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Research Article

Antimicrobial and Antioxidant Properties of Phenolic Extracts from *Haloxylon articulatum* Bioss Growth in Oued Souf – Algeria

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ABSTRACT
This study intends to investigate plants that grow in southeast Algeria namely *Haloxylon articulatum Bioss.* which are commonly used by medical science for a treatment. More especially this study shed light on the antioxidant effect and biological activity of the plant whereby extraction of the active ingredients phenol is taken into account. The active ingredients were identified through the scanning device using High-performance liquid chromatographic (HPLC). In-order to get deep insight into the body of knowledge towards extraction process; current study utilized both qualitative and quantitative approach. The data was analyzed separately on the anti-bacterial activity and oxidation of the phenolic extracts. The obtained results revealed a significant effect on the proliferation of some bacterial strains and free radical. In addition, of the phenolic extracts have shown less effective than the antibiotic chosen "polymyxin B" on bacteria; *Staphylococcus aureus ATCC 6816 and Staphylococcus aureus methicillin* resistant. As phenolic extracts have shown greater efficacy than the antibiotic chosen "polymyxin B" on bacteria *Bacillus cereus ATCC 14579*. Hence, based on the empirical evidences it can be stated that from various concentrations approach; the sensitivity of bacteria *Bacillus cereus ATCC 14579* against phenolic extracts.

Keywords: *Haloxylon articulatum* Bioss, extract, polyphenol, antioxidant, antimicrobial.

INTRODUCTION

Haloxylon articulatum Bioss. It called in the region of south east Algeria in the name *« homaire »*, it is a small red pigment which is usually found in the crust of the roots of an herbaceous plant in the region of south Algeria. The size of the homaire does not exceed 25 cm. It is covered with stiff bristles of the latter turn into a semblance of thorns thin when they reach the plant and the beginning of drying *Haloxylon articulatum Bioss.* elongated leaves and do not have a clear neck. The flowers are yellow in color and collects in Nurat apical dense[1]. Herbal treatment for curing certain medical diseases is a common practice in Africa, Statistically it is estimated that over 80% of the total population produced a wide array of phytochemical; most of which are used, from the plan. The main reason for preferring herbal treatment is to avoid the undesirable secondary effects which are commonly known as unwanted side-effects of some synthetic chemical drugs[2].

Moreover, research has indicated that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease[3]. Two synthetic antioxidants namely butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are more used in the food industry and also considered as a major contributor towards liver damage and carcinogenesis or toxic[4,5,6]. Thus, in order to avoid certain unhealthy circumstances, it is necessary to focus on others natural antioxidant extracts from plants. Several chemical compounds extracted from plant leaves, however, the most important is the flavonoids. It is secondary metabolites ubiquitously distributed in all higher plants[7]. Flavonoids from the Latin word flavus meaning yellow, their color in nature. Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6[8,9].

Contemporary studies confirmed the antimicrobial activity of flavonoids occurring in vegetable foods and medicinal plants. These antimicrobial activities facilitate in diverse ways such as anti-allergic, antimicrobial, anti-inflammatory, vasoprotector and anti-tumour agents respectively[10].

The plants of the Sahara is rich with phytochemicals like phenolic acids, sterols, proanthocyanidins, flavanoïds, carotenoids and anthocyanin. Research has also revealed that *Haloxylon articulatum Bioss.* are beneficial in a biological and pharmacological viewpoint. Because it present several biological activity antiviral, antibacterial,

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anti-inflammatory, antitumor, these activities strength the immunity system, also antioxidant activity[1,11,12,13,14,15].

Although all studies conducted in appointments, in our knowledge, there is no scientific information and empirical evidence on the study of the plant, Antioxidant and antibacterial activity on the *Haloxylon articulatum Bioss*. There the current the study was conducted to estimate the phytochemical composition, the effect of an antimicrobial and antioxidant extract from the *Haloxylon articulatum Bioss* effective flavonoid growth in the southeast of Algeria. It is expected that the obtained results can be taken as a guideline and might considered as a new source of agent antioxidant and antimicrobial.

