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Bioactive compounds and biological activities of *Eucalyptus cladocalyx* Muell. leaves grown in Bainem forest of Algiers

[Compuestos bioactivos y actividades biológicas de las hojas de *Eucalyptus cladocalyx* Muell. cultivadas en el bosque de Bainem de Argel]

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Abstract: This study aimed to evaluate the phytochemical composition, antioxidant properties, and antibacterial activities of the essential oil and extracts from *Eucalyptus cladocalyx* using leaves collected from the Baïnem forest. Essential oil was extracted via hydrodistillation with a Clevenger apparatus, and the methanolic extract was prepared using a rotary evaporator. Prior to analysis, 0.1 µL of the essential oil was diluted in 1:10 hexane solution for GC-MS analysis, which identified 30 constituents, with 1,8-cineole and p-cymene as the primary components. The antioxidant activity assessment demonstrated that the methanolic extract effectively neutralized DPPH, achieving 55% inhibition at a concentration of 500 µg/mL. Furthermore, the essential oil exhibited excellent antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella oxytoca* as tested using the aromatogram method, while the methanolic extract showed notable antibacterial activity specifically against *Klebsiella oxytoca*. These findings suggest that the essential oil of *E. cladocalyx* has potential applications as a disinfectant in the pharmaceutical industry, while the extracts may serve as a food additive.

Keywords: Antibacterial activity; Antioxidant activity; Essential oil; Extracts; GC-MS

Resumen: Este estudio tuvo como objetivo evaluar la composición fitoquímica, las propiedades antioxidantes y las actividades antibacterianas del aceite esencial y los extractos de *Eucalyptus cladocalyx* utilizando hojas recolectadas del bosque de Baïnem. El aceite esencial se extrajo mediante hidrodestilación con un aparato Clevenger, y el extracto metanólico se preparó utilizando un evaporador rotatorio. Antes del análisis, se diluyó 0.1 µL del aceite esencial en una solución de hexano 1:10 para el análisis por GC-MS, que identificó 30 constituyentes, siendo 1,8-cineol y p-cimeno los componentes principales. La evaluación de la actividad antioxidante demostró que el extracto metanólico neutralizó eficazmente el DPPH, logrando un 55% de inhibición a una concentración de 500 µg/mL. Además, el aceite esencial mostró excelente actividad antibacteriana contra *Staphylococcus aureus*, *Bacillus subtilis* y *Klebsiella oxytoca* según se probó mediante el método del aromatograma, mientras que el extracto metanólico mostró una notable actividad antibacteriana específicamente contra *Klebsiella oxytoca*. Estos hallazgos sugieren que el aceite esencial de *E. cladocalyx* tiene aplicaciones potenciales como desinfectante en la industria farmacéutica, mientras que los extractos pueden servir como aditivos alimentarios.

Palabras clave: Actividad antibacteriana; Actividad antioxidante; Aceite esencial; Extractos; GC-MS.

INTRODUCTION

Eucalyptus, a genus native to Australia, was first introduced to Algeria in 1856 by Ramel with the aim of sanitizing swampy areas (Ali-Dellile, 2013). Around 800 species of the *Eucalyptus* genus have been acclimatized globally, with approximatively 500 species commonly used in the industry. The widespread use of these species can be attributed to their richness in bioactive compounds, particularly 1,8-cineole (Goldbeck *et al*., 2014), along with other compounds such as α-pinene (Chamali *et al*., 2022; Grewal *et al*., 2022), (-)-spathulenol, o-cymene and (+-)-cryptone (Bourakna *et al*., 2022).

Beyond essential oil components, *Eucalyptus* species are also known to contain significant amounts of polyphenols and tannins, particularly in organic extracts (Ben Hassine *et al*., 2022). Their diverse phytochemical profile makes *Eucalyptus* a valuable resource in various fields, including pharmacology, timber wood, and agriculture (Salem *et al*., 2015). Recent studies have focused on the composition and biological activities of Eucalyptus essential oils (Umereweneza *et al*., 2019; Boukhalfoun *et al*., 2020), and extracts (Jerbi *et al*., 2017; Ben Hassine *et al*., 2022; Ouldkiar *et al*., 2023).

