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Research Article

SCREENING OF PHYTOCHEMICAL, EVALUATION OF PHENOLIC CONTENT, ANTIBACTERIAL AND ANTIOXYDANT ACTIVITIES OF *EPHEDRA ALATA* FROM THE ALGERIAN SAHARA

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ABSTRACT. *Ephedra alata* is a medicinal plant that has a long history of usage in folk medicine for the treatment of several diseases such as bronchial asthma, digestive system disorders and cancer. The present study aimed to elucidate the chemical constituents of the crude methanolic and hydro-methanolic extracts of *Ephedra alata* aerial part and to assess their antibacterial and antioxidant activities. In this study, the percentage yield of crude methanolic and hydro-methanolic extracts was 12.92% and 15.01% respectively. Phytochemical screening investigations using standard method showed that *E. alata* aerial part extracts was rich in flavonoids, reducing sugars, phenols and tannins and quantitative analysis using spectrophotometric assay revealed that the methanolic extract had the highest phenols and flavonoids contents (214.92 mg AGE/g extract and 30.74 mg CE/g extract, respectively). The results of the antibacterial activity by the agar well diffusion and microdilution assays showed that the both extracts displayed antibacterial effect, especially on gram positive strains with *S. aureus* being the most sensitive. Antioxidant activity was examined by the DPPH radical scavenging method. The methanolic extract has a significant inhibitory activity (IC_{50}= 226.58 µg/mL). These findings revealed that *E. alata* extracts could be a natural source of antioxidants and antibacterials that can prevent several diseases.

Keywords: *Ephedra alata, phytochemical, antibacterial activity, antioxidant, crude extract.*

INTRODUCTION

Plant-based traditional medicine has been around for millennia. Today, this medicine is still widely used, especially in developing countries [1], not only for poverty and cultural reasons, but also for the ineffectiveness of several existing drugs and reduced side effects of medicinal herbs [2]. The chemical diversity of natural products from plants offers unlimited possibilities for new drug discovery [3]. These phytochemicals act as therapeutic agents in large number of diseases [4], where phenolic compounds being one of the most important phytochemical groups [5]. These compounds play a major role due to their pharmacological properties such as cardioprotective, anti-inflammatory, anticancer, anti-allergy, antiviral, vasodilatory and antimicrobial activities [6, 7], as well

as protective activity against oxidative damage diseases [3]. However, bacterial infections and the prevalence of resistance to certain antibiotics have become difficult to treat and we are increasingly seeing therapeutic dead ends. Faced with this problem, the search for new active substances has become a necessity. Algeria has an important richness and variety of flora estimated at about 3000 plant species of which 15% are endemic and belong to different botanical families [8]. Several of these plants of Algeria have been little studied, but have various pharmacological effects. This is the case of *Ephedra alata*.

E. alata (Alanda in Arabic) a perennial species belonging to the *Ephedraceae* family and order Gnetales, is a light green, densely branched, dioecious and small that grow to be about 50–100 cm tall, has a strong pine odor and astringent taste[1]. This ephedra species is endemic to Iran, Palestine, Saudi Arabia, Algeria, Morocco, Egypt, Tunisia, Libya, Lebanon, Jordan and Iraq, and grows profusely in arid conditions on gravely rocky, sandy, and clay soil, frequently near shifting sand dunes [5]. *E. alata* is one of the herbs used in traditional medicine, particularly its stems decoction, for the treatment of so many disorders such as kidney, bronchial asthma, circular system, digestive system disorders, cancer, fungal and bacterial infection [9, 10]. Local populations in Algeria use *E. alata* to treat influenza, colds and respiratory disorders mainly in herbal tea [13]. Some researches *in vivo* and *in vitro* about different extracts from this plant revealed several biological activities depend on the phytoconstituents such as antibacterial, anti-inflammatory, antioxidant, antiobesity, antidiabitic, antiviral and anticancer effects [2]. Danciu et al. [12] reported that the 70% ethanol extract of *E. alata* present anticancer effect in breast cancer cells line (MCF-7), as well as antimicrobial and antioxidant activities. Moreover, the methanolic fraction of this plant present anti-obesity and antioxidant activities [1]. Furthermore, it has also shown anti-diabetogenic effects *in vitro* and *in vivo* [13]. Health beneficial effects of *E. alata* plant extracts are related to its richness in chemical active compounds, like phenolic acids, tannins, flavonoids, cardiac glycoside, alkaloids, reducing sugars and saponins [14].