MATERIAL AND METHODS

Chemicals and reagents

Methanol and ultra pure water were purchased from VWR Merk (France), folin-ciocalteu reagent, Diphenyl-1 picrylhydrazyl (DPPH), BHT and chlorogenic acid were procured from Sigma–Aldrich Inc (Paris, France). All other chemicals and reagents were analytical-reagent, sodium carbonate (Na_2CO_3), gallic acid, sodium nitrate ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), catechin, hydrochloric acid (HCl), quercetin, linoleic acid, sodium phosphate, tricloroacetic acid (CCl_3COOH), ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4) and ammonium molybdate. The following reagents were used for the microbial activity namely Nutrient agar and sabouraud dextrose agar.

Plant material

The aerial parts of *Haloxylon articulatum* Bioss. in March 2014 from Douilatte located in Wilaya of El-Oued south east Algeria (33° 07" 00" N 7° 11' 00" E). This species was identified by Pr. Gerraf Noureddine Laboratory of Biomolecules and Plant, Breeding, Larbi Ben M'hidi University, Oum El Bouaghi. Prior using the extraction of the plant for medical purpose. The leaves were dried in well-ventilated spaces at room temperature, after powdered and sifted in a sieve (0,750 µm).

Extraction method

After drying in a dry, ventilated area, away from sunlight, the plant is crushed and then be weighed (M = 100g). Plant material obtained is then maceration in a hydro alcoholic mixture (methanol /water; 80/20; V/ V). This maceration process repeated in three times with solvent renewed every after forty-eight hours. After went through the process of filtration and concentration in vacuum, the methanolic extract is then diluted with water distilled at 50 ml per 100 g of dry matter, the rest is left in solution overnight then filtered. After filtration, the solution has undergone successive liquid-liquid extractions type using solvents of increasing polarity starting with chloroform and ethyl acetate and finally with n-butanol. [16] [17] [18] [19]

High-performance liquid chromatography (HPLC)

Phenolic compounds have been separated and identified by liquid chromatography system high performance reverse phase mark (Agilent Technologies 1260, Germany) equipped with a UV diode array detector (DAD) and equipped with a chromatographic column filled with a grafted silica gel, octadecyl type ZorbaxEclipse XDB- C18 (4.6 x 100 mm, 3.5 microns).

For the various extracts, a conventional chromatographic condition is usually adapted. Indeed, the detector (DAD) is adjusted to a scan of scanning from 200 to 400 nm, whereby the column temperature was maintained at 25 ° C. The volume injected is 20 µl and the mobile phase used is made up of two solvents A and B: Solvent A (Methanol), Solvent B (MilliQ water containing 0.1% formic acid). The speed of this phase is set at 0.4 ml / min. The separation method adopted is the gradient elution in which the program is shown in the Table 1.

Identification of polyphenol and flavonoids compounds was performed by comparing the retention times of peaks obtained for those flavonoids standards injected in the same chromatographic conditions.

Determination of total polyphenol content (TPC)

Total phenolic content of each extract method was determined with the Folin-Ciocalteu reagent (FCR)[17,18,19,20]. Briefly, a dilute solution of each extract in ethyl acetate (1 mL) was mixed with 1 mL of Folin-Ciocalteu reagent, followed by 1 mL of a $CaCO_3$ (10 % w/v) after 4 min. The reaction mixture was incubated for 30 min at room temperature. The absorbance of reaction mixture at 700 nm was calculated, the blanks prepared with the same procedure described above except that the samples solution was substituted by 1 mL of ethyl acetate. The concentration of total phenolic in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight. All results presented are means (±SEM) and were analyzed in three replications.

Determination of total flavonoids (FVT)

Two reagents were used namely sodium nitrite colorless solutions ($NaNO_2$, 5%) and aluminum chloride ($AlCl_3$, 10%). The principle of the method is based on the oxidation of the flavonoids by these reagents; it leads to the formation of a brownish complex, which absorbs at 510 nm. Comparing the OD observed to that obtained by a known concentration of catechin standard used to evaluate the total content of flavonoids. The total flavonoids are measured calorimetrically in a flask of 10 ml were introduced successively 250 µl of extract of known concentration in leaves and 75 µl of a solution of $NaNO_2$ (5%). After 6 minutes was added 150 µl of $AlCl_3$ (10%) and 500 µl of NaOH (1N) and in 1525 µl of distilled water was added to the mixture successively. A calibration curve is prepared at different concentrations with standard solutions of catechin. The absorbance of the mixture obtained is directly measured by UV-visible spectrophotometer at 510 nm and the results are expressed in mg catechin equivalent / g of dry matter (EC / g DM)[21,22,23,24,25]. The data was analyzed with three separate experiments. The obtained correlation coefficient of the calibration curve was $R^2 = 0.998$ while the result of the obtained result is presented as mean (± SEM).