Among these species, *Eucalyptus cladocalyx* commonly known as sugar gum and native to South Australia, is widely used in traditional medicine. Its essential oil is recognized for its ability to relieve colds and flu (Bruneton, 2009). Moreover, *Eucalyptus* leaf extracts have long been used to treat influenza, respiratory issues, and skin rashes. The vapor from the leaves is also inhaled to fight inflammation (Damjanovic-Vratnica *et al*., 2011).

The essential oil yield of *Eucalyptus cladocalyx* varies significantly depending on the region, ranging from 0.3 to 5.2% (Farah *et al*., 2002; Elaissi *et al*., 2021). Several studies have explored the chemical composition of *E. cladocalyx* leaves, with monoterpenes being the primary constituents (Foudil-Cherif *et al*., 2000). Research by Elaissi *et al*. (2011), found that essential oil contained high levels of p-cymene, a-terpineol and borneol. Additionally, Fouad *et al*. (2015), identified spathulenol and 1,8-cineole as the major components of *E. cladocalyx* essential oil, followed by p-cymene.

Oxygenated monoterpenes such as 1,8-cineole and a-terpineol have been reported to possess strong antimicrobial properties against range of pathogens (Bakkali *et al*., 2008). *E. cladocalyx* essential oil has demonstrated promising antibacterial activity, particularly against Escherichia coli, and showed the lowest MIC value against H. influenza (Elaissi *et al*., 2021).

Despite its recognized antimicrobial activity, limited information is available regarding its antioxidant properties, particularly in solvent extracts. However recent research by Koursaoui *et al*. (2023), revealed that *E. cladocalyx* essential oil exhibits a strong DPPH radical scavenging activity, with an inhibition percentage of 80.46% at high concentration (160 mg/mL), The aim of the present work is to (i) identify the constituents of Eucalyptus cladocalyx essential oil grown in north Algeria and to (ii) evaluate its biological activities using both essential oil and solvent extracts.

MATERIAL AND METHODS

Plant material and environmental conditions

Fresh leaves of *Eucalyptus cladocalyx* were collected in July 2018 at the flowering stage from the forest of Bainem (Algiers, Algeria), located in a sub-humid bioclimatic zone at an altitude of 50 m (36°45'37" N 3°25'55"E). The region is characterized by moderate cold, wet winters and hot, dry summers. Annual rainfall averages around 650 mm, primarily occurring during the winter. Summer temperatures can reach up to 30°C, while in winter, they drop to 8°C. In July, there was no rainfall, and the temperature was balanced from 24.6 to 28.3°C. The soil in Bainem forest is predominately sandy-loamy, providing good drainage and ranging from moderately acidic to neutral (National Institute of forestry research of Bainem, Algiers). The collected plant material was air-dried in the shade at room temperature for two weeks. A voucher specimen has been placed in the herbarium of National Institute of Forestry Research of Bainem (Algiers, Algeria) following the standards of the Australian National Herbarium (**https://www.anbg.gov.au/cpbr/herbarium**).

Essential oil extraction and solvent extracts preparation

Essential oil was extracted through hydrodistillation using a Clevenger apparatus for 3 hours, repeated three times (3 x 200 g). The oil was dried over anhydrous sodium sulfate and stored at 4°C. The essential oil yield was calculated based on dry weight.

Dried leaves were reduced to a fine powder, and 50 g of *Eucalyptus cladocalyx* powder was macerated in 166.66 mL of methanol and distilled water for extract preparation. The mixtures were stirred for 4 days, filtered, and concentrated at 65°C

using rotary evaporator. Extracts were stored in the dark at 4°C.

Chemical composition of essential oil by GC-MS

The essential oil was analyzed on a Hewlett-Packard gas chromatograph with an HP5MS column and coupled to a quadrupole mass spectrometer. Key parameters included an injector temperature of 300°C, oven temperature ramping from 50°C, and a helium carrier gas flow rate of 1 mL/min. The components of the essential oil were identified by matching their mass spectra with those in the NIST and Willy libraries and confirmed by comparison of their retention time (Adams, 2007). Quantification of different constituents, expressed in percentage, was done by peak area normalization measurements.

