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Antimicrobial activity of *Rosmarinus eriocalyx* essential oil and polyphenols: An endemic medicinal plant from Algeria

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ABSTRACT

Objective: To evaluate the antimicrobial potency of *Rosmarinus eriocalyx* (*R. eriocalyx*) essential oil and total polyphenols against pathogenic microorganisms.
Methods: Antimicrobial activity of *R. eriocalyx* extracts was assessed by disc diffusion method and minimum inhibitory concentrations determination. Essential oil obtained from endemic rosemary by hydrodistillation was analysed by gas chromatograph/retention index and gas chromatograph-mass spectrometer.
Results: An interesting antimicrobial activity was shown by *R. eriocalyx* extracts. Polyphenols, constituted mainly by flavonoids, were the most effective extract with very low minimum inhibitory concentrations values, ranged between 0.06 and 8.00 mg/mL, while essential oil was less efficient. It should be noted that antimicrobial activities of both *R. eriocalyx* extracts were more directed against fungi and Gram-positive bacteria than Gram-negative ones, in which *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans* were the most sensitive strains. Concerning chemical composition of *R. eriocalyx* essential oil, camphor (37.8%), 1,8-cineole (17.4%), camphene (13.3%), and α-pinene (10.9%) were the major compounds.
Conclusions: The findings of the present study indicate that *R. eriocalyx* extracts possess significant bactericidal and fungicidal activities. Because of its richness in essential oil, and especially flavonoids, *R. eriocalyx* may be a source for effective and safe antimicrobial agents.

1. Introduction

Rosemary, *Rosmarinus officinalis* L. (*R. officinalis*), is a well-known medicinal plant for its various therapeutic properties[1], as well as its uses in traditional cuisine as an additive and flavor[2]. Several interesting biological activities were highlighted in rosemary, especially antimicrobial activity[3] and antioxidant activity[4], which were attributable to its terpenoids and polyphenols. Other medicinal properties were also found in rosemary extracts, such as anti-depressant effect[5], reducing cholesterol[6], anti-inflammatory activities[7], and even an activity against cancers[8]. These cited characteristics make rosemary an important plant for valuation, very required for pharmaceutical, cosmetic, and food industries[9], as source for bioactive molecules.

Botanically, there are three species belong to the *Rosmarinus* genus, which are *R. officinalis*, as well as two endemics, namely *Rosmarinus tomentosus* Hub.-Mor. & Maire, and *Rosmarinus eriocalyx* Jord. & Fourr. (*R. eriocalyx*)[10]. Previously known as *Rosmarinus tournefortii* (Noë ex Jord. & Fourr.) Jahand. & Maire, and described as exclusively endemic in Algeria[11], *R. eriocalyx* is actually defined as an endemic plant in North-West of Africa and Southern Spain[12]. *R. eriocalyx* epithet means woolly calyx, refers to its double hairy calyx, one short type and the other consists of long erect glandular hairs at the top[10,12]. In Algeria, *R. eriocalyx* growth wildly in the mountainous regions, in which it's a well reputed medicinal plant, widely used by local populations in treatments of several pathologies, including infectious diseases.

In recent decades, health care-associated infections, also known as nosocomial infections, are global problems which are responsible for high morbidity and mortality rate in hospitals[13,14]. The main cause of these intractable infections is due to the multidrug resistance of pathogenic microorganisms to the used antibiotics[15]. Against this serious problem, research for alternative antibiotics must be continued and all possible strategies should be explored[16]. Among the available solutions, plant secondary metabolites are promising and

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wealthy sources for new effective antimicrobials, which are without side effects[17], unlike most of presently used antibiotic.

