

Evaluation of Antibacterial Activity of some Medicinal Plants Extracts Commonly Used in Algerian Traditional Medicine against some Pathogenic Bacteria

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ABSTRACT

Introduction: The objective of this study was to evaluate the antibacterial activity of hydro-methanolic extracts of *Berberis vulgaris*, *Cassia angustifolia*, *Cinnamomum cassia*, *Cistus monspeliensis*, *Nigella sativa*, *Punica granatum*, *Rhus tripartita*, *Withania frutescens* and *Zingiber officinale* against different Gram-positive and Gram-negative reference bacterial strains. **Methods:** The evaluation of antibacterial activity for different extracts of each plant was carried out using the disc diffusion method and determination of minimum inhibitory concentration (MIC). **Results:** The plant extracts showed zone of inhibition ranging from 0.60 to 23.0 mm against one or more tested bacteria, and their efficacy in terms of MICs where ranged from 0.1 to 12.8 mg/mL. The Refluxed and Macerated extracts of these plants have shown relatively similar results in terms of diameters of inhibition and MICs. The extracts of *B. vulgaris*, *C. monspeliensis* and *P. granatum* demonstrated relatively high activity as compared to the other plant extracts mainly against *S. aureus*, *E. faecalis* and *E. cloacae*.

Conclusion: Findings of this study indicate that hydromethanolic extracts of these plants have antibacterial activity against the different tested bacterial strains. This activity supports their use in treatment of infections caused by such resistant bacteria.

Key words: Medicinal plants, Hydromethanolic extracts, Antibacterial activity, Inhibition zones, MICs, Bacterial resistance.

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INTRODUCTION

The uses of antibiotics are widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes,¹ this has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages.² Natural products are typically secondary metabolites, produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely used in the pharmaceutical industry for their remarkable structural diversity and range of pharmacological activities.³

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents.⁴ Several works have been done to examine the antimicrobial effects of herbal plants extracts, including roots, stem, leaves or flowers.^{5,6} Many countries in Africa and other parts of the world have continued to encourage screening programs of plants used in traditional medicine in order to authenticate their antimicrobial activities and possible inclusion in primary health care.⁷ Like the other countries of the Maghreb and Africa, the empirical use of

medicinal plants continues to retain great popularity in Algeria. In some rural areas, resorting to natural remedies with "miraculous" plants is preferred to modern medicine.⁸ The present study was aimed to determine the potential antibacterial activities of hydromethanolic extracts from nine selected medicinal plants organs belonging to different families on human pathogenic bacteria, in order to valorize them in the light of previous works for further application in food and pharmaceutical industries as natural valuable products. All of plants assayed in this study are commonly used as medicinal plants in different localities of Tlemcen, Algeria, and other parts of the world. Their medicinal properties are described in Table 1.

MATERIALS AND METHODS

Plant Material

The selected plants were either collected from the field in different regions of Algeria or purchased from the local market of Tlemcen, Algeria, in the period of March 2013 (Table 1). They were identified by the authors and voucher specimens have been deposited in the Laboratory of Natural Products,

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Table 1: Medicinal plants tested for their antibacterial activity in the study.

Plant	Family	Parts used	Voucher specimen	Origin	Traditional uses
<i>Berberis vulgaris</i> L.	Berberidaceae	Root bark	LPN/BV-M 21	Market	Used for treatment of internal injuries, removal of kidney stones, sore throat and fever and also used for tanning skin. ^{9,10}
<i>Cassia angustifolia</i> Vahl.	Fabaceae	Leaves	LPN/CA-M 22	Market	Used as purgative, astringent, anthelmintic, expectorant and febrifuge, jaundice and typhoid fever. ¹¹
<i>Cinnamomum cassia</i> L.	Lauraceae	Peels	LPN/CC-M 23	Market	Used as a spice and in traditional medicine for treatment of diarrhea, rheumatism and coldness, digestive complaints and antiseptic. ¹²
<i>Cistus monspeliensis</i> L.	Cistaceae	Aerial part	LPN-CM 1	Tlemcen	Traditionally used for arthrosis, asthma, bacterial and fungal infections, diarrhea and inflammation. ¹³
<i>Nigella sativa</i> L.	Ranunculaceae	Seeds	LPN/NS-M 24	Market	Commonly used for culinary and medicinal purposes as a remedy of hypertension and diabetes, and as hypoglycemic, anti-inflammatory, antiulcer and bronchodilator. ¹⁴
<i>Punica granatum</i> L.	Punicaceae	Fruit peels	LPN-PG 2	Tlemcen	Commonly used for treating diarrhea, dysentery and used as astringent. Used also for prevention of atherosclerosis and as antidiabetes, anticancer and with antimicrobial properties. ¹⁵
<i>Rhus tripartita</i> (Ucria) Grande	Anacardiaceae	Aerial part	LPN-RT 3	Bechar	Treatment of diarrhea, dysentery, diabetes, inflammatory diseases, hemorrhoids and fever. ¹⁶
<i>Withania frutescens</i> L.	Solanaceae	Leaves	LPN-WF 4	Tlemcen	Used for treatment of dysentery and ulcers, tooth pain, and as diuretic. Root juice, instilled with honey clarifies the view. ^{17,18}
<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Rhizome	LPN/ZO-M 25	Market	Ginger is widely used both as a spice and for its medicinal properties to treat stomach aches, diarrhea, nausea, respiratory disorders and has hypoglycaemic and hypolipidaemic effects. ^{19,20}

