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Comparative study of chemical composition Antioxidant and Antimicrobial activity of Methanolic Extracts of *Mentha rotundifolia*

Estudio comparativo de la composición química, actividad antioxidante y antimicrobiana del extracto metanólicos de *Mentha rotundifolia*

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ABSTRACT

Mentha rotundifolia is a member of the Lamiaceae family and is distributed mainly in traditional medicine in Algeria and the Mediterranean locality. This study aimed to demonstrate how the extraction technique can determine the type of active ingredients obtained, as well as their potential pharmacological and therapeutic effects. The methanolic extracts were obtained by maceration and Soxhlet apparatus. The study of the chemical composition was determined by colourimetric methods and liquid chromatography–mass spectrometry (LC–MS/MS); the antioxidant activity was evaluated *in vitro* by three tests: DPPH test, ferrous ion chelating test and reducing power test. The antimicrobial effect of extracts was evaluated by agar diffusion assay against six microbial strains. The results demonstrate that the best yield of extraction, Total phenolic and flavonoid contents, and antioxidant activity were found to be higher in the extract obtained by Soxhlet than those obtained by maceration. The phytochemical screening tests identified polyphenols, flavonoids, tannins, terpenoids, and quinones, and anthraquinones and saponins were absent in both extracts. The LC–MS/MS revealed the presence of several phenolic compounds, the predominant in both extracts was rosmarinic acid. The antimicrobial activity shows that both extracts have no effect. In conclusion, this study reveals that extraction by Soxhlet is more suitable than extraction by maceration for this plant.

Key words: Antioxidant; extraction; LC/MS/MS**;** maceration; *Mentha rotundifolia*; Soxhlet

RESUMEN

Mentha rotundifolia es miembro de la familia Lamiaceae y se distribuye ampliamente en la medicina tradicional en Argelia y la localidad mediterránea. El objetivo de este estudio fue demostrar cómo la técnica de extracción puede determinar el tipo de principios activos obtenidos, así como sus potenciales efectos farmacológicos y terapéuticos. Los extractos metanólicos se obtuvieron mediante maceración y aparato Soxhlet. Se determinó el estudio de la composición química mediante métodos colorimétricos y espectrometría de masas por cromatografía líquida (LC–MS/MS). La actividad antioxidante se evaluó *in vitro* mediante tres pruebas: prueba de DPPH, prueba de quelación de iones ferrosos y prueba de poder reductor. El efecto antimicrobiano de los extractos se evaluó mediante un ensayo de difusión en agar contra seis cepas microbianas. Los resultados demuestran que el mejor rendimiento de extracción, el contenido de fenólicos y flavonoides totales, la actividad antioxidante fue mayor en los extractos obtenidos por Soxhlet que los obtenidos por maceración. Las pruebas de detección fitoquímica permitieron identificar polifenoles, flavonoides, taninos, terpenoides, quinonas y la ausencia de antraquininas y saponinas en ambos extractos. La LC–MS/MS reveló la presencia de varios compuestos fenólicos, el predominante en ambos extractos fue el ácido rosmarínico. La actividad antimicrobiana muestra que ambos extractos no tienen ningún efecto. En conclusión, este estudio revela que la extracción mediante Soxhlet es más adecuada que la extracción por maceración para esta planta.

Palabras clave: Antioxidante; extracción; LC/EM/EM; maceración; *Mentha rotundifolia*; Soxhlet

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INTRODUCTION

Due to its richness in secondary metabolites, plants can be considered a rich source of therapeutic compounds. Variation in extraction methods usually depends on the duration of the extraction period, the solvent used, the pH of the solvent, temperature, the particle size of the plant, and the solvent–to–sample ratio. The majority of extraction methods involve the separation of medicinally active compounds of plants from the inactive components by using selective solvents [1, 2].

The solubility of compounds in the solvent depends on their chemical natures, which vary for compounds of others; this structural diversity is responsible for diversity in molecules' physicochemical properties. For this reason, it is difficult to develop a universal method for extracting all effective compounds from plants [3].

Oxidative stress can be defined as the state in which the free radicals in our body outnumber our antioxidant defenses. An antioxidant is a molecule capable of inhibiting the oxidation of another molecule. Antioxidants break the free radical chain of reactions by sacrificing their own electrons to feed free radicals without becoming free radicals themselves [4]. Thus, it is essential to develop effective and natural antioxidants that can protect the body from free radicals and retard the progress of many chronic diseases [5].