Determination of total phenolics and hydrolyzable tannins

Total phenolic content in methanolic and aqueous extracts of *E. cladocalyx* was determined according to the Folin-Ciocalteu procedure (Skerget *et al*., 2005). The results are expressed in mg of gallic acid equivalent per g of dry matter (mg EAG/g). While the content of hydrolyzable tannins was measured using $FeCl_3$ reagent according to the method described by Price *et al*. (1978). The results were expressed in mg equivalent of tannic acid per g of dry matter (mgEAT/g of extract).

DPPH scavenging capacity assay

The antioxidant activity of the extracts was evaluated by the DPPH method described by Sánchez-Moreno *et al*. (1998), measuring the ability of extracts to neutralize DPPH radicals. The IC_{50} was calculated, indicating the concentration needed for 50% of inhibition.

Evaluation of antibacterial activity

The antibacterial activity was evaluated following the method of agar disk diffusion as reported by Abdelli *et al*. (2016), against 5 ATCC strains, including *Staphylococcus aureus* ATCC29213, *Enterococcus feacalis* ATCC29212, *Bacillus subtilis* ATCC6633, *Listeria innocua* ATCC74915, *Escherichia coli* ATCC25922 and 4 strains clinically isolated from the urine of patients such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aerugenosa*. Sterile white discs (ϕ = 6 mm) were soaked in extracts or essential oil of *E. cladocalyx* and then deposited on the surface of the agar. Gentamicin (10 µg/mL) was used as a positive control. The preparation was incubated at 37°C for 24 h. The Minimum inhibitory concentration (MIC) was determined following the same steps that were shown in the evaluation of the antibacterial activity, by varying concentrations of the essential oil (1-8 mg/mL).

Data analysis

The data was subjected to ANOVA using Statistica software Version 8 (2007). The comparison of the means was done according to the Fisher test referring to the least significant difference (LSD). Results were considered statistically significant at $p<0.05$, highly significant at $p<0.01$, and very highly significant at $p<0.001$. All tests were repeated three times.

RESULTS

Yield and chemical composition of essential oil

The mean value of essential oil yield of the *Eucalyptus cladocalyx* was at 0.49%. The chemical composition analysis of *E. cladocalyx* essential oil identified 30 constituents in order of elution (Table No. 1), divided into five (5) biochemical classes. A rate of 98.2% of the constituents was identified, of which 66.1% are monoterpenes dominated by eucalyptol (19.0%), p-cymene (18.0%), β-pinene (14.3%) and α-pinene (12.1%). Terpene alcohols (16.4%) are mainly represented by trans-P-mentha-1(7), 8-dien-2-ol (5.3%). The ketone compounds (8.7%) are constituted mostly of pinocarvone (4.2%), cryptone (3.9%). The sesquiterpenes represent 6.7% and are dominated by globulol, spathulenol and caryophyllene oxide. The aldehydic compounds (1.3%) are composed of myrtenal (0.7%) and (1R)-(-)-myrtenal (0.6%).

Total phenolics and hydrolyzable tannins content

The methanolic extract showed significantly higher total phenolic content ($p<0.05$), with 531 mg GAE/g compared to the aqueous extract, which contained 309 mg GAE/g. Similarly, the level of hydrolyzable tannins was 2.76 mg EAT/g in methanolic extract while the aqueous extract showed 1.6 mg EAT/g.

DPPH scavenging capacity assay

The antioxidant activity was notably different between extracts ($p=0.001$), with the methanolic extract exhibited a strong free radical-scavenging capacity, indicated by an IC_{50} = 340 µg/mL, which was lower than that of the aqueous extract, and significantly lower than ascorbic acid, which had an IC_{50} = 160 µg/mL (Figure No. 1). This would be due to the high content of phenolic compounds (531 mg GAE/g) in methanolic extract.