To date, the available literature does not show any reference to previous work about the phytochemical contents of *E. alata* that grows in Southwestern of Algerian sahara desert. Therefore, the present work aims to investigate the phytochemical composition of the methanolic and hydro-methanolic aerial part extracts of *E. alata*. Additionally, the antibacterial and antioxidant properties of the both extracts were also evaluated.

MATERIALS AND METHODS

Plant Material and Extracts Preparation

Fresh *E. alata aerial parts* were harvested during December 2018, in a field located from Southwestern of Algeria (Bechar) (30°50’27’’N, 1°59’53’’W), at the altitude of 593 m. Identification were performed by the botanist Prof. Belhaçaini Hachimi, at the Microbiology and Plant Biology Laboratory at Abdelhamid Ibn Badis University-Mostaganem, Algeria (voucher series: A.h a ONA 2018). The plant materials were dried for 21 days then pulverized to obtain a fine powder and preserved at room temperature from light [8].

20 g of the dried, grounded aerial part were macerated separately in 200 mL of methanol and methanol-water at 7:3 (V/V) for 72 h in a shaker device at room temperature. Each extract was filtered and concentrated in rotary evaporator at 60°C. All

crude extracts were conserved at 4 °C until use. Yield of extracts was calculated as the following formula:

$$\% \text{ Yield} = (\text{Weight of } E.\ alata \text{ extract/weight of dry } E.\ alata) \times 100\%$$

Phytochemical Screening

The presence of major chemical constituents in the *E. alata* extracts was performed using standard procedures as described by Pandey and Tripathi [15]. The presences of flavonoids were tested by hydrochloric acid and magnesium turnings. Ferric chloride was needed to search for phenolic compound and tannins. Acetic anhydride and sulfuric acid were used to test the sterols and triterpenoïdes. The presence of persistent foam indicates the presence of saponins. Characterization of alkaloids was performed by Dragendorff. Ferric chloride and sulfuric acid was used to search for glycosides. Fehling's reagent allowed the characterization of reducing compounds.

Determination of Total phenolic Contents (TPC)

The concentration of phenolic content in the extracts was assessed using the Folin-Ciocalteu Reagent (FCR) method [16]. Briefly, the extracts were diluted to a concentration of 1 mg/ml, then 0.5 mL of extract or standard acid gallic solution (0.025–0.125 mg/mL) are mixed with 2.5 mL FCR (10%). After 3 min at 25 °C, 2mL Na_2CO_3 (7.5%) was added and mixed well, the whole was left for 2 h at room temperature before recording its absorbance at 765 nm. The calibration curve of acid gallic standard was plotted and the total phenolic content was presented as mg acid gallic equivalents (AGE)/g extract.

Determination of Total Flavonoid Contents (TFC)

The content of total flavonoid compounds was evaluated using the aluminum chloride ($AlCl_3$) colorimetric assay [17]. Briefly, 50 µL of each diluted extract (1 mg/mL in methanol) or standard catechin solution (0.2–1 mg/mL), 1250 µL distilled water and 75 µL 5% $NaNO_2$ solution was mixed. After 6 min of reaction time, 150 µL of freshly prepared $AlCl_3$ solution (10%) was added and the mixture was stood for 5 min. Afterward, 500 µL NaOH solution (1M) was combined. The absorbance of this preparation was measured at 510 nm. The calibration curve of catechin standard was plotted and the total flavonoid content was presented as mg catechin equivalents (CE)/g extract.

Antibacterial Activity

Antibacterial effect of the ME and HME was evaluated by using *B. cereus* ATCC 10876, *S. aureus* ATCC 25923, *E. feacalis* ATCC 29212, *S. typhi* ATCC 14028, *E. coli* ATCC 29522, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, and *K. pneumoniae* isolate bacterial strains obtained from the Algerian Pasteur institute except *Klebsiella pneumoniae* isolate which was obtained from the Laboratory of bacteriology (Bedj Sisters Hospital, chlef-Algeria), this Isolate strain was identified by morphological and biochemical tests, followed by antibiotic sensitivity assays.