Mentha rotundifolia is one of the Lamiaceae species that is widely distributed in Algeria. It is used as a condiment, and it has been applied in traditional medicine for a wide range of actions: stimulative, tonic, stomachic, carminative, analgesic, antispasmodic, anti–inflammatory, sedative, hypotensive and insecticidal [6]. This plant was also the subject of several scientific studies, which made it possible to determine its therapeutic effect as an antioxidant [7], anti–inflammatory [8], and antimicrobial [9]. The objective of this study was to investigate the effect of temperature in the extraction of phenolic compounds from *M. rotundifolia* and its antioxidant and antimicrobial activity.

MATERIAL AND METHODS

Plant materials

The plant *M. rotundifolia* was collected in the region of Djemila Wilaya of Setif (Algeria) during the period of plain Florissant. Professor Laouer H, a botanist in the laboratory of Botanical Sciences, Ferhat ABBES Setif–1 University, Algeria, carried out the botanical identity of the plant (family, genus, and species). The aerial parts were air–dried in the shade at room temperature away from humidity and then powdered by an electric grinder.

Extraction

Extraction by maceration

Twenty g of plant powder was put into a maceration bottle and filled with 96% methanol for 48 hours (h), with the solvent renewed after 24 h. The ratio between plant powder and ethanol was 1:10. Afterwards, the filtrate separated from the residue using Watman filter paper was collected and evaporated using a rotary evaporator (Rotavator, Büchi; Swiss) at 45°C until a dry extract was obtained.

Soxhlet extraction

Twenty g of aerial parts powder was packed in a Watman filter paper and placed in the Soxhlet extractor (Büchi; Swiss). Then, 200 ml of methanol was poured into the roundbottom flask. The solvent was heated using the dismantle, which began to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the plant extract. The process was made to run for a total of 6 h. Finally, the extract was collected, and the methanol was evaporated using a rotary evaporator (BUCHI rotavap Suiss) at 45°C. The extract was stored at room temperature for further use.

Phytochemical screening

Phytochemical tests were performed on methanolic extracts to verify the presence of some compounds (polyphenols, flavonoids, tannins, terpenoids, quinines, and saponins). Their detection is achieved using the methods described by Bagre *et al.*[10], Khaldi *et al.*[11], Vijayalakshmi *et al.* [12].

Determination of total phenolic compounds

The polyphenols in extracts was quantified using Folin–Ciocalteu reagent according to the method describedby Li *et al.*[13]. Briefly, an aliquot of 200 µL of the extract was mixed with 1 ml of Folin–Ciocalteu reagent for 4 min, followed by the addition of 800 µL of Na_2CO_3 aqueous solution (7,5%). The absorbance was measured (SECOMAN Spectrophotometer, French) at 765 nm after two hours of incubation. The polyphenol content was expressed as mg gallic acid equivalent (GAE)·g extract^{-1}.

Determination of total flavonoids

The total flavonoid content of each extract was determined by a colourimetric method as described by Kosalec *et al.* [14]. In brief, 1 ml of each extract was added to 1 ml of aluminum chloride ($AlCl_3$) methanolic solution (2%) and allowed to stand for 30 min, the absorbance of the mixture was measured at 430 nm. The total flavonoid content was reported as mg of quercetin equivalent (QE)·g extract^{-1}.

Identification and quantification of phenolic compounds in extracts using liquid Chromatography/ Mass Spectrometry (LC/MS/MS)

Methanol was used to dissolve the extracts and standard at a rate of 1.0 mg·mL^{-1}. For every analysis, a volume of 20 µL was continuously injected at a flow rate of 1.0 mL·min^{-1} onto an Agilent Zorbax 150 mm × 4.6 mm C18 column. A gradient solvent system was used for the study, with aqueous–formic acid (0.10%) serving as solvent (A) and acetonitrile (100%) serving as solvent (B). A 35–min run time was allocated to a five–step linear gradient elution, wherein solvent A was reduced to 10% and solvent B was increased to 90%.