Determination of the antioxidant activity of the extracts.

The measurement of antioxidant potential was carried out by determining the products resulting from the oxidation or by assessing the ability to trap reaction models radicals.

Table 1: Gradient elution.

Temps (min)	Solvant A (%)	Solvant B (%)
0	10	90
5	20	80
10	30	70
30	50	50
40	60	40
45	70	30
50	90	10
55	50	50
60	10	90

Table 2: Mass yield, phenolic content and flavanoïds of methanol 80% of extract from *Haloxylon articulatum Bioss.*

dry weight extract g/100 g of leaves powder	Yield (%) w/w	Total phenolic content (mg EAG /g)	Flavanoïds (mg EC/g)
3.9 ± 0,07	3.9 ±0,07	114.3±0.0254	12.025± 0.245

The first mode requires prior knowledge of the compounds from oxidation. Indeed these methods seek some functional groups (aldehydes, ketones, dicarbonyl) in the derivatives of the original components. The second mode links the amount of trapped radicals of used antioxidan. These dual modes and forms of expression are preferred to use the percent inhibition (IC) and / or equivalence standards polyphenols obtained by UV-Visible spectroscopy. The percent inhibition for assessing antioxidant activity of a sample is determined using the following formula: $IC (\%) = [(A-B) / (A)] \times 100$

Where ; a = absorbance of the oxidized solution in the absence of antioxidant agents,

b = absorbance of the oxidized solution in the presence of antioxidant agents.

Evaluation of the ability of the compound (extract) to trap free radicals is therefore to measure its ability to scavenge free radicals and therefore slow or inhibit the creation of free radicals

In the case of the evaluation of the antioxidant activity according to the equity standard polyphenols, the method comprises of comparing the absorbance of targeted samples to that of a calibration straight line, which connects the absorbance to the concentration in standard. The types of radicals that are used to evaluate the antioxidant activity of extracts of the leaves are reducing power, the radical ABTS test, the radical OH, and the DPPH radical[25,26,27,28].

Total antioxidant activity

During this test, hydrogen and electron are transferred from the reducing compound (extract-antioxidant) to the oxidizing complex (PPM). This transfer phenomenon depends on the redox potential of the medium pH and on the structure of the antioxidant compound. The test is based on the reduction of the molybdenum of the oxidation stage (VI) the oxidation state (V). This reduction is materialized by forming a greenish complex (phosphate / Mo (V)) at an acidic pH which measures the decrease in the coloration of molybdenum (VI) complex in the presence of antioxidant. Unlike other tests, this test makes it possible not only to quantify the contribution of the antioxidant activity of the polyphenols but also other antioxidants such as vitamins. The method comprises introducing into an Eppendorff tube 100 .mu.l of the extract of the leaves mixed with 1 ml of a reactive compound of H_2SO_4 (0.6 M), NaH_2PO_4 (28 mM) and ammonium molybdate (4 mM). The tube is then closed and incubated at 95 °C for 90 minutes. After being cooled, the absorbance is measured at 695 nm. The control consisted of 100 .mu.l of methanol mixed solvent with 1 ml of the reagent mentioned above. The calibrators, controls and samples are incubated under the same conditions. The results are expressed in mg of gallic acid equivalents per gram of dry matter (E AG mg / g DM). This curve is established using gallic acid as a reference and therefore, the results are expressed in mg equivalent gallic acid per gram of dry matter (mg EGA / GMS). The calibration curve is established with a correlation coefficient $R^2 = 0.998$. [29,30]