The agar well diffusion assay described by Ahmad and Beg [18] was used to study the antibacterial activity. In brief, 100 µL of each bacterial suspension (10^8 CFU/mL) were spread on to Mueller Hinton Agar (MHA) surface and wells (6 mm) were punched

aseptically in plates. Then, 50 µL of the test extracts at concentrations of 50 mg/mL were poured into the wells. Similarly, 10% DMSO was prepared as negative controls to dissolved the latex extracts while Tobramycine (10µg), Ampicillin (30 µg) and Doxycycline (30µg) served as positives controls. All the petri dishes treated were then putted at 4°C for 2 h before incubating overnight at 37°C. The diameter zone of inhibition (DIZ) was measured and given in millimeters (mm).

The minimum inhibitory concentration (MIC) was performed according to the microdilution method, using a 96-well microtitration plate [19]. 100µL of the ME and HME at different concentrations (0.78 to 50 mg/mL) and 5µl of the bacterial suspension to final concentrations of 5×10^5 CFU/mL were added to 95 µL of MH broth in each well of the plate. The Positive growth control wells consisted of bacteria only in their adequate medium while 10% DMSO served as the negative control. The plates were then incubated for 18 h at 37 °C. The lowest concentration that inhibits 90% of microorganism form growing was recorded as the MIC. The minimum bactericidal concentrations (MBC) were determined by streaked 50 µL aliquots from the clear wells with no visible growth after MIC assay, on agar plates and incubated for 18h at 37 °C. MBC was determined as the lowest concentration of extracts that exhibited no bacterial growth.

Antioxidant Activity

The procedure described by Thaiponga et al. [20] was followed to evaluate the free radical scavenging effect of *E. alata* extracts on DPPH radical. Volumes of 150 µL of extracts at various dilutions (0.0156-0.5 mg/mL) were mixed with 2850 µL DPPH working solution (24 mg/100 mL in methanol was diluted to obtain an absorbance of 1.1 ± 0.02 at 515 nm) for 30 min. The absorbance was then recorded at 515 nm. Ascorbic acid (0.003–0.12 mg/mL) and Trolox (0.006–0.2 mg/mL) were used as standards at the same conditions. The % inhibition of the extracts was calculated based on the following formula:

$$\% \text{ DPPH inhibition} = [(A_{contol} - A_{sample})/A_{control}] \times 100.$$

The concentrations of samples providing 50% of antioxidant effect (IC_{50}) were determined using the graphs of antioxidant effect percentages against samples concentrations [5].

Data Analysis

Data analysis was assessed using one-way ANOVA by Tukey test in SPSS statistics (v. 25.0 program). All assays were evaluated in triplicates. The results are presented as means ± standard deviation (SD) and differences are considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction Yield

The extraction yield of the methanol (ME) and hydro-methanol (HME) extracts obtained for the aeriel parts of *E. alata* are given in Table 1.

The highest yield was observed with the HME (15.01%) compared to the ME (12.92%). The difference in yield between the two extracts can be attributed to the extraction capacity of each solvent, each of which is capable of extracting well-defined

phytochemicals existing in *Ephedra alata* aerial part. The yield of the ME and HME of *E. alata* is lower than those found by Jaradat et al [1] in the different extracts of the fruits of *E. alata* collected in the Jenin area of Palestine, which are of the order of 29.0% in the methanolic extract. However, it is difficult to compare our findings with those of the literature, because the yield of extract varies depending on the extraction method and conditions, the solvent and the organ used, the geographical origin, the conditions and duration of storage, as well as the period of harvest [21].

Table 1. *Yield, total polyphenols (TPC) and flavonoids (TFC) content of aerial parts extracts of E. alata*

***E. alata* aeriel part extracts**	**Yield %**	**TPC (mg AGE/ g extract)±SD**	**TFC (mg CE/ g extract) ±SD**
ME	12.92^{a}	214.92 ± 0.01^{a}	30.74 ± 0.008^{a}
HME	15.01^{b}	157.54 ± 0.05^{b}	21.86 ± 0.002^{b}

Results expressed are means ± SD (n=3). The letters (a and b) indicate a significant difference in the same column according to the Tukey test ($p<0.05$).