University of Tlemcen, Algeria. The plants were dried at room temperature for two weeks.

Preparation of plant extracts

The dried plants parts were ground and then 10 g of the dry plant powder of each plant was immersed in 100 mL of methanol/water (80/20) and submitted to extraction under reflux for 3 h, or extracted by maceration by overnight constant shaking at room temperature. After, the extracts were filtered and concentrated under reduced pressure at 45°C for preparation of Refluxed Methanolic Extract (RME) and Macerated Methanolic Extract (MME) respectively. The obtained residues were kept in dark and stored at 4°C until use.

Bacterial strains

Following standard bacterial strains were used in this study belonging to Gram positive and Gram negative species: *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 700603) and *Pseudomonas aeruginosa* (ATCC 27853). They were obtained from Natural Products laboratory (University of Tlemcen, Algeria). Because of their ability to survive in harsh conditions and their multiple environmental habitats, these bacterial organisms including Gram positive and Gram negative are the main source to cause severe infections in humans.²¹

Disc diffusion method

Antibacterial activity of tested plants parts was carried out by the disc diffusion method.²² First, the different extracts of plant parts tested were dissolved in DMSO at a concentration of 100 mg/mL and filtered through 0.45 µm sterile filter membranes. Then, 100 µL of bacterial inoculums

containing 10⁸ CFU/mL were spread over plates containing Mueller Hinton agar, and discs (6 mm in diameter) impregnated with 10 µL of the extracts solutions (1 mg/disc) were placed on the surface of the media. Two control discs were used containing DMSO and Gentamicin (10 µg/disc) as negative and positive controls, respectively. The plates were incubated for 24 h at 37 °C, and the experiments were performed in duplicate. The diameters of inhibition zones were measured and antibacterial activity was considered for diameters of inhibition zone greater than 9 mm.²³

Determination of the minimum inhibitory concentration (MIC)

The broth micro-dilution method was used to determine the MIC according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST).²⁴ The tested extracts were dissolved in 10% DMSO and diluted to the higher concentration. Then, a serial ½ dilutions of extracts were prepared directly in a microtiter plate containing Mueller Hinton broth to obtain concentrations from 0.0125 to 12.8 mg/mL. The bacterial inoculum was added to give a final concentration of 5 × 10⁵ CFU/mL in each well. The positive control was used containing Gentamicin as standard drug at final concentrations from 0.125 to 128 µg/mL. The plate was covered with a sterile sealer and incubated for 24 h at 37°C. The MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth. The lower the MIC, the higher the activity of the extract.

RESULTS

Yields of extracts

The preparation of extracts from different parts of selected plants was performed using two types of extraction. The yields obtained for different extracts are shown in Table 2.

Table 2: Extraction yields of the studied plants.

Plants	Yield (%)	
	RME	MME
<i>Berberis vulgaris</i>	17,98	17,23
<i>Cassia angustifolia</i>	12,60	17,75
<i>Cinnamomum cassia</i>	24,94	24,39
<i>Cistus monspeliensis</i>	26,60	27,42
<i>Nigella sativa</i>	16,03	12,22
<i>Punica granatum</i>	35,07	38,19
<i>Rhus tripartita</i>	15,91	12,22
<i>Withania frutescens</i>	21,29	18,06
<i>Zingiber officinale</i>	17,32	06,74

The two types of extracts of all different plants parts studied show substantially similar yields, with the exception of *Z. officinale* dry rhizome where the yield of RME is approximately three times greater than MME. This latter difference may be due to the extraction method used and the biochemical composition of the plant.