Table No. 1
Chemical composition of the essential oil of *E. cladocalyx*

N°	Class/Compounds	RI determined	RI reported	Content %
	Monoterpene hydrocabons			
1	α-thujene	937	930	0.3
2	α-pinène	941	939	12.1
3	Sabinéne	975	975	0.3
4	β-pinène	979	980	14.3
5	Myrcéne	992	990	0.3
6	β-phéllandrène	1003	1005	0.7
7	p-cyméne	1027	1026	18.0
9	Cis-ociméne	1038	1040	0.3
10	γ-terpinéne	1054	1059	0.4
	Oygenated monoterpenes			
8	Eucalyptol	1031	1033	19.0
11	Linalool	1086	1096	0.3
12	Trans pinocarvéol	1136	1139	0.5
13	Trans sabinol	1140	1140	0.7
14	Pinocarvone	1160	1162	4.2
15	Terpinen-4-ol	1174	1177	1.2
16	4-Terpineol	1178	1177	0.7
17	p-Cymene-8-ol	1182	1182	1.2
18	Cryptone	1185	1182	3.9
19	α-terpinéol	1186	1189	1.4
20	L-α-Terpinéol	1187	1189	1.0
21	Myrténal	1196	1196	5.3
22	(1R)-(-)-Myrtenal	1198	1198	0.7
23	Trans-P-mentha-1(7),8-dien-2-ol	1224	1229	0.6
24	Cis-P- mentha-1(7) ,8-dien-2-ol	1225	1230	1.3
25	Piperitone	1245	1245	0.5
26	Trans piperitol	1248	1248	2.3
	Sesquiterpenes			
27	Caryophylléne oxide	1555	1555	0.2
28	(-)-Spathulenol	1572	1572	0.6
29	Spathulenol	1577	1576	4.7
30	Globulol	1578	1578	1.2
	Monoterpene hydrocabons			46,7
	Oygenated monoterpenes			44,8%
	Sesquiterpenes			6,7%
	Total			98,2%

Antibacterial activity

When evaluating antibacterial activity, the methanolic extract showed very highly significant effects (p<0.001) against various bacterial strains, with inhibition zones ranging from 6 to 11.33 mm. The strongest inhibition was registered only in *K. oxytoca* at the concentration of 200 mg/mL. The aqueous extract also demonstrated highly significant differences (p=0.001), with maximum inhibition zone of 10.66 mm against *S. aureus* ATCC 29213 at the same concentration. In comparison, the positive control (Gentamicin) showed effective inhibition across all strains, with diameters ranging from 24.66 to 42.6 mm (Table No. 2A & 2B).

Figure No. 1
Antioxidant activity of methanolic and aqueous extracts of *Eucalyptus cladocalyx*. C1: 500 µg mL-1, C2: 250 µg mL-1, C3: 125 µg mL-1, C4: 62.5 µg mL-1, C5: 31.25 µg mL-1. F = 278.9, *p*=0.001

