*, 2023). However, previous research has documented the effects of solar distillation on the quality of EO (Yen and Lin, 2017; Afzal *et al.*, 2021; Nannaware *et al.*, 2022). Consequently, the literature review indicates no research regarding the antimicrobial effect of EO extracted using solar distillation system. The present research examines the potential of EOCS extracted by SD against various microbial strains at different dilutions, elucidating their antimicrobial activities by measuring the inhibition zones through a well-diffusion assay.

Materials and methods

Plant material

The inflorescences of CS plants were purchased from a local market in Timiaouine, Bordj Badji Mokhtar (southwest Algeria: 20°26'41.0" and N 1°47'55.0"E, Altitude 577 m) (figure 1). According to the book of Quézel and Santa (1962), the botanical identity and authentication were verified by Mr. Bouallala M'hammed, an expert at the Laboratory of Saharan Natural Resources (LRNS), University of Adrar, Algeria.

Essential oil extraction process

In June 2023, during clear weather and high solar radiation intensity (ranging from 891.570 W.m⁻² to 10262.247 W.m⁻²), the EO was extracted by steam distillation using a SD system at the LRNS. This system (figure 2) consists of:

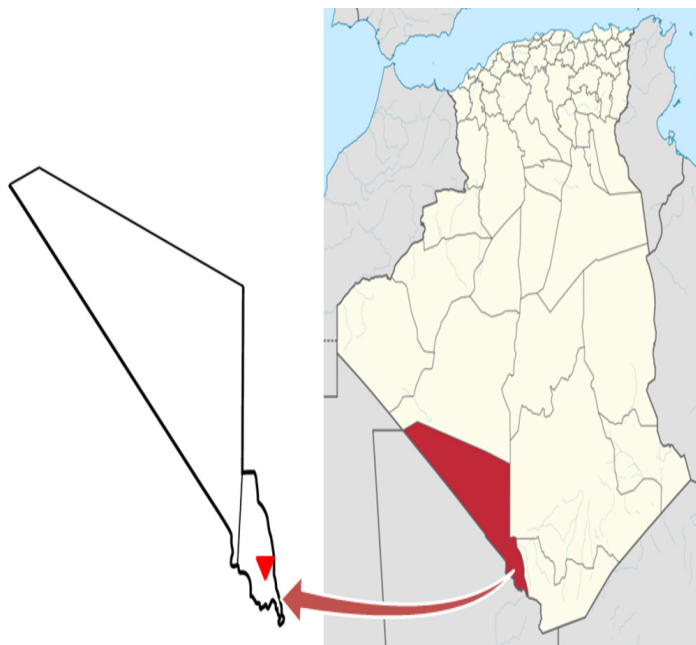


Figure 1. Localisation of Timiaouine region from Algeria.

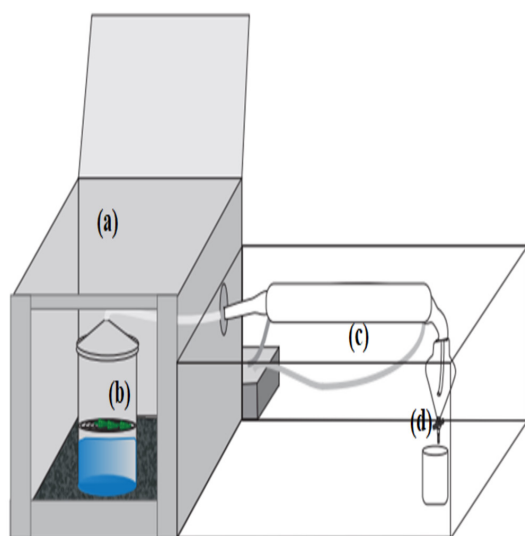


Figure 2. Schematic of solar energy distillation system. (a): insulating box, (b): distillation still, (c): condenser, (d): Florentine flask.

An insulating box with a transparent glass lid (a): is hexahedral in shape (length 65 cm, width 56 cm, height 53 cm and 46 cm);

A distillation still equipped with a grid (b): Stainless steel, 5 L capacity;

A condenser (c), and a Florentine flask with capacity of 500 mL (d).