Antibacterial activity

Evaluation of the antibacterial activity of hydromethanolic extracts of the studied plants was determined initially by the disc diffusion method against different bacteria. These bacterial strains are Gram-positive and Gram-negative species frequently encountered in infectious diseases. The results of the diameters of inhibition zones are shown in the Table 3.

It can be noted that all plants parts extracts, except for those of *Cassia angustifolia* leaves, *Nigella sativa* seeds and *Zingiber officinale* dry rhizome, exhibited varying degrees of antibacterial activity against all bacterial strains tested. The macerated extracts of *Berberis vulgaris* root barks presented a strong activity against *S. aureus* with diameter of inhibition zone of 23.0 mm, a weak activity against *E. faecalis* (13.0 mm diameter)

and no activity toward other strains. The extracts of *Cistus monspeliensis* aerial parts and *Punica granatum* fruit peels showed a relatively moderate activity mainly against *S. aureus* (16.0, 17.0 mm and 20.0 mm, respectively). These plants parts extracts showed a low activity particularly against *K. pneumoniae* (10.0 mm) for *C. monspeliensis* aerial parts and against *P. aeruginosa* (12.0 mm) for *P. granatum* fruit peels.

The plant extracts of *Cinnamomum cassia* peels, *Rhus tripartita* aerial parts and *Withania frutescens* leaves showed a low activity with diameters of about 11.0 and 12.0 mm mainly against *S. aureus* and of 11.0, 12.0 and 10.0 mm against *E. coli*, *E. cloacae* and *P. aeruginosa*, respectively for *C. cassia* peels. The refluxed and macerated extracts of these plants showed diameters were close.

Minimum Inhibitory Concentrations

The effectiveness of the extracts on tested bacterial strains was determined by measuring the minimum inhibitory concentration (MIC) (Table 4).

The MICs of hydromehanolic extracts of individual plants parts varies against different tested strains. MICs values obtained from extracts of *B. vulgaris* root barks were of 0.4 mg/mL against *S. aureus* and 1.6 mg/mL against *E. cloacae*. In addition, *Cinnamomum cassia* peels extracts showed MICs of 0.1 and 0.8 mg/mL against *S. aureus* and *E. faecalis* and 1.6 mg/mL against *E. coli* and *E. cloacae*. The extracts of *C. monspeliensis* aerial parts and *P. granatum* fruit peels showed MICs of 0.1 and 0.2 mg/mL (RME and MME respectively) against *S. aureus*. MICs against *E. faecalis*, *E. coli*, *E. cloacae* and *P. aeruginosa* were 1.6 mg/mL by *C. monspeliensis* aerial parts and were of 0.8 mg/mL against *E. cloacae* by MME *P. granatum* fruit peels extracts.

Contrary to what was expected, MICs of *R. tripartita* aerial parts extracts were 0.2 and 0.8 mg/mL for MME and RME respectively against *S. aureus* and 1.6 mg/mL against *K. pneumoniae*. MICs values of *W. frutescens* leaves extracts were 1.6 mg/mL against *S. aureus*. Other results were between 3.2 and 12.8 mg/mL. It can be noted that overall there is no difference between the results of the RME and MME in terms

Table 3: Antibacterial activity (inhibition zones, mm) of the tested extracts.

Plants		<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Berberis vulgaris</i>	RME	12.0	–	–	–	–	–
	MME	23.0	13.0	–	07.5	–	–
<i>Cassia angustifolia</i>	RME	–	–	–	–	–	–
	MME	–	–	–	–	–	–
<i>Cinnamomum cassia</i>	RME	11.0	07.0	07.0	08.5	10.0	07.5
	MME	11.0	08.0	11.0	12.0	07.0	10.0
<i>Cistus monspeliensis</i>	RME	17.0	–	–	08.0	07.0	08.0
	MME	16.0	09.5	08.5	08.0	10.0	09.0
<i>Nigella sativa</i>	RME	07.5	–	–	–	–	–
	MME	–	–	–	–	–	–
<i>Punica granatum</i>	RME	20.0	–	07.0	08.5	09.5	08.0
	MME	20.0	–	09.0	09.0	08.0	12.0
<i>Rhus tripartita</i>	RME	08.7	08.0	–	–	–	–
	MME	12.0	–	09.0	–	–	08.0
<i>Withania frutescens</i>	RME	12.0	–	–	–	–	–
	MME	11.0	–	–	–	–	–
<i>Zingiber officinale</i>	RME	–	–	–	–	–	–
	MME	–	–	–	–	–	–
<i>Gentamicin (10µg)</i>		24.0	14.0	25.0	24.0	16.0	22.0

— : No inhibition zone observed, RME