A triple–quadrupole mass spectrometer (API 3200; MDS Sciex, Concord, ON, Canada) received the full flow from the high–performance liquid chromatography (HPLC). The mass spectrum information was collected in negative ion mode with a capillary voltage of 4500 V, an Electrospray Ionization (ESI) ion source, a cone voltage of 70 V, a collision energy of 35 eV, a drying temperature of 650°C, N_2 as the drying gas at a flow rate of 4.0 L·min^{-1}, and Analyst software version 6. The eluted samples and standards were found at 280 nm [7].

Antioxidant activity

Diphenyl–1–picrylhydrazyl (DPPH•) radical scavenging assay

All substances that can donate a hydrogen atom or an electron to DPPH can be considered antioxidants and, therefore, radical

scavengers. The degree of discolouration of the violet colour of DPPH as it gets reduced indicates the radical scavenging potential of the antioxidant [15]. The capacity of extracts to scavenge DPPH was determined following the method of Sarikurkcu *et al.* [16]. Briefly: 0.1 mL of extracts of different concentrations was mixed with 1 ml DPPH solution (0.008% in ethanol). The mixture was shaken and left to stand for 2 h at room temperature. The absorbance was measured at 515 nm. The scavenging activity of extracts was compared with that of Butylated hydroxytoluene (BHA) as standard. The capability to scavenge the DPPH• radical was calculated using the following equation:

$$I\% = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

Where A_0 is the absorbance of the control, and A_1 the absorbance of the sample.

Metal ion chelating assay

Transition metal species such as ferrous iron (Fe^{2+}) can facilitate the production of ROS within animal and human systems; the capacity of compounds to chelate iron can provide a valuable antioxidant capability [17]. The Metal ion chelating effect of the extract was estimated using the method reported by Le *et al.* [17]. Briefly, one ml of each extract at a different concentration was mixed with 1 ml of $FeCl_2$ (0.6 mM). After 5 min, the reaction was initiated by adding 1 ml ferrozine (5 mM). The tubes were vortexed and allowed to stand for 10 min at room temperature. Absorbance was measured at 562 nm. The ratio of ferrozine–Fe^{2+} complex formation inhibition was calculated as follows:

$$I\% = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

The EDTA was used as a positive control.

Reducing power assay

The plant extract components' reducing power might be a significant indicator of its potential antioxidant activity [18]. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [17]. The reducing power of extracts was assessed by the method described by Beyhan *et al.* [17], 1 ml of extracts was mixed with 2.5 ml phosphate buffer (pH=6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The mixture was incubated at 50°C for 20 min, and then 2.5 mL of 10% Trichloroacetic acid was added. After vigorous agitation, 2.5 mL of this solution was mixed with 2.5 mL of distilled water and 0.5 ml ferric chloride ($FeCl_3$) (0.1%). The absorbance was measured at 700 nm. The reducing power of the extracts was compared with that of Butylated Hydroxyanisole (BHA) as a positive control. Higher absorbance indicates higher reducing power.

Antimicrobial activity

The study of antimicrobial activity was performed by the method of agar diffusion against two Gram–negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, one gram–positive bacteria *Staphylococcus aureus* ATCC 25923 and three fungi (*Aspergillus niger* 2CA936, *Aspergillus flavus* NRRL391, *Candida albicans* ATCC1024). Sterilized Whatman paper discs of 6 mm diameter were impregnated with 20 µl of solution of extracts (200–500 mg·ml^{-1}), and the discs were then placed in Petri dishes previously inoculated by Muller–Hinton agar for bacteria, Potato Dextrose Agar (PDA) for fungus, and Sabouraud + Chloramphenicol for *Candida albicans*. Different standard antibiotics (according to the type of bacteria) (Gentamicin (GEN), Imipenem (IMP), Cefoxitin (CX), Pristinamycin (RP), Vancomycin (VA), Piperacillin (PRL), Ciprofloxacin (CIP), Clindamycin (CD)) and antifungal (Amphotericin B (AM), Chloramphenicol (CTR), Nystatin (NY)) were used as positive control.

The Petri dishes are incubated (Memmert ovens, Germany) at 37°C·24 h^{-1} for bacteria, at 37°C for 48 h for yeast, and at 27°C for 72 h for fungi. The negatif control was an impregnated disk DMSO (Dimethyl sulfoxide) alone. Different antibiotic and antifungal disks were used as positive control. Three repetitions were performed for each test [19].