Huge number of biomolecules are produced by plant organisms, substances which have several vital roles in their development, such as protection from ultraviolet radiation[18], and defences against parasites[19]. Through many studies published in the last years, researchers have find that among these molecules designated in plant immunity, some class of secondary metabolites possess a biocidal activity towards microorganisms. Essential oils (terpenoids) and polyphenols are ones of those substance made by plant metabolism, and serve as protection against pathogens[20], which explain their antimicrobial potency with interesting minimal inhibitory concentrations (MICs)[21].

The need of antimicrobials agents is not limited only to the human medicine, these bioactive biomolecules are in increasing demand in several domains, including food industries for foodstuffs preservation[22], and in dentistry[23]. So, there is a continuous requirement for the discovery of new antimicrobial agents. Amongst research areas, endemic plants appear as a probable reservoir for new bioactive molecules, even which are endowed with biocidal activity against pathogenic microorganisms.

In the same research, axis for highlight new substances provided with biocidal activity against photogenic microorganisms, this study aims to evaluate for the first time the antimicrobial potency of polyphenols and essential oil obtained from endemic rosemary, the *R. eriocalyx*.

2. Materials and methods

2.1. Plant material

Aerial parts of *R. eriocalyx* were collected in June 2012 during full inflorescence from mountainous station called Sebdou, which located 25 km south of Tlemcen (west of Algeria) at 1 000 m of altitude. Identification of the species was confirmed by the Laboratory of Ecological Management of Natural Ecosystems, University of Tlemcen. A voucher of specimen was deposited in Laboratory of Applied Microbiology in Food, Biomedical and Environment, University of Tlemcen under code RTS-BF150412. The plant material used for polyphenols extraction was washed and dried by spreading in open air and away from sun light for 10 days.

2.2. Phytochemical screening

In order to highlight the chemical composition of *R. eriocalyx*, phytochemical tests were performed in three solvent extracts, which were chloroform (Riedel de Haën®, Germany), methanol (Scharlau®, Spain) and distilled water. Extraction with these three solvents was carried out successively by a Soxhlet apparatus according to their degree of polarity. This screening involves the detection of different families existing in the plant. The tests were performed by precipitation reactions or staining using specific reagents to each family of compounds. Phytochemical screening was performed according to methods described by Harborne[24,25].

2.3. Obtaining the essential oil

Essential oil extraction was realised by hydrodistillation using Clevenger apparatus. The essential oil was recovered from fresh plant material as recommended by Benbelaïd *et al.*[26]. After four h of distillation, essential oil extraction gave a yield of 1.9% (v/w) of pale yellow extract. The obtained essential oil was recovered and dried using magnesium sulphate, then conserved at 4 ˚C away of light until analysis.

2.4. Chemical analysis of the essential oil

The chemical composition of *R. eriocalyx* essential oil was determined by gas chromatography/retention index (GC/RI) and gas chromatography coupled with mass spectrometry (GC/MS). For GC/RI analyses, which served to determinate the percentages of essential oil components, we have used Perkin Elmer Autosystem GC-type chromatograph, equipped with two flame ionization detectors, for the detection of volatile compounds, one injector/splitter, and two polar (Rtx-Wax, polyethylene glycol) and nonpolar (Rtx-1, polydimethylsiloxane) columns (60.00 m × 0.22 mm inner diameter, film thickness 0.25 µm). The carrier gas was helium (1 mL/min) with a column head pressure of 25 psi. The injector temperature was 250 ˚C and that of the detector was 280 ˚C. The temperature was programmed to increase from 60 to 230 ˚C at the rate of 2 ˚C/min, and then maintained constant for 45 min at a level of 230 ˚C. The injection was done by split mode with a split ratio of 1/50. The amount of essential oil injected was 0.2 µL. Quantification was made by direct electronic integration of peak areas.

For the GC/MS, analysis was performed using a Perkin Elmer Autosystem XL chromatograph coupled with a Perkin Elmer TurboMass mass detector. Chromatographic conditions were the same with GC/RI. While detection was carried out by a quadrupole analyzer which consisted of an assembly of four parallel electrodes with cylindrical section. The source temperature was 150 ˚C. The device functioned in electron impact and fragmentation was performed at an electric field of 70 eV. The resulting mass spectra were acquired over the mass range of 35–350 Da.