This system aims to collect the maximum amount of solar radiation by regulating its inclination to concentrate the rays on the reflector, which then reflects them onto the distillation still. This method was used because of its simplicity and distillation efficiency.

For the extraction of the EO, 50 grams of dry plant material were placed onto the grid. The distillation still was filled with 400 mL of water under the grid level. The water was heated during the experiments until it reached the boiling point. The steam was passed through the plant, charged with essential oil, and moved into the

condenser for phase change. After 6 h of distillation, the oil and water mix was collected in a Florentine flask. Then, the EO was dried over anhydrous sodium sulfate and then stored at 4 °C in hermetically sealed amber bottles.

Yield (Y %) was calculated using the following formula:

$$\text{Yield \%} = (m_o / m_f) \times 100.$$

Where m_o and m_f are the masses of the extracted oils and the CS, both are expressed in the same unit (g).

Essential oil's physical properties

The density was determined gravimetrically using a one-milliliter pycnometer at 20 °C, and the refractive index was determined using an Abbe refractometer at 20±1 °C (ISO, 1998).

Antimicrobial activity

The antimicrobial effect of EOCS was assessed by the agar well-diffusion test (CLSI, 2012).

Biological Material

We used one reference strain of the yeast *Candida albicans* ATCC 14053 (CA), three Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 (PA), *Klebsiella pneumoniae* ATCC 13883 (KP), and *Escherichia coli* ATCC 2592 (EC), as well as one Gram-positive bacterium, *Staphylococcus aureus* ATCC 25923 (SA). All studied strains are pathogenic to humans, resulting in a multitude of severe illnesses.

Inoculum Preparation

Bacterial cultures were cultivated on an agar medium and incubated at 37 °C for 24 h. Then, they were mixed with a solution containing 9 % NaCl, adjusting their opacity to 0.5 McFarland turbidity (108 CFU.mL⁻¹).

Well Diffusion Assay

In this experiment, Petri plates were used as containers for Sabouraud dextrose agar medium, supplemented with 2 % glucose for yeasts and Mueller Hinton agar for bacteria. The plates were inoculated with a suspension of 10⁶ cells.mL⁻¹ derived from a young culture of yeast or bacteria using an aseptic swab. After the plates dried, the agar was perforated in the central region using the upper section of a Pasteur pipette (the cavity diameter was 6 mm). These wells were then filled with 60 µL of EO at various dilutions: 1/3, 1/4, 1/8, and 1/12, after 24 h incubation at 37 °C for bacteria and 48 h at 37 °C for yeast. The test was conducted in triplicate.

Determining the sensitivity

The inhibitory action was observed through the formation of a distinct halo region around the hole. The antimicrobial activity was assessed by measuring the inhibitory zone in millimeters. These were assessed following the parameters suggested by Ponce *et al.* (2003):

- Non-sensitive (-) or less sensitive: diameter of 8 mm;
- Sensitive (+): diameter between 9-14 mm;
- Very sensitive (++): diameters between 15-19 mm;
- Extremely sensitive (+++): diameters >20 mm.

Statistical analysis

The results of the antimicrobial activity of EOCS were subjected to a two-factor analysis of variance (ANOVA2) using XLSTAT.2019.2.2. The statistical analysis was carried out to evaluate the effect of different essential oil dilutions on the tested microorganisms, using a comparison against the diameters of the zones of inhibition taken as variables. We used Fisher's least significant difference (LSD) test in this context. The significance level was set at $p < 0.05$.

Results and discussion

The extraction yield

The EOCS obtained by the SD method is liquid, yellow-golden, and penetrating with a strong odor. It was obtained with a yield of $0.97\% \pm 0.13$, which is similar to that reported by Sawadogo *et al.* (2022) at 0.95 ± 0.15 , and lower than those cited by various researchers ranging between 1.11 % and 4.45 % (Khadri *et al.*, 2008; Naima *et al.*, 2016; Yagi *et al.*, 2016; Pavlović *et al.*, 2017; Bellik *et al.*, 2019; Aous *et al.*, 2019; Malti *et al.*, 2020). Other studies reported by Yen and Lin (2017), Al-Hilphy *et al.* (2022), and Nannaware *et al.* (2022) showed that conventional and solar extractions provide comparable yields with insignificant deviation. This contrasts with the findings of Munir *et al.* (2014), who documented variations in both the quantity and quality of essential oil. However, the solar extraction method showed a better yield compared to the conventional method.