Statistical analysis

The results were presented as the mean ± standard deviation (SD). Statistical data analysis was performed using the GraphPad Prism 5 program with the Dunnett test; the level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Phytochemical analyses

In the present study, methanolic extracts of *M. rotundifolia* were evaluated for their phytochemical screening, polyphenolic contents, antioxidant, and antimicrobial activity.The best extract yield was recorded by the Soxhlet apparatus with 16.05 % against 9.2 % for the maceration. The phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, quinones, and terpenoids. However, saponins and anthraquinones were absent in both extracts.

Amounts of total polyphenols and flavonoids

The contents of total polyphenols, and total flavonoids were determined in plant extracts by using Folin–Ciocalteu and $AlCl_3$ reagents respectively, the results are shown in TABLE I. As can be seen, the Soxhlet apparatus seems to be significantly ($P<0.05$) better than the maceration to extract total polyphenols and flavonoids.

TABLE I
Amounts of total phenolic compounds and flavonoids in *Mentha rotundifolia* extracts

Extract	Polyphenols [(a)]	Flavonoids [(b)]
Methanolic extract obtained by maceration (MEM)	141.571 ± 0.143	15.636 ± 0.030
Methanolic extract obtained by Soxhlet (MES)	168.642 ± 1.642	33.045 ± 0.76

[(a)]: Expressed as mg galic acid equivalent (GAE) per gram of extract, [(b)]: Expressed as mg quercitin equivalent (QE) per gram of extract

Identification and quantification of phenolic compounds using LC/MS/MS

The results of this study demonstrate that among the 24 standards used, 17 phenolic compounds were identified in MEM and 15 in MES. Among the phenolic compounds identified: phenolic acids (Vanilic acid, Rosmarinic acid, Ellagic acid, Syringic acid…), flavonols (Myrtillin, Quercetin, Rutin, Isoquercitin), flavone (Apigenin, Luteolin) and anthocyanins (Cyanin chloride).The predominant compound in both

extracts was Rosmaric Acid (18900 and 7690 ng·mL^{-1} for MEM and MES respectively)(TABLEII). Furthermore, the study showed that the amounts of the majority of phenolic compounds identified are higher in MEM than in MES. However, the quantities of syringic acid, vanillic acid, and trans-cinnamaldehyde were higher in MES.

The medicinal value of the plant is due to the phytochemical constituents they produce, which exhibit certain physiological actions on human body [20].The result of this study shows the presence of polyphenols, flavonoids, tannins, quinones, and terpenoids*M. rotundifolia* extracts. This diversity of compounds can lead to a diversity of plant therapeutic properties. The determination of the amount of total polyphenols and flavonoids demonstrate the richness of both extracts on this compounds with a predominance in the extract obtained by Soxhlet. In a study carried out by Boussouf *et al.* [8] on the leaves of the same plant from the northern Algeria, the methanolic extract obtained by maceration gave respective contents of polyphenols and flavonoids of 350.10±0.96 mg galic acid equivalent (GAE·g^{-1}) of extract and 79.44±0.76 mg quercitin equivalent (EQ·g^{-1}) of extract. These differences with our study may be due to several factors that can influence the contents and the nature of phenolic compounds in extracts, such as the region and period of the harvest of the plants, the part used, the time and the extraction temperature, the polarity of the solvent [1]. In order to identify the phenolic compounds of extracts, LC-MS/MS was carried out. Based on comparing their chromatographic profiles and retention times with those of the standards used, several phenolic compounds with therapeutic interest are identified. Rosmarinic acid was the major compound and has been the subject of numerous scientific studies demonstrating its therapeutic effects, such as antioxidant properties [21], anticancer [22], anti-inflammatory [23].

Antioxidant activity

DPPH radical scavenging assay

The IC50 for DPPH radical-scavenging activity reported in TABLE III demonstrates that the radical-scavenging activity of extracts increased with increasing concentration of extract. The methanolic extract obtained by the Soxhlet apparatus had shown better (P<0.05) scavenging activities than those obtained by maceration. However, the activity of BHA was much more marked (P<0.001) than extracts.