Identification of component of *R. eriocalyx* essential oil was carried by two methods, which are Kovats index[27] and comparison of mass spectra with those of the bibliography[28,29].

2.5. Extraction and fractionation of polyphenols

Total polyphenols extraction and fractionation was carried out according to Basli *et al.*[30] and Bencheraiet *et al.*[31]. A total of 100 g of *R. eriocalyx* dried powder was macerated in 70% methanol for 48 h at room temperature. After that, the extract was filtered with Whatman filter paper and evaporated to dryness using Büchi Rotavapor R110-type rotary evaporator (Büchi, Switzerland). For fractionation of the polyphenols fractions, residues obtained were recovered in hot water (50 ˚C) then separated by 1-butanol (Riedel de Haën®, Germany) and ethyl acetate (Prolabo®, France), successively. Then, the two fractions were dried using rotary evaporator. Residues obtained were recovered in dimethyl sulfoxide (Sigma–Aldrich®, Germany), sterilized by filtration trough syringe filter (0.2 µm) and conserved in dark at 4 ˚C until testing.

2.6. Flavonoids dosage

Total flavonoid contents were determined using the aluminium

chloride colorimetric method[32]. Mixtures were prepared by adding 0.5 mL of extracts (1-butanol or ethyl acetate fractions) to a solution containing 0.1 mL of aluminium chloride (10%), 0.1 mL potassium acetate (1 mol/L), and 4.3 mL of distilled water. After incubation at room temperature for 30 min, the content of flavonoids in mixture was determined by absorbance at 415 nm using a spectrophotometer. Catechin was used to make the calibration curve.

2.7. Microbial strains

In order to assess the antimicrobial potential of *R. eriocalyx* extracts, 10 reference strains provided by our laboratory were used in this study. These pathogenic species represent various infection sources, including fungal strains (yeast) which are *Candida albicans* (*C. albicans*) ATCC 10231 and *C. albicans* IPP 444. Whereas other strains were bacteria, three Gram-negative ones which are *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Salmonella montevideo* ATCC 3581 (*S. montevideo*), *Salmonella enteritidis* ATCC 2453 (*S. enteritidis*) and five Gram-positive species which are *Enterococcus faecalis* ATCC 29212 (*E. faecalis*), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus subtilis* ATCC 6633 (*B. subtilis*), *Bacillus cereus* ATCC 11778 (*B. cereus*), and *Listeria monocytogenes* ATCC 19115 (*L. monocytogenes*).

2.8. Antimicrobial assay

2.8.1. Preparation of inocula

Standardized suspension of each strain was used for microbiological tests. The preparation of inoculums was carried out from well purified cultures of each microbial species, in which isolated colonies were taken and inoculated into Mueller-Hinton broth (Fluka®, India) for bacteria or Sabouraud broth (Fluka®, India) for yeasts. After incubation at 37 ℃ for 24 h, the suspension standardizing was made by cultures dilution until 0.5 McFarland, which corresponds an optical density situated between 0.08 to 0.13 at 625 nm wavelengths. The inoculum final concentration was 10^8 CFU/mL[33].

2.8.2. Disc diffusion method

Preliminary evaluation of the antimicrobial activity was carried out by modified Kirby-Bauer's agar method[17,34]. In Petri dishes casted by solid medium, Mueller-Hinton agar (Fluka®, India) for bacteria or Sabouraud agar (Fluka®, India) for yeasts, and pre-inoculated by swabbing of standardized microbial suspension (10^8 CFU/mL), Whatman filter paper discs (6 mm diameters) impregnated with 10 µL of extracts were placed on the surface of agar. Essential oils were deposited directly, pure as they were, while polyphenols fractions were put at a concentration of 100 mg/mL, which corresponded to 1 mg per disc. Gentamicin (10 µg per disc) (Oxoid, England) and amphotericin B (100 µg per disc) (Cypress Diagnostics, Belgium) were used and served as positive controls for bacterial and fungal strains respectively. After incubation at 37 ℃ for 24 h, the results were read by measuring the diameter of inhibition zones in millimetres (mm) by vernier scale. All tests were performed in triplicate.