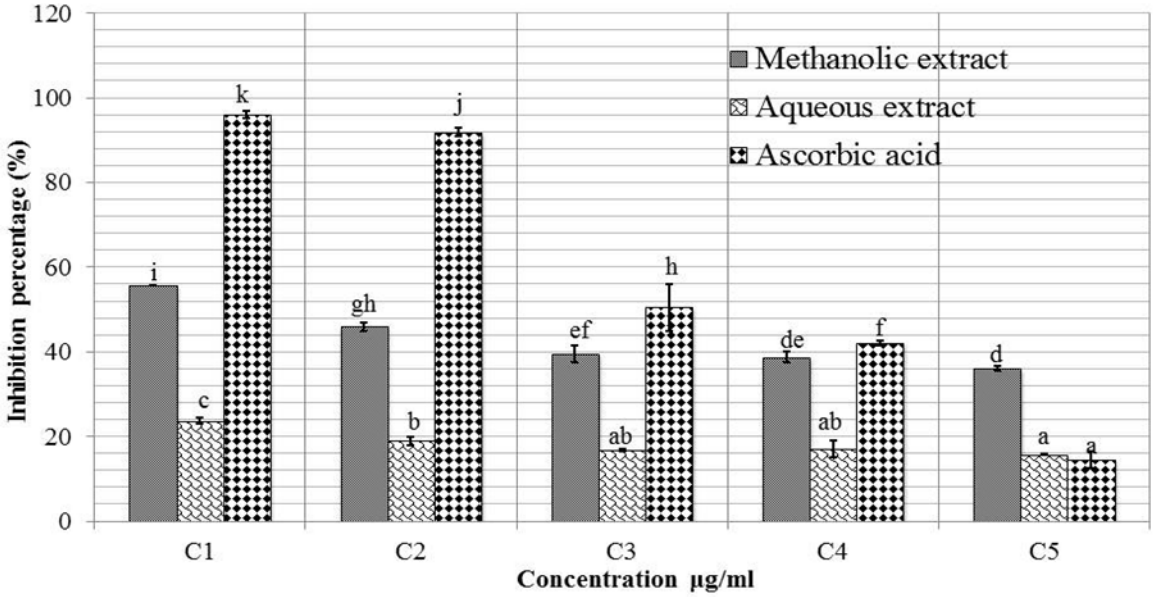

Table No. 2A
Antibacterial activity of essential oils and extracts of *Eucalyptus cladocalyx*; Inhibition zone (IZ) in diameter

		Strains				
		Gram-positive				
		Staphylococcus aureus	***Staphylococcus aureus* (isolated)**	***Bacillus subtilis***	***Enterococcus feacalis***	***Listeria innocua***
Extracts	**Dose**					
Methanolic extract	C1	$6.67^{a} \pm 0.66$	$10.00^{a} \pm 1.00$	$10.33^{a} \pm 0.88$	$8.00^{a} \pm 0.00$	$6.33^{a} \pm 0.33$
	C2	$8.67^{a} \pm 1.76$	$6.67^{a} \pm 0.66$	$6.33^{a} \pm 0.33$	$7.00^{a} \pm 0.00$	$6.00^{a} \pm 0,00$
	C3	$8.33^{a} \pm 1.85$	$6.33^{a} \pm 0.33$	$7.67^{a} \pm 0.88$	$6.00^{a} \pm 0.00$	$6.33^{a} \pm 0,33$
	C4	$9.33^{a} \pm 1.66$	$6.00^{a} \pm 0.00$	$7.33^{a} \pm 0.88$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$
	PC	$31.33^{ef} \pm 0.88$	$29.66^{de} \pm 3.71$	$37.00^{f} \pm 1.52$	$17.66^{bc} \pm 1.45$	$23.66^{cd} \pm 0.33$
Aqueous extract	C1	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$9.33^{ab} \pm 0.33$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$
	C2	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$
	C3	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.67^{a} \pm 0.33$	$6.00^{a} \pm 0.00$
	C4	$8.00^{ab} \pm 0.33$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$	$6.33^{a} \pm 0.33$	$6.00^{a} \pm 0.00$
	PC	$29.66^{d} \pm 0.33$	$28.00^{d} \pm 0.57$	$41.66^{e} \pm 1.66$	$24.00^{b} \pm 0.66$	$24.00^{b} \pm 0.57$
Essential oil	C1	$31.50^{gh} \pm 0.86$	$6.00^{a} \pm 0.00$	$32.33^{h} \pm 1.45$	$28.00^{fg} \pm 2.00$	$18.00^{bc} \pm 1.00$
	C2	$29.50^{fgh} \pm 0.28$	$6.00^{a} \pm 0.00$	$23.33^{cde} \pm 3.28$	$25.00^{de} \pm 0.57$	$20.33^{cd} \pm 0.33$
	C3	$27.00^{f} \pm 0.00$	$6.00^{a} \pm 0.00$	$25.67^{ef} \pm 1,45$	$25.00^{de} \pm 1.00$	$19.00^{bc} \pm 0.57$
	C4	$25.50^{ef} \pm 0.28$	$6.00^{a} \pm 0.00$	$25.00^{de} \pm 0.00$	$24.67^{de} \pm 0.66$	$18.33^{bc} \pm 0.88$
	PC	$28.66^{fgh} \pm 0.88$	$29.66^{fgh} \pm 0.33$	$33.33^{h} \pm 3.33$	$31.66^{gh} \pm 1.66$	$33.66^{h} \pm 1.20$

C1: 200 mg mL^{-1}**; C2**: 100 mg mL^{-1}; **C3**: 50 mg mL^{-1}; **C4**: 25 mg mL^{-1} (For aqueous and methanolic extracts); **C1**: 8 mg/mL; **C2:** 4 mg mL^{-1}**; C3**: 2 mg mL^{-1}; **D4:** 1 mg mL^{-1} (For essential oil). PC: Positive Control. Mean values showing different letters (a,ab,b,c…) are significantly different at $p<0.001$ for all extracts as well as the essential oil