Phytochemical Screening

In this work, the qualitative phytochemical analysis of the ME and HME of the aerial part of *E. alata* showed the presence of phenols, reducing sugars, flavonoids and tannins in abundance while alkaloids, triterpenoïdes and steroids were scarcely present. The search for saponins and glycosides were revealed negative in both extracts (Table 2). The phytochemical study carried out on the *E. alata* plant showed results which are confirmed with other works, namely the presence of certain chemical families. On the other hand, we note that there is absence of other chemical families. This can be explained by a difference at the level of several parameters either geographical, physicochemical or biological parameters such as: the difference of the site of harvesting including the environment, light, precipitation, topography, season, soil type, harvesting period, genetic heritage the extraction procedure used and the part of the plant studied [22].

Table 2. *Phytochemical screening test of E. alata aerial parts*

Phytochemicals	**Reagent**	**ME**	**HME**
Flavonoids	Mg, HCl	+	++
Phenols	$FeCl_3$ 2%	+++	+++
Tannins	$FeCl_3$ 1%	++	++
Steroids	$(CH_3CO)_2O$, H_2SO_4	+	-
Triterpenoïdes	H_2SO_4	-	+
Saponins	H_2O (mousse)	-	-
Alkaloids	Dragendorff	+	+
Glycosides	H_2SO_4, $FeCl_3$	-	-
Reducing sugars	Fehling	++	++

-: not detected; +: minimum content; ++: moderate content; +++: maximum content.

Determination of TPC and TFC

The contents of total phenols and flavonoids are determined from the equations of the linear regression of each calibration curve: $y = 7.598x$ ($R^2 = 0.998$) and $y = 4.391x$ ($R^2 = 0.99$), respectively. Results obtained for the ME and HME are summarizes in table 1.

Variable contents are found in the both plant extracts studied. The highest concentration of phenols (214.92 mg AGE/g extract) and flavonoids (30.74 mg CE/g extract) was measured in the ME, significantly different from HME ($p<0.05$) (Table1). The TPC are higher than those found by Jaradat et al. [12] in methanolic extract from the aerial part of Palestinian *E. alata* (47.62 mg AGE/g extract). Another study shows that the contents of total phenols in hexane, Ethyl acetate and ethanol extracts of Tunisian *E. alata* is 0.15, 58.47 and 164.58 mg AGE/g extract, respectively [23]. The work of Danciu et al. [16] performed on a hydroalcoholic extract of the same species revealed almost similar amounts of total phenols (156.226 mg AGE/g extract) compared to our HEM (157.54 mg AGE/g extract). More recently, work carried out on *E. alata* fruits reveals the presence of a higher amount of total flavonoids (98.95 mg RUE/g extract) than ours [1], in another work, the TPC and TFC found in the aerial parts is lower than our results (125.73 mg AGE/g and 27.89 mg QE/g extract, respectively) [2]. It is difficult to compare our findings with this work, because the use of different plant organ, different extraction method, different extraction solvents, and calibration curves (cathechin, quercetin, rutin) reduce the reliability of the comparison between studies.

Antibacterial Activity

The antibacterial activity of the ME and HME of E. *alata* is done by the well diffusion and the microdilution methods. The obtained data are given as DIZ (Table 3), MICs and MBCs (Table 4). ME and HME demonstrated a dose-dependent antibacterial effect against selected pathogenic bacteria. Based on diameters of the inhibition zone values, both extracts were effective on gram-negative and especially on gram-positive bacterial strains (Table 3, Fig. 1). The MIC and MBC were 12.5 mg/mL and 50.0 mg/mL, respectively, For *E. coli*. In fact, the results showed that ME was effective, especially on *S. aureus* (MIC= 4.17 mg/mL, MBC= 12.5 mg/mL). According to a research developed by Danciu et al. [12]**,** the hydroalcoholic extract for the Tunisia *E. alata aerial parts* showed a bactericidal activity, particularly against *S. aureus* and *E. feacalis* as Gram (+) bacteria, as well as against Candida spp with low concentration of MIC and MBC. Furthermore, the antibacterial effects obtained for the methanolic extract of *E. alata* are highest than ethylic and hexanic extracts [24]. Also, Ziani et al. [5] reported that the methicillin resistant and susceptible *S. aureus* (MRSA and MSSA), *E. coli* ESBL and *E. coli* strains were the most sensitive to the hydroethanolic extract of *E. alata* collected from Algeria. Recently, Dbeibia et al. [2] showed the significant activity of *E. alata* extracts against *S. aureus*.