The DPPH test

DPPH test (2, 2-diphenyl-1-picrylhydrazyl) is a method widely used in the analysis of an antioxidant activity. Indeed, the DPPH is characterized by its ability to generate stable free radicals. This stability is due to the relocation of the free electrons in the molecule. The presence of DPPH radicals resulted in a dark purple color of the solution, which absorbs at about 517 nm. The reduction of DPPH radicals by an antioxidant agent results in a discoloration of the examined solution. Evaluation of the antioxidant capacity is performed as follows: in 250µ l of a solution of DPPH methanolic (7.8 mg DPPH in methanol 100 ml) was mixed 1 ml of the extract of leaves. The resulting mixture was then kept sheltered from the light at room temperature for 30 minutes. The absorbance is measured at 517 nm same a control compound of 250µ l of the solution of DPPH and 1 ml of solvent methanolic.

Sample preparation and the control is carried out under the same operating conditions. The decrease in the absorbance is measured spectrophotometrically and IC% (percent inhibition) is calculated using the formula below;

$\% IC = [(At\ 0 - At\ 30) / At\ 0 \times 100)]$

Where; At_0: absorbance of control (containing no antioxidant) 30 minutes

At_{30}: excerpts absorbance measured after 30 minutes.

The activity of antiradical is usually expressed in IC 50 (Mcg / ml), the dose antiradical required to cause 50% inhibition. All results presented are averages (± SEM) and analyzed with three repetitions. By varying the concentration of the extracts and calculating the percentage concentration for each corresponding IC, a linear regression was determined between the different concentrations and percentage IC. Prediction also leads towards deduction of the corresponding IC_{50} value. [31,32]

Table 3: Quantification of polyphenols and flavonoids compounds in methanolic 80% extract from *Haloxylon articulatum* Bioss.

Retention time (min)	Air	Identification	Quantification µg/ml
8.577	32.5	Gallic acid	2.997
10.462	16.25	Catechin	1.498
15.860	241.3	Resorcinol	22.25
27.657	1024.1	trans- hydroxycinnamic acid	94.44
29.397	852	luteolin 7-O glucoside	76.077
31.212	221.8	Hyperosid	20.45
32.192	1363.4	Nobiletin	125.7
34.334	470.2	Myricetin	43.356