2.8.3. MIC determination

MICs of extracts were determined by broth micro-dilution method modified from Wiegand *et al*.[35]. For essential oil[34], 10 initial concentrations, which ranged between 400.0 and 0.8 (mg/mL), were prepared in sterilized tubes by ½ dilution in mixture medium/Tween 80 at (1%), Mueller–Hinton broth (Fluka®, India) for bacteria or Sabouraud broth (Fluka®, India). Then, in a 96-well microplate, 10 final concentrations were reached by combining 10 µL from each concentration with 90 µL of inoculum at 5×10^5 CFU/mL (prepared by 1/200 dilution of 10^8 CFU/mL inoculum), giving a final range of concentrations situated from 40.00 to 0.08 (mg/mL), and a final concentration of Tween 80 at 1% in each well. While for polyphenols[17], 10 initial concentrations were prepared similarly, excepting for the concentration fork was started from 80.00 to 0.15 (mg/mL), and we have used dimethyl sulfoxide at 10% instead of Tween 80 as emulsifier. So, in a 96-well microplate, the 10 final concentrations were defined between 8.000 and 0.015 (mg/mL).

After incubation of the microplates at 37 ℃ for 24 h, MICs were determined as the lowest concentration of the extract (essential oil or polyphenols) inhibiting visible growth. Furthermore, positive control was performed by using gentamicin and amphotericin B (Calbiochem®, Germany) for bacterial and fungal strains, respectively. All tests were performed in triplicate[36].

3. Results

3.1. Phytochemical screenings and flavonoids dosage

The phytochemical analyses have revealed high content of tannins, flavonoids, terpenoids and steroids in *R. eriocalyx* extracts. Contrariwise, several chemical families were found missing such as alkaloids, anthraquinones, xanthones, mucilages and saponins. All results are presented in Table 1. Regarding the dosage of flavonoids, it's observed that *R. eriocalyx* contains an acceptable quantity of such polyphenols (flavonoids), in which 1-butanol has extracted the high yield of flavonoids (more than 48 mg quercetin/g equivalent), while ethyl acetate fraction includes less flavonoids, which does not exceed 25 mg quercetin/g equivalent.

Table 1
Phytochemical screening of principal chemical families contained in *R. eriocalyx*.

Phytochemicals	Solvents		
	Chloroform	Methanol	Water
Alkaloids	nt	-	-
Anthocyanins	nt	-	-
Anthraquinones	-	-	-
Essentials oils	+	-	-
Flavonoids	nt	+	+
Mucilages	nt	nt	-
Saponins	-	-	-
Steroids	+	-	-
Tannins	nt	+	+
Terpenoids	+	-	-
Xanthones	nt	-	-

+: Presence; -: Absence; nt: Not tested (non-miscible reagents).

3.2. Chemical composition of the essential oil

Chemical composition of *R. eriocalyx* essential oil is presented in Table 2. It's found that *R. eriocalyx* essential oil is richly composed by oxygenated monoterpenes, in which camphor (37.8%) is the major component, followed by 1,8-cineol (17.4%), camphene

(13.3%), and α-pinene (10.9%), respectively. Other compounds exists in small quantity in essential oil, namely β-pinene (2.4%), borneol (2.3%), and *p*-cymene (1.9%), and the rest of oil constituents are presents in traces.

Table 2
Chemical composition of *R. eriocalyx* essential oil.