This variability of results for the same plant species can be attributed to various factors, such as the genetics and physiology of the species (Sellami *et al.*, 2009; Kpoviessi *et al.*, 2014), the geographical origin of the plant (Halla *et al.*, 2020), environmental conditions (Verma *et al.*, 2013; Mc Gaw and Skeene, 2021), and extraction methods (Sellami *et al.*, 2009; Bellik *et al.*, 2019). Notably, the radiation intensity can also affect the solar extraction yield, which depends on geographical location, weather conditions, regional characteristics, and other parameters (Al-Hilphy *et al.*, 2022).

Physical constants

The evaluation of the physical properties of EOCS extracted by SD revealed a density of 0.8490 and a refractive index of 1.4850, which aligned with the standard norms of ISO and those reported by Katiki *et al.* (2011), Mahboub *et al.* (2019), and Boukhalfa *et al.* (2023). These studies reported that density varied from 0.8575 to 0.9266, while the refractive index ranged from 1.4820 to 1.4890. On the other hand, the research of Bellik *et al.* (2019) and Al-Hilphy *et al.* (2022) on the impact of various techniques on the physical properties of EO from different plants revealed slight variations, which may be due to alterations in the chemical composition of the EO.

However, the presence of water in oil reduces the refractive index of EO due to the natural properties of water that promote oil refraction (Nannaware *et al.*, 2022).

Antimicrobial activity of EOCS

The results of the antibacterial activity of the EOCS, tested against five pathogenic germs, are presented in figure 3 and table 1.

Antimicrobial activity results reveal a highly significant difference between the microbial strains tested. Therefore, the EOCS inhibited the growth of Gram-negative bacteria (EC and KP) at dilutions ranging from 1/3 to 1/12, showing a zone of inhibition varying from 9 to 15 mm and 7 to 12 mm, respectively. Note that both EC and KP recorded the highest diameter inhibition zones at the 1/3 concentration, measuring 15 mm and 12 mm, respectively. This is comparable with the results of numerous researchers who observed inhibition zones ranging from 8 mm to 14 mm for KP (Bellik *et al.*, 2019; Malti *et al.*, 2020) and from 10 mm to 19 mm for EC (Naima *et al.*, 2016; Hashim *et al.*, 2017; Aous *et al.*, 2019; Malti *et al.*, 2020). Conversely, Bellik *et al.* (2019) showed that the EC was the most resistant strain, with no zone of inhibition noted.

Figure 3. Antimicrobial activity of essential oil against strains. a: *E. coli*, **b:** *K. pneumoniae*, **c:** *P. aeruginosa*, **d:** *S. aureus*, **e:** *C. albicans*.

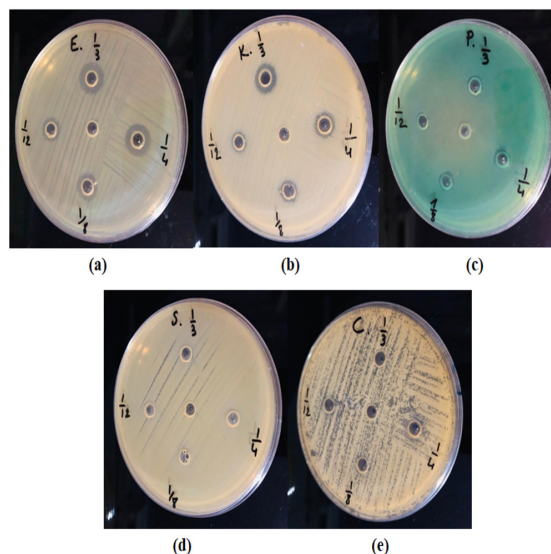


Table 1. Diameter of colony inhibition of microorganisms treated with the different dilutions of *Cymbopogon schoenanthus* essential oil.