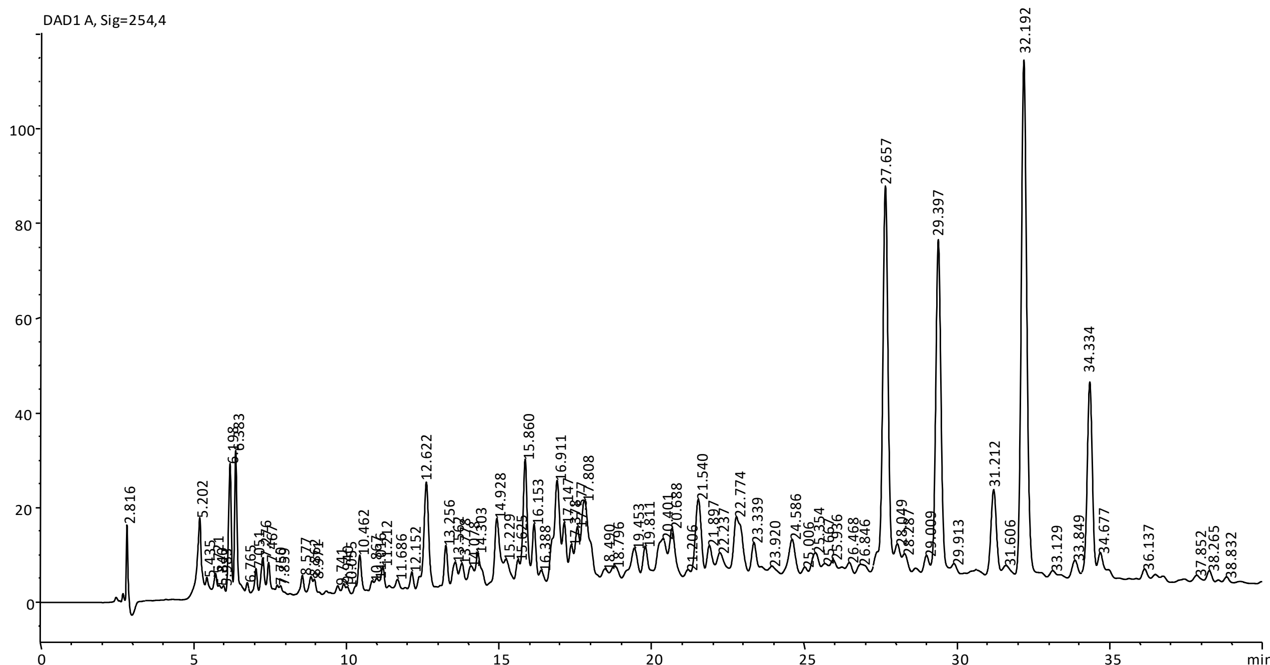

Figure 1: Chromatographic profile of extract recorded in UV at 254 nm.

Antimicrobial activity assays

Microorganisms

The bacterial cells used in this study it is three gram-positive. The bacterial cells assayed included four gram-positive that were Staphylococcus aureus ATCC 6816, Staphyloccocus aureus resistant methicillin, Bacillus cereus ATCC 14579[33,34,35]. All strains were obtained from the Laboratory of Bioactive Substances, Center of Biotechnogy, Borj Cedria (CBBC), and BP 901- Hammam Lif - 2050, Tunisia.

Incubation conditions

Nutrient agar was used culture medium for bacteria which was incubated for 24 h at the temperature of 37 °C and yeasts were cultured in sabouraud dextrose agar (SDA; 4% dextrose, 2% neopeptone and 1,7% agar) for 24-48 hours at the temperature of 30°C[36,37].

Disc diffusion assay

Methanolic aqueous extracts *Haloxylon articulatum Bioss.* were dissolved in methanol/water for a final concentration 10 mg/ml and filter-sterilized through a 0.45 membrane filter. The antimicrobial activity was estimated by method of disc diffusion, 100 µl of suspension for each microorganism 108 colony-forming units (CFU)/ml containing 20 ml of nutrient agar for bacteria, after were placed in the petri sterilized filter paper disc (6mm in diameter). Later it was soaked with 15 µl of the 50 mg/ml of each methanolic extracts (150 µg/disc). The methanol 50% was used as a negative control whereas polymyxine B was taken as the positive control. However both positive and negative control was prepared with the same procedure. The detailed described on the procedure was mentioned-earlier except for the methanol extract which was substituted by 15 µl of positive control at 50 mg/ml. The diameter of the inhibition zone around each disc was measured for three replicates.

Statistical analysis

Data was analyzed using statistical tests whereby the obtained results were presented in mean values, and standard deviations (SD). Since, all measurements were carried out in three experiments therefore, the all the analyses in the present study were analyzed three times determinations). Statistical calculations were carried out by OriginPro version 8 software (Prolab), correlations were obtained by Pearson correlation coefficient using bivariate correlations test. P value were set at 0.05.

RESULTS AND DISCUSSION

Table 3: Total antioxidant activity (mg GAE/g), DPPH radical scavenging and standards pf extract of *Haloxylon articulatum* Bioss and standards (BHT).

DPPH (IC_{50} µg/ml)	BHT(IC_{50} µg/ml)	Total antioxidant activity (mg GAE/g)
2.49 ± 0.067	11.7 ± 0.30	114.3±0.0254

Table 4: Diameter of zone inhibition of methanolic extract of *Haloxylon articulatum* Bioss (50 mg/ml).

Bacteria	Diameter of zone inhibition(mm)	
	Extract	polymyxine B
Staphylococcus aureus ATCC 6816	10 ± 0.3	12.5 ± 0.5
Staphyloccocus aureus méthicilline résistant	13 ± 0.5	11.0 ± 0.4
Bacillus cereus ATCC 14579	17 ± 0.6	8.5 ± 0.1

Extract yield

The methanol is a solvent extract significant amount of alkaloids compounds and recently used in several studies. It is considered as the best solvent of antimicrobial substances compared with the other solvents. The results of extract yield for each variety of *Haloxylon articulatum Bioss.* are mentioned in Table 2, which shows the extraction yield (g/100 g dry weight), the mass yield obtained for methanolic extract of leaves *Haloxylon articulatum Bioss.* found 3.9 % for methanolic extract of *Haloxylon articulatum Bioss.*

Results expressed as the mean and ±standard deviation of three independent experiments.