#	Component	nRI	pRI	%	ID
1	Tricylene	921	1020	0.5	RI, MS
2	α-Pinene	931	1022	10.9	RI, MS
3	Camphene	943	1066	13.3	RI, MS
4	β-Pinene	970	1110	2.4	RI, MS
5	Myrcene	979	1159	0.5	RI, MS
6	α-Phellandrene	997	1164	0.1	RI, MS
7	δ-3-Carene	1005	1147	0.5	RI, MS
8	*p*-Cymene	1011	1268	1.9	RI, MS
9	1,8-Cineole	1020	1209	17.4	RI, MS
10	γ-Terpinene	1047	1243	0.4	RI, MS
11	(Z)-Sabinene hydrate	1051	1451	0.1	RI, MS
12	Fenchone	1071	1401	0.2	RI, MS
13	Terpinolene	1078	1280	0.3	RI, MS
14	Linalool	1081	1544	0.3	RI, MS
15	Camphor	1123	1517	37.8	RI, MS
16	Borneol	1148	1698	2.3	RI, MS
17	Terpinen-4-ol	1161	1600	0.9	RI, MS
18	α-Terpineol	1179	1700	0.5	RI, MS
19	Bornyl acetate	1269	1515	0.1	RI, MS
20	Eugenol	1330	2171	0.7	RI, MS
21	α-Ylangene	1375	1476	0.2	RI, MS
22	Neryl acetone	1410	1825	0.4	RI, MS
23	Germacrene D	1478	1710	0.1	RI, MS
24	Cadinene D	1516	1725	0.4	RI, MS
25	Caryophyllene oxide	1576	1980	0.3	RI, MS
26	τ-Cadinol	1632	2169	0.1	RI, MS
27	α-Cadinol	1645	2231	0.7	RI, MS
28	α-Bisabolol	1672	2217	0.9	RI, MS
	Total identified (%)			94.2	
	Monoterpene hydrocarbons			30.5	
	Oxygenated monoterpenes			59.9	
	Sesquiterpene hydrocarbons			0.7	
	Oxygenated sesquiterpenes			2.4	
	Others			0.7	

Results are in percentage (%) of components for *R. eriocalyx* essential oil. Percentages and elution order of individual components are given on nonpolar column. Retention indices nRI and pRI are given respectively on nonpolar (Rtx-1) and polar (Rtx-Wax) columns. ID: Identification method by comparison of retention indices (RI) and mass spectra (MS).

3.3. Antimicrobial activity of extracts

Results of *R. eriocalyx* antimicrobial activity are shown in Table 3. In diffusion method, essential oil has shown a significant activity against studied pathogens, contrarily to polyphenol fractions, resulting in broad inhibition zones. *C. albicans* strains were the most sensitive microorganisms to the essential oil, with inhibition zones that exceeded 50 mm. Large inhibition zones are also noted against Gram-positive bacteria, especially *S. aureus* (40 mm), *B. cereus* (32 mm), and *B. subtilis* (27 mm). Regarding the rest of pathogens, *R. eriocalyx* essential oil has shown moderate antimicrobial activity in particular against Gram-negative bacteria. While *R. eriocalyx* polyphenol fractions showed less antimicrobial activitities, in which their inhibition zones against studied microorganism did not go beyond 18 mm. Such as essential oils, *C. albicans* (strains 1 and 2) was the most sensitive species to both polyphenol fractions with inhibition zones larger than 14 mm, followed by *B. cereus*, *S. aureus*, *B. subtilis*, and *E. faecalis*, respectively. Remaining species have been over at least more resistant towards *R. eriocalyx* polyphenols. It should also be noted that among polyphenol extracts, 1-butanol polyphenols fraction, was more effective than ethyl acetate.