Determination of metal chelating activity

The results of this test also demonstrate that the extract obtained by maceration has a significantly smaller chelating

TABLE II
Phenolic profile of methanolic extracts obtained by maceration and Soxhlet, identified by LC- MS/MS

Compounds	C_1(ng·ml^{-1}) MEM	C_2 (ng·ml^{-1}) MES	RT	Q1	Q3	MWT
Apigenin	213.00	2.99	8.40	269.000	151.0	270.120
Isoquercitrin	57.60	nt	7.00	464.900	300.0	464.400
Catechol	nt	nt	5.50	109.000	109.0	110.110
Epicatechin	nt	nt	6.57	289.100	108.8	290.300
Gallic Acid	152.00	nt	1.53	169.000	124.6	170.120
Procyanidin B_2	nt	nt	6.40	577.100	407.0 / 289.0	578.520
Quercetin 3-O-Galactoside	328.00	60.00	7.00	463.000	301.0	464.379
Luteolin	275.00	118.00	8.00	285.000	217.0	286.240
Chlorogenic Acid	357.00	96.50	5.94	353.155	190.4 /84.8 / 93.1	354.310
Epigallocatechin Gallate	nt	nt	6.66	456.579	168.2 / 168.5 / 124.8	458.372
Cyanin Chlorid	304.00	203.00	8.00	286.198	133.4 /132.8 / 150.6	287.100
Myrtillin	65.40	8.62	7.01	462.178	299.8 / 270.8 / 254.7	500.800
Quercetin	nt	nt	8.01	300.604	150.4	302.200
Rutin	337.00	168.00	6.84	609.419	299.0 / 299.9 / 270.9	610.520
Caffiec Acid	156.00	95.10	6.50	178.465	134.2 / 106.4 / 89.1	18.160
Ellagic Acid	7.98	nt	8.01	300.703	149.7 / 150.6 / 149.9	302.197
Ferulic Acid	109.00	56.00	7.20	192.807	133.9 / 133.4 / 177.8	194.180
Hydroxybenzoic Acid	198.00	84.70	6.48	134.779	88.4 / 106.8 / 89.1	135.120
P-Cumaric Acid	178.00	153.00	7.06	162.756	118.8 / 118.1 / 92.7	164.160
Rosmaric Acid	18900.00	7690.00	7.38	358.319	160.7 / 161.0 / 132.7	360.320
Syringic Acid	116.00	126.00	6.48	196.718	120.4 / 120.7 / 152.3	198.170
Transcinamaldehyde Acid	nt	975.00	7.95	131.797	103.7 / 102.8 / 101.9	132.160
Vanilic Acid	206.00	221.00	6.38	166.660	107.8 / 151.2 / 151.7	168.150
Hypericin	nt	nt	10.53	503.000	405.0	504.450

Q1: compound molecular weight, Q3: fragment molecular weight, MWT: molecular weight, nt: not found

power capacity (IC50=3417 ± 0.011 µg·ml^{-1}) than the Soxhlet extract (IC50=2194 ± 0.038 µg·ml^{-1}) (TABLE III).

Reducing power

In this assay, The EC50 values indicated that the BHA showed a good reducing power (EC50= 89 ± 0.285 µg·ml^{-1}); the EC50 values of extracts demonstrate that the activity of the extract obtained by maceration is significantly lower (P<0.001) than of Soxhlet extract (TABLE III).

TABLE III
IC_{50} Values of *Mentha rotundifolia* Extracts and standard for DPPH Scavenging Activity, Metal chelating activity and EC_{50} for reducing power

Test	MES (µg·ml^{-1})	MEM (µg·ml^{-1})	BHA (µg·ml^{-1})	EDTA (µg·ml^{-1})
DPPH scavenging	133.160 ± 11.346	265.491 ± 2.221	20.701 ± 0.065	NT
Metal chelating activity	2194.000 ± 0.038	3417.000 ± 0.011	NT	11.100 ± 0.060
Reducing power	468.000 ± 1.000	550.325 ± 3.613	89.000 ± 0.285	NT

NT: not tested

Phenolic and flavonoid compounds are responsible for the antioxidant activity of plant materials [24]. Polyphenols' antioxidant activity was attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers, some of which show metal chelation properties [25, 26]. However, some plant polyphenols may generate reactive secondary radicals during the cycling process. Furthermore, phenoxyl radicals produced in the course of radical scavenging by some phenolic compounds are capable of oxidizing both proteins and lipids [2].