Identification by HPLC

The identification of compounds polyphenol extract the majority of plant extract *Haloxylon articulatum* Bioss. HPLC was carried out based on the comparison of their retention times with those obtained for the same standard compounds. This comparison allowed us to confirm the presence of two-majority flavonoids resorcinol with a retention time of 15.860 min, and luteolin 7-*O*glucoside with a retention time 29.397 min. Hyperoside with a retention time 31.212 min, Nobiletin with a retention time 32.192 min, Myricetin with a retention time 34.334 min show in Fig. 1 and table 3. The concentration of phenolic respectively 22.25 µg, 76.077 µg, 20.45 µg, 125.7 µg / 100 g of plant extract and we find a Catechin compound with a retention time 10.462 min.

Minor peaks were also recorded with retentions times ranging from 8.577 min probably are phenolic compounds "gallic acid" with a rate of 2.997 µg / 100g and compounds trans- hydroxycinnamic acid a 27.657 min time of retention with a rate of 94.44 µg / 100 g of plant extract. Flavonoids rates are determined in plant extracts according to the calibration curve (peak areas as a function of the concentration of the standards).

Total phenolic and flavanoïds

The extracts technique methods of aerial parts of *Haloxylon articulatum* Bioss differ to each other considerably with respect to the total phenolic. The amount of total phenolic content it is 114.3±0.0254 mg EAG /g and the content of flavanoïds in catechin equivalent it is 12.025± 0.245 mg EC/g Ms. The results of the quantitative analyzes of polyphenols and flavonoids in the extracts of the leaves of the variety *Haloxylon articulatum* Bioss. are reported in Table 2.

These results indicate that the extract from aerial parts of *Haloxylon articulatum* Bioss. is rich in polyphenols and flavonoids.

DPPH radical scavenging activity

The activity Radical trapping DPPH leaves of methanol extract of Saharan plants of the *Haloxylon articulatum* Bioss. is obtained Their Highest value (IC_{50} = 2.49 ± 0.067 ug / ml). There antioxidant capacity of different varieties of plants such as *Haloxylon articulatum Bioss.* is higher than even the standard BHT (IC_{50} = 11.7 ± 0.3 µg / ml) to head antioxidant capacity from radical trapping DPPH related the high amount of polyphenols in our extracts.

The results strong confirmed the high antioxidant activity of extract of plant of the region Oued Souf; the results are presented in Table 3.

Antimicrobial activity

The results of the antibacterial activity of methanol extracts of studied tree are against a set of Gram-positive (Staphylococcus aureus ATCC 6816, Staphyloccocus aureus méthicilline résistante, Bacillus cereus ATCC 14579. The diameter of zone inhibition are summarized in Table 4.

CONCLUSION

We think that the present study is the first investigation and comparing the phytochemical composition, antioxidant and antimicrobial activity of extracts of three varieties of *Haloxylon articulatum* Bioss. growth in Southeast Algeria. This study showed that considerable variance exists between the three extracts of leaves for plant *Haloxylon articulatum Bioss.* "polyphenol". We found highest amount of flavonoids. On the other hand, the results of antioxidant activity tests present the strong capacity of extracts of leaves for plant *Haloxylon articulatum Bioss.*", higher than the standards antioxidants (BHT). Finally, all extracts appears the high antimicrobial activity for the microorganisms tests (bacteria) exceeded most of the time the positive control. A strong correlation was found between activity and phytochemical contents indicates that the effects observed could be attributed to polyphenol compounds. This data suggests a strong potential of this extracts as a natural source of polyphenol compounds, antioxidant and antimicrobial and may be considered in future to replace synthetic preservatives and drugs in pharmaceutical and food industry. After the results obtained in the previous study, from various concentrations, we can determine the sensitivity of each type of bacteria against each extract: And as end conclusion, we can say that they extracts

polyphenol to have an medium effect on bacteria; Staphylococcus aureus ATCC 6816, and active effect on bacteria : Staphylococcus aureus methicillin resistant and bacteria Bacillus cereus ATCC 14579 and have shown greater efficacy than the antibiotic chosen "polymyxin B".

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