Regarding quantitative analyses (MICs) of the antimicrobial activities of *R. eriocalyx* extracts, it's noticed that polyphenol fractions are more efficient than the essential oil as contrary to diffusion method in which their MICs are much smaller. In the other hand, there is an accord between the two methods that Gram-positive bacteria and yeasts are the most sensitive pathogens to essential oils and polyphenols. Among the results obtained by micro-dilution method, *E. faecalis* was the most sensitive bacterium to extracts, as well as the essential oil (10 mg/mL) and polyphenol fractions, 1-butanol (0.17 mg/mL) and ethyl acetate (0.25 mg/mL). Moreover, Gram-positive bacteria were likewise sensitive to essential oils (between 10 and 20 mg/mL) and polyphenol fractions (between 0.25 to 0.50 mg/mL). While Gram-negative bacteria appear the most resistant strains to *R. eriocalyx* extracts. From the fungal strains, *C. albicans* IPP 444 was the most sensitive strains in this study, especially toward polyphenols, which had the lowest MIC (0.06 mg/mL) in both fractions. At last, we found that 1-butanol polyphenols fraction was more effective than ethyl acetate fraction with remarkably low concentrations.

Table 3
Antimicrobial activities of *R. eriocalyx* essential oil and flavonoids fractions.

Strains	Essential oil		Polyphenols				Antibiotics			
			1-Butanol		Ethyl acetate		Gentamicin		Amphotericin B	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
B. cereus	32 ± 2	1.25 ± 0.00	15 ± 0	0.21 ± 0.07	12 ± 1	0.41 ± 0.11	21 ± 1	4.00 ± 0.00	na	na
B. subtilis	27 ± 1	1.25 ± 0.00	14 ± 1	0.25 ± 0.00	12 ± 0	0.50 ± 0.00	19 ± 1	2.00 ± 0.00	na	na
C. albicans	49 ± 1	1.25 ± 0.00	16 ± 1	0.13 ± 0.00	13 ± 0	0.33 ± 0.15	na	na	19 ± 1	1.00 ± 0.00
C. albicans	52 ± 2	0.83 ± 0.36	16 ± 2	0.06 ± 0.00	14 ± 1	0.06 ± 0.00	na	na	20 ± 1	1.00 ± 0.00
E. faecalis	14 ± 1	2.50 ± 0.00	14 ± 1	0.17 ± 0.07	10 ± 1	0.25 ± 0.00	20 ± 1	8.00 ± 0.00	na	na
L. monocytogenes	11 ± 1	10.0 ± 0.00	09 ± 1	1.00 ± 0.00	9 ± 1	1.67 ± 0.58	20 ± 1	2.00 ± 0.00	na	na
P. aeruginosa	8 ± 1	40.0 ± 0.00	08 ± 1	4.00 ± 0.00	8 ± 1	8.00 ± 0.00	18 ± 0	1.33 ± 0.58	na	na
S. enteritidis	9 ± 1	20.0 ± 0.00	09 ± 1	2.00 ± 0.00	8 ± 0	4.00 ± 0.00	20 ± 1	2.00 ± 0.00	na	na
S. montevideo	10 ± 1	20.0 ± 0.00	10 ± 1	2.00 ± 0.00	9 ± 1	4.00 ± 0.00	18 ± 1	2.67 ± 1.15	na	na
S. aureus	40 ± 1	0.63 ± 0.00	14 ± 1	0.25 ± 0.00	13 ± 1	0.50 ± 0.00	21 ± 2	0.25 ± 0.00	na	na

Values are expressed as mean ± SD. All tests were performed in triplicate. IZ: Inhibition zone; MIC: Minimal inhibition concentration; na: Not applicable.