Due to the varied characteristics of phytochemicals, the antioxidant activity of plants could not be properly assessed using either one antioxidant assay method [27]. In the present study, three different assays were employed to determine the mode of action and compare the antioxidant properties of *M. rotundifolia* extracts.

The antioxidant results demonstrate a correlation between the amount of total polyphenols and flavonoids in extract and the antioxidant activity; a high antioxidant activity in all tests was observed in Soxhlet extract in comparison with those obtained by maceration. This correlation was observed by other researchers [28, 29, 30].But, for others, no correlation existed [31, 32].

No correlation was noted between levels of some phenolic compounds determined by HPLC/ MS–MS and antioxidant activity; this may be, explained by the presence of other compounds not identified in this study in soxhlet extract with a good antioxidant effect.

Antimicrobial activity

The antimicrobial activity of methanolic extracts was evaluated against two Gram–negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, one Gram–positive bacteria *Staphylococcus aureus* ATCC 25923 and three fungus (*Aspergillus niger* 2CA936, *Aspergillus flavus* NRRL391, *Candida albicans* ATCC1024). The results indicated that *M. rotundifolia* extracts have no significant antimicrobial activity against all microbial strains tested (TABLE IV).

Infectious diseases caused by pathogenic microorganisms affect millions of people worldwide [33]. This study did not report a significant antimicrobial effect.

According to Gulluce *et al.* [34], the methanolic extract of *Mentha longifolia* L. ssp. *longifolia* was inactive against 38 different microorganisms (bacteria, fungus, and yeast). Moreover, the essential oil showed a high activity against all microorganisms. Adiguzel *et al.* (2009) [35] studied the antimicrobial activity of other Lamiaceae species (*Nepta quataria*) against 40 different microorganisms (24 bacteria, 15 fungi, and yeast); the methanolic extract of this species was inactive against 28 microorganisms (*Pseudomonas aeroginosa* ATCC–9027, *Staphylococcus aureus* ATCC–29213, *Streptococcus pyogenes* ATCC–176…).

The inactivity of our extracts may be due to the absence of phenolic oligomers. According to Karou *et al.* (2005) [36], the mechanism of toxicity of polyphenols towards microorganisms is either through the deprivation of metal ions such as iron or by non–specific interactions such as the establishment of hydrogen bonds with cell wall proteins or enzymes. However, an important factor that governs the antimicrobial activity of polyphenols is their molecular weight; monomers are too small to establish enough hydrogen bonds, while high molecular weight polymers are too large to cross the bacterial cell wall. Therefore, the ideal molecular weight would be that of oligomers.

CONCLUSION

The results of this study reveal the riches of *M. rotundifolia* in polyphenols and flavonoids. The extract obtained by Soxhlet had the highest amount of total phenolic compounds and the best antioxidant activity compared to the extract obtained by maceration. The results of the antimicrobial assay show that both extracts have no antimicrobial activity. Further studies are needed to determine the toxicity and other biological properties of this plant.

TABLE IV
Antimicrobial activities of *Mentha rotundifolia* extracts against the bacterial and fungal strains tested

	Escherichia coli **ATCC 25922**	***Pseudomonas aeruginosa*** **ATCC 27853**	***Staphylococcus aureus*** **ATCC 25923**	***Aspergillus niger*** **2CA936**	*Aspergillus flavus* **NRRL391**	***Candida albicans*** **ATCC1024**
MEM 200 and 500 (µg·ml^{-1})	NI	NI	NI	NI	NI	NI
MES 200 and 500 (µg·ml^{-1})	NI	NI	NI	NI	NI	NI
	27 (CX)	34 (PRL)	30 (RP)	9 (AM)	8 (AM)	19 (AM)
Antibiotics	28 (GEN)	39 (CIP)	30.5 (CD)	11 (NY)	11 (NY)	21 (NY)
	37 (IMP)	38 (IMP)	16 (VA)	20(CTR)	24 (CTR)	33 (CTR)

NI: No inhibition zone observed around the discs (6 mm) impregnated with 20 µl of methanolic extracts.

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Conflict of interest

The authors declare no conflict of interest.

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