Table 3. *Diameter of inhibition (mm±SD) of E.alata extract*

Bacteria	Extracts (50 mg/ml)		Positive Control		
	ME	HME	TOB	AMP	DXT
E. coli ATCC	11.00 ± 1.00^{a}	8.50 ± 0.87^{a}	17.60 ± 0.17^{a}	-	-
S. typhi ATCC	$10.00\pm1.31^{a'b}$	$8.00\pm0.73^{a'b}$	$18.67\pm0.76^{a'b}$	-	-
K. pneumoniae ATCC	$8.00\pm1.00^{b'c}$	7.10 ± 0.29^{b}	$19.83\pm1.04^{b'c}$	-	-
K. pneumoniae isolate	7.57 ± 0.81^{c}	7.07 ± 0.51^{b}	$00.00^{c'd}$	-	-
P. aeruginosa ATCC	12.08 ± 0.49^{d}	13.03 ± 1.70^{c}	21.97 ± 1.67^{d}	-	-
S. aureus ATCC	17.00 ± 1.73^{d}	16.03 ± 1.00^{c}	20.50 ± 0.50^{d}	-	-
B. cereus ATCC	15.50 ± 1.80^{e}	8.33 ± 0.58^{d}	33.53 ± 1.27^{e}	25.33±0.76	-
E. feacalis ATCC	11.55 ± 0.64^{e}	8.70 ± 0.72^{d}	13.67 ± 0.46^{e}	-	35.00±0.50

Results expressed are means ± SD (n=3). The letters (a–e) indicate a significant difference in the same column according to the Tukey test ($p<0.05$)

The high content of phenolic compounds in the aerial part extracts of *E. alata* could explain the observed bioactivities. These compounds are known as the most dynamic substances, and they are responsible for the biological properties of a wide range of plant species. Currently, medicinal herbs and its phenolic substances has become an effective alternative with particularly low toxic effects for Biological control of pathogens [2].

Table 4. *Minimum inhibitory and bactericidal concentration values of E. alata extracts*

Bacteria	MIC (mg/mL)		MBC (mg/mL)	
	ME	**HME**	**ME**	**HME**
E. coli ATCC	12.5±0.0	50.0±0.0	50.0±0.0	>50
S. typhi ATCC	50.0±0.0	50.0±0.0	>50	>50
K. pneumoniae ATCC	25.0±0.0	>50	>50	>50
K. pneumoniae isolate	50.0±0.0	50.0±0.0	>50	>50
P. aeruginosa ATCC	8.33±3.61	50.0±0.0	>50	>50
S. aureus ATCC	4.17±1.80	25.0±0.0	12.5±0.0	50.0±0.0
B. cereus ATCC	8.33±3.61	>50	>50	>50
E. feacalis ATCC	25.0±0.0	>50	>50	>50