4. Discussion

For the purpose of the identification of new alternative antimicrobial agents or products, which are in high demand for several areas, including food industries, pharmacy and human medicine, we have interested in the evaluation of antimicrobial activity of an endemic rosemary growth wild in Algeria. According to obtained results, *R. eriocalyx* extracts have shown excellent antimicrobial potency, especially polyphenol fractions, mainly when we compared finds obtained with those of other rosemary species in previous studies. In particular, 15 µL of *R. officinalis* essential oil, valuated by diffusion method, was less active on *L. monocytogenes* and *S. aureus* compared with 10 µL of *R. eriocalyx* essential oil[37]. In the same way, MICs of *R. officinalis* on *S. aureus* and *B. subtilis* was higher than those of *R. eriocalyx*[1], which means that Algerian rosemary is more powerful. Furthermore, solvents extracts of *R. officinalis* were also less antimicrobial active relative to *R. eriocalyx* polyphenols, for example the ethanol extract of rosemary evaluated by Oh *et al*.[38] was weakly effective on *L. monocytogenes* (minimum lethal concentration = 10 mg/mL), and in a study realised by Celiktas *et al*.[39], they found that *R. officinalis* methanol extract exhibited very low antimicrobial activity compared to its essential oil.

Concerning secondary metabolites contained in *R. eriocalyx*, and its relationship with antimicrobial activities, phytochemical screening has indicated that essential oils and polyphenols, especially flavonoids, are present in high percentage in Algerian rosemary, such as *R. officinalis*[40]. Based on GC and GC/MS analyses of *R. eriocalyx* essential oil, the major compound identified was an oxygenated monoterpene, camphor, which logically are the molecule responsible for the antimicrobial activity of essential oil, since biocidal activity of essential is typically attributable to major compound[41]. However, synergetic effect between components of essential oil against microorganisms was signalled in certain studies[42,43]. Thus, the effective antimicrobial activity showed by *R. eriocalyx* essential oil is portably attributable to the combination of its molecules, which signified that the use of essential oil as antimicrobial agent is butter than use of terpenoids alone, and signed also the importance of this endemic plant.

In the other hand, and among most important results obtained in this study, polyphenols extracted from *R. eriocalyx* by 1-butanol and ethyl acetate, which constituted principally by flavonoids, have demonstrated a strong antimicrobial activity against studied strains. Exceeded that of essential oil, the antimicrobial activity of *R. eriocalyx* polyphenols was very interesting with low MICs, in which 1-butanol fraction was the most active. The outweigh activity of 1-butanol fraction can be attributed to the high content of flavonoids. However, sugars play a very important role in the activity of antimicrobial agents[44], which may differentiate between flavonoids glycosides and aglycones. Flavonoid glycosides have a high affinity for alcohols such as 1-butanol than ethyl acetate[45], the outweigh activity of the 1-butanol fraction may also be explained due to the amount of flavonoid glycosides.

It should be mentioned that all *R. eriocalyx* extracts were effective against Gram-positive bacterial and fungal strains than Gram-negative ones. It is known that the difference between bacteria is personified in their cell-wall, in which Gram-negative species are characterised by a complex cell-wall constituted by double membranes contrary to Gram-positive which have just one membrane[46]. Therefore, the less sensitivity of Gram-negative bacteria to *R. eriocalyx* extracts, even to any plant antimicrobial, is attributable to their cell-wall. While *C. albicans* strains are most sensitive to *R. eriocalyx*, which is explained by the fact that fungi are the natural enemy of plants, where most of plant diseases are caused by fungus[47]. So, it is logical that the specificity of the antimicrobial activity of plants secondary metabolites, including essential oil and polyphenols, is significantly oriented against fungi than other parasites.

In summary, evaluation of *R. eriocalyx* extracts against pathogenic microorganisms has shown excellent results. Among secondary metabolites tested, essential oil and polyphenols obtained from Algerian rosemary possess a very interesting antimicrobial potency. Therefore, findings of this study indicated that endemic rosemary can be a source for antimicrobial agents, especially flavonoids, which may be used as a new pharmacologically acceptable antibiotics in the future[48]. Essential oil, composed by the combination of monoterpenes, has an interesting antimicrobial activity, which makes it a specific oil and interesting for evaluation.

Conflict of interest statement

We declare that we have no conflict of interest.

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