Table No. 2B

Strains					
Gram negative					
		Escherichia coli	***Escherichia coli* (isolated)**	***Klebsiella oxytoca* (isolated)**	***Pseudomonas aerugenosa* (isolated)**
Extracts	**Dose**				
Methanolic extract	C1	$9.00^{a} \pm 0.00$	$9.33^{a} \pm 0.33$	$11.33^{ab} \pm 0.88$	$8.67^{a} \pm 0.88$
	C2	$9.00^{a} \pm 0.00$	$9.67^{a} \pm 0.88$	$10.33^{a} \pm 0.33$	$7.67^{a} \pm 0.88$
	C3	$10.00^{a} \pm 0.00$	$10.67^{a} \pm 0.66$	$9.33^{a} \pm 0.88$	$6.67^{a} \pm 0.66$
	C4	$9.00^{a} \pm 0.00$	$8.67^{a} \pm 0.88$	$9.33^{a} \pm 1.33$	$6.00^{a} \pm 0.00$
	PC	$28.00^{de} \pm 1.52$	$26.33^{de} \pm 0.66$	$31.00^{ef} \pm 2.30$	$25.00^{de} \pm 2.51$
Aqueous extract	C1	$9.33^{ab} \pm 1.20$	$8.00^{ab} \pm 0.57$	$7.33^{ab} \pm 0.88$	$6.00^{a} \pm 0.00$
	C2	$6.00^{a} \pm 0.00$	$7.33^{ab} \pm 0.33$	$6.67^{a} \pm 0.66$	$6.33^{a} \pm 0.33$
	C3	$6.00^{a} \pm 0.00$	$6.67^{a} \pm 0.66$	$6.00^{a} \pm 0.00$	$6.00^{a} \pm 0.00$
	C4	$6.00^{a} \pm 0.00$	$6.67^{a} \pm 0.33$	$6.33^{a} \pm 0.33$	$6.00^{a} \pm 0.00$
	PC	$27.66^{cd} \pm 1.45$	$24.33^{bc} \pm 2.18$	$29.00^{d} \pm 0.57$	$27.00^{c} \pm 0.00$
Essential oil	C1	$28.00^{fg} \pm 1.52$	$6.00^{a} \pm 0.00$	$29.00^{fgh} \pm 1.52$	$16.50^{bc} \pm 2.02$
	C2	$28.00^{fg} \pm 1.00$	$6.00^{a} \pm 0.00$	$26.00^{ef} \pm 1.52$	$11.50^{ab} \pm 0.86$
	C3	$24.00^{de} \pm 0.57$	$6.00^{a} \pm 0.00$	$24.33^{de} \pm 1.66$	$8.33^{a} \pm 2.33$
	C4	$24.00^{de} \pm 0.57$	$6.00^{a} \pm 0.00$	$25.67^{ef} \pm 1.20$	$7.33^{a} \pm 1.33$
	PC	$31.33^{fgh} \pm 3.52$	$31.33^{fgh} \pm 0.33$	$30.66^{fgh} \pm 2.33$	$42.33^{e} \pm 1.85$

C1: 200 mg mL^{-1}**; C2**: 100 mg mL^{-1}; **C3**: 50 mg mL^{-1}; **C4**: 25 mg mL^{-1} (For aqueous and methanolic extracts); **C1**: 8 mg/mL; **C2:** 4 mg mL^{-1}**; C3**: 2 mg mL^{-1}; **C4:** 1 mg mL^{-1} (For essential oil). PC: Positive Control. Mean values showing different letters (a,ab,b,c…) are significantly different at $p<0.001$ for all extracts as well as the essential oil

The essential oil *E. cladocalyx* revealed highly significant antibacterial effects (p=0.001, Table No. 2A & 2B), with isolated *E. coli* and isolated *S. aureus* revealing the lowest zone of inhibition of 6 mm at all concentrations tested. The highest inhibition was recorded against *B. subtilis* at 8 mg/mL, recording an inhibition zone of 32.33 mm, followed by *S. aureus* ATCC29213 at the same concentration (31.5 mm). The minimum inhibitory concentration (MIC) values of the essential oil ranged from 0.007 to 0.06 mg/mL, with *P. aerugenosa* showing the highest MIC. In contrast, various bacterial species such as: *E. coli* ATCC 25922, *S. aureus* ATCC29213, *K. oxytoca*, *L. innocua* and *B. subtilis* had the lowest MIC values.