Strain tested	Types of microorganisms	Diameter of inhibition zone (mm)			
		1/3	1/4	1/8	1/12
<i>E. coli</i> ATCC 25922(A)	Gram-	15 (++)	13 (+)	10 (+)	9 (+)
<i>K. pneumoniae</i> ATCC 13883 (B)	Gram-	12 (+)	9 (+)	7 (+)	7 (+)
<i>P. aeruginosa</i> ATCC 27853(C)	Gram-	NA (-)	NA (-)	NA (-)	NA (-)
<i>S. aureus</i> ATCC 25932(C)	Gram+	NA (-)	NA (-)	NA (-)	NA (-)
<i>C. albicans</i> ATCC 14053 (C)	Yeast	NA (-)	NA (-)	NA (-)	NA (-)

*NA = No activity; Inhibition zone diameter from 9-14 mm: (+); from 15-19 mm: (++); >20 mm (+++); <6 mm: (-) (Ponce *et al.*, 2003), $R^2 = 0.998$

Although the antibacterial activity decreases as the essential oil concentration decreases, KP shows the lowest value at 1/8 and 1/12 (7 mm). At the 1/12 concentration, the essential oil exhibits less antibacterial activity against EC (9 mm). This is consistent with the research conducted by Boutabia *et al.* (2016) on rosemary EO, indicating that this influence could be due to the diffusion of the oil's components by high dilution.

The other strains resisted EOCS at all dilutions, their diameters measured less than 6 mm. This is inconsistent with studies where inhibition zones varied from 12 mm to 38 mm for CA (Naima *et al.*, 2016; Aous *et al.*, 2019; Malti *et al.*, 2020), 11 mm to 32 mm for SA (Yagi *et al.*, 2016; Naima *et al.*, 2016; Hashim *et al.*, 2017; Bellik *et al.*, 2019; Aous *et al.*, 2019; Malti *et al.*, 2020), and between 8 mm and 7 mm for PA (Yagi *et al.*, 2016; Malti *et al.*, 2020). However, Hashim *et al.* (2017) observed PA as the most resistant strain, and no zone of inhibition was observed. This agrees with our findings.

Reichling *et al.* (2009) concluded that the oil had bacteriostatic properties, effectively inhibiting the growth of SA at relatively high concentrations. Additionally, the oil caused inefficiencies in metabolism at lower doses due to energy waste in the form of heat.

The observed variation in resistance levels between Gram-negative and Gram-positive bacteria is because of the complex action of EO on microorganisms, which is associated with the differentiation of their hydrophilic or lipophilic characteristics, the structure of their outer membranes, and the composition of the microbial cells. Generally, the entry of EO into these bacteria inhibits the formation of DNA, RNA, proteins, and polysaccharides (Kalemba and Kunicka, 2003). Furthermore, it disrupts their different layers, leading to increased permeability and the depletion of essential cellular components (Bakkali *et al.*, 2008), ultimately causing the death of microorganisms.

Additionally, the selectivity of EO for specific microorganisms can vary based on several factors, including the pathogen studied (Dorman and Deans, 2000), the EO used (Deans and Ritchie, 1987), the concentration of the oil (Boutabia *et al.*, 2016), and the extraction method (Wojdylo *et al.*, 2007). Furthermore, the absence of antimicrobial activity for certain strains may be explained by the lack of specific components in the composition of EO that demonstrate effectiveness against these strains (Bellik *et al.*, 2019; Malti *et al.*, 2020).

The differences in the plant organ, the origin of the plant, the type of bacteria, and the extraction technique of EOCS could influence its antibacterial activity.

Consequently, the EOCS extracted by the SD method exhibits antimicrobial activity against certain studied pathogens, making it a viable alternative natural agent in the cosmetics, food, and pharmaceutical industries.

Conclusion

In summary, this paper indicates that the EOCS obtained from Timiaouine, Algeria, and extracted using the SD technique was found to have an antibacterial effect against the gram-positive bacteria EC and KP. This is a positive point for its potential domestication. Therefore, additional research is necessary to evaluate the possible impact of this oil as a natural agent against various microorganisms. It is also necessary to study the chemical composition using both conventional and SD methods.

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