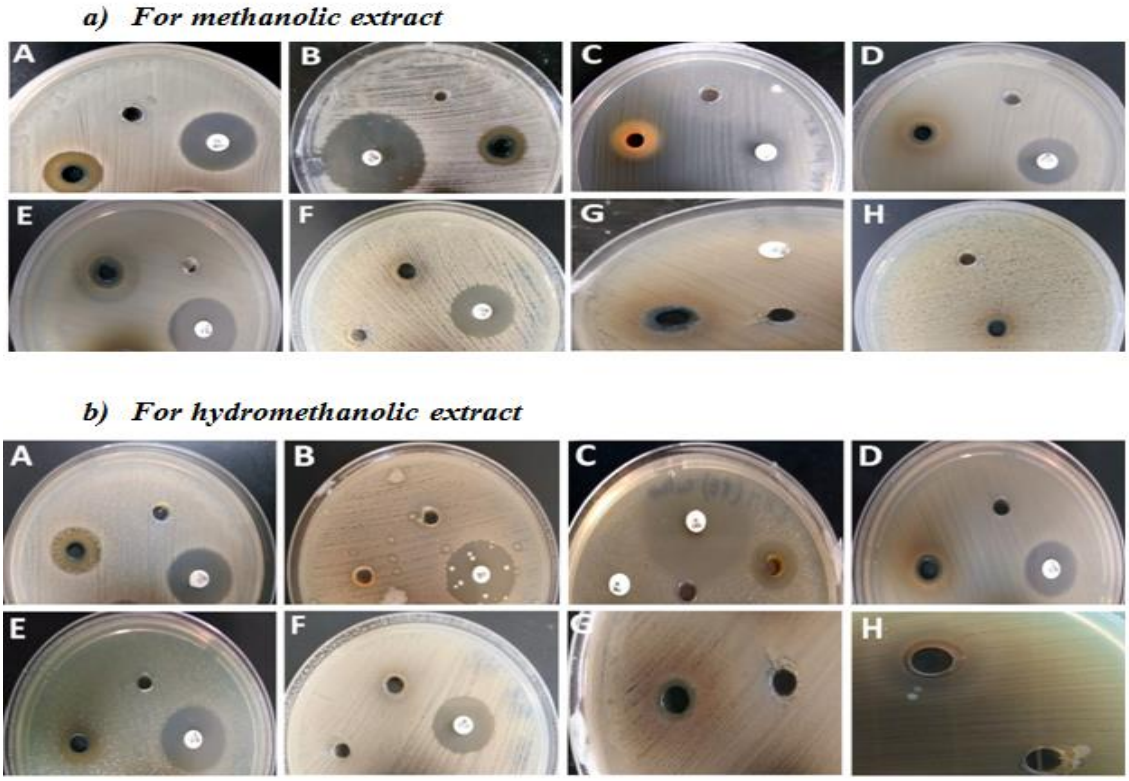

Fig. 1. *Antibacterial activity of E. alata aerial parts extracts against pathogenic bacteria. A) S. aureus; B) B. cereus; C) E. feacalis; D) E. coli; E) P. aeruginosa ; F) K. pneumoniaeü; G) K. pneumoniae (isolate); H) S. typhi*

Antioxidant Activity

The antioxidant power of extracts, Trolox and ascorbic acid is shown in Table 5. The antiradical activity of ME was the most potent (IC_{50}=226.25 µg/mL), considerably height than the HEM. Whereas the both extract demonstrated the significantly low antioxidants effect ($p<0.05$) compared to references standards.

According to Jaradat et al. [10] and Jerbi et al. [24], the aerial part of Palestinian *E. alata* and stem of Tunisinian *E. alata* for the methanolic extract have a significant free radical scavenger DPPH (IC_{50}=16.03 µg/mL and 27.0 µg/mL, respectively). Moreover, Al-rimawi et al. [25] showed that the hydroethanolic extract of Palestinian *E. alata* had the highest radical scavenger DPPH activity (78 µg/mL) when compared to pure ethanolic and aqueous extract. There was a significant correlation between DPPH and TPC in all extracts (hydroethanolic, aqueous and ethanolic). Also, Danciu et al. [12] reported strong antioxidant activity (7453 µmol Trolox/g) in the hydroethanolic extract of *E. alata*. The difference of the bioactive substances present in different plant extracts and their contents could be explained the discordance among the antioxidant effect of plant extracts [26].

Table 5. *DPPH radical scavenging assay of E. alata extracts*

Extracts	R^2	IC_{50} (µg /mL)
ME	0.992	226.58±0.003[a]
HME	0.986	266.0±0.003[b]
Trolox	0.997	71.56±0.004[c]
Ascorbic acid	0.999	48.83±0.002[d]

Results expressed are means±SD (n=3). The letters (a–d) indicate a significant difference according to the Tukey test ($p<0.05$)

CONCLUSION

Medicinal plants contain active ingredients that can be used to treat disease. The antioxidant and antibacterial effects of secondary metabolites in plants could explain their therapeutic properties. Qualitative and quantitative phytochemical analysis showed that crude extract (ME and HME) are a sources of various metabolites compounds with interesting biological properties. In fact, the both extracts demonstrated antibacterial activities in a dose dependant – manner, especially against Gram-positive bacteria, as well as antioxidant effect. *Ephedra alata* aerial part can be an important source in search of natural bioactive substance with antioxidant and antibacterial properties.

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Conflict of Interest. The authors declared that there is no conflict of interest.

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