DISCUSSION

The essential oil yield of *Eucalyptus cladocalyx* varies depending on the region. in the current research, a low yield was obtained, which aligns with the finding of Farah *et al*. (2002). However, Elaissi *et al*. (2021), found higher yield, reaching up to 5%. This variability suggests that environmental factors, such as climate and soil conditions, may play a significant role. Multiple studies have been reported on the chemical composition of the essential oils of other *Eucalyptus* species belonging to different regions, in which the variation in chemical composition is increasingly important. In fact, 1,8-cinéole, α-pinene were the major compounds (Boukhalfoun *et al*., 2020; Chamali *et al*., 2021; Elaissi *et al*., 2021; Grewal *et al*., 2022). These significant differences in terms of yield, and chemical composition of the essential oil could be attributed to the genetic potentialities within or between species, the stage of the plant's growth, the time of harvest (Boukhebti *et al*., 2011), the method of extraction (Chamali *et al*., 2021), time of harvesting, organ used (Bencheqroun *et al*., 2012), and season variation (De Oliveira *et al*., 2008).

The total phenolic content measured in the current work was higher than 148.68 mg GAE/g reported by Ashraf *et al*. (2015), for *E. camaldulensis* methanolic extract, reaching 531 mg GAE/g in this research. Furthermore, this amount is similar than that found by Jerbi *et al*. (2017), for *E. globulus* methanolic extract. Both of previous studies have shown that the total phenolic content in polar subfractions of methanolic extract was higher than nonpolar ones. In this context, the solubility of phenolic compounds depends mainly on the polarity of the solvent used for the extraction, the degree of polymerization of the molecules to be extracted, the length of the carbon chains, and the number and position of the hydroxyl groups (Illoki-Assanga *et al*., 2015). Moreover, factors such as genotype, part of plant, environmental conditions can affect the number of phenolic compounds.

The richness of hydrolyzable tannins in both *E. cladocalyx* extracts suggests the ability of this plant to play a major role as antimicrobial and antioxidant agents (Bruneton, 2009). Methanol was found to solubilizes hydrolyzable tannins more effectively than distilled water. Chavan *et al*. (2001), reported that the extraction of tannins depends on their chemical nature and the solvent used.

The antioxidant activity of methanolic extract of *E. cladocalyx* can be attributed to its high phenolic content (531 mg GAE/g), compared to the aqueous extract. In addition, the synergy between compounds may further enhance the antioxidant activity (Miguel, 2010). Lower IC_{50} values were found compared to other species (Ashraf *et al*., 2015; Illoki-Assanga *et al*., 2015; Jerbi *et al*., 2017). Considering the adverse effects associated with synthetic chemical compounds and the growing consumer demand for natural food additives and preservatives (Bearth *et al*., 2014), The food industry is increasingly focused on identifying natural antimicrobial and antioxidant agents, particularly those derived from plant. There is rising interest in extracting antioxidants from natural sources through multiple techniques, with numerous aimed at identifying compounds that can serve as effective antioxidants and replace synthetic food additives (Gachkar *et al*., 2007).

The antimicrobial sensitivity tests indicated that both methanolic and aqueous extracts of *E. cladocalyx* had inhibitory effects against all tested pathogenic microorganisms (Table No. 2A & 2B). The extracts showed a dose-dependent inhibition, in fact, the methanolic extract showed greater antibacterial activity than the aqueous extract. At the highest concentration (200 mg/mL), *K. oxytoca*, *E. coli* (isolated and ATCC), *B. subtilis* ATCC6633 and *S. aureus* ATCC29213, were most sensitive to inhibition by methanolic extract, while, *E. coli* ATCC and *B. subtilis* ATCC6633 were more inhibited by the aqueous extract. At lower concentrations, the aqueous extract showed no bacterial activity, whereas the methanolic extract maintained the same levels of activity compared to that at higher concentrations against specific strains such as *E. coli* (isolated and ATCC), *K. oxytoca*, and *S. aureus* ATCC29213. Notably, *E. feacalis* ATCC29212 and *L. innocua* ATCC74915 were the most resistant strains against both extracts, as well as the positive control. In comparison, Rahimi-Nasrabadi *et al*. (2012), reported modest antibacterial activity of *Eucalyptus procera* against *S. aureus*, *E. coli*, and *B. subtilis*. Conversely, Boukhalfoun *et al*. (2020), revealed a strong antibacterial effect of *Eucalyptus blakelyi* against *B. subtilis*, *E. feacalis* and *S. aureus*. Vaghasiya *et al*. (2008), found that the aqueous extract of *Eucalyptus citriodora* showed low antibacterial activity against *Staphylococcus subflava* and *Bacillus megaterium*, emphasizing that antimicrobial activity is related to the polarity of bioactive substances. Additionally, Sarker *et al*. (2005), suggested that the extract's effectiveness might stem from the synergy of its components, which can become inactive when isolated.

The essential oil of *E. cladocalyx* exhibited notable antibacterial activity against *B. subtilis* ATCC6633 and *S. aureus* ATCC29213 (32.33 mm and 31.5 mm, respectively). Interestingly, Gentamicin showed lower antibacterial efficacy than the essential oil against *S. aureus* ATCC29213 at both of concentration (8 and 4 mg/mL). At lower concentrations, the essential oil maintained a closer activity compared to that at higher concentrations against some strains, including *S. aureus* ATCC29213, *K. oxytoca*, *B. subtilis* ATCC6633, *E. feacalis* ATCC29212, and *E. coli* ATCC25922. Notably, the isolated strains such as: *E. coli*, *S. aureus*, and *P aerugenosa* were the most resistant strains. The strong antibacterial activity of *E. cladocalyx* essential oil may be attributed to the isolated or conjugated action of its bioactive molecules. The ineffectiveness of the essential oil against clinically isolated bacteria could be due to acquired resistance. GC-MS of the *E. cladocalyx* essential oil revealed a significant presence of monoterpenes known for their antimicrobial activities. This finding is in agreement with Grundy & Still (1985), who reported that cyclic monoterpenes could integrate into cell membrane,

leading to membrane expansion and increased fluidity, ultimately facilitating the efflux of intracellular components and causing microbial death. Additionally, minority compounds may interact in a synergistic, additive, or antagonistic manner (Bakkali *et al*., 2008; Wang *et al*., 2008). Similarly, Bardaweel *et al*. (2014), demonstrated that essential oil from *E. sargentii* exhibited strong activity against *B. subtilis* (21 mm) and *S. aureus* (18 mm), contrasting with *E. torquate*, which showed only modest antibacterial activity against the Gram-negative strains, including *S. aureus*, *S. epidermidi*, and *B. subtilis*. Overall, the essential oil of *E. cladocalyx* demonstrates antibacterial effect against both Gram-positive and Gram-negative bacteria across various bacterial families, showcasing a broad spectrum of action for this purpose. Dorman & Deans (2000), highlighted the independence of antibacterial activity from Gram classification, while Karaman *et al*. (2003), indicated that Gram-positive bacteria tend to be more sensitive to essential oils and plant extracts than Gram-negative bacteria. It should be noted that while isolated strains exhibited resistance to the essential oil, they remained sensitive to the methanolic extract, particularly at high doses.

CONCLUSION

Analysis of the essential oil by GC-MS reveals that *E. cladocalyx* is very rich in chemical compounds, especially in the monoterpenes class. The richness of the chemical compounds found in the essential oil could strengthen its activity. The antioxidant activity evaluated by the DPPH test shows that the methanolic and aqueous extracts of *E. cladocalyx* are effective at the concentration of 500 µg/mL. The results of bacterial activity reveal the effectiveness of the methanolic extract compared to the aqueous extract. Moreover, the essential oil of *E. cladocalyx* has a good antioxidant activity. The essential oil has a powerful antibacterial potential in the majority of its concentrations against almost all bacteria tested, reaching in most cases the effect of the Gentamicin used as a positive control. The results of the present study suggest that the essential oil of *E. cladocalyx* could be used as a disinfectant in the pharmaceutical industry; it would be interesting to target the compound(s) responsible for the antimicrobial effect. This involves the use of more efficient purification and identification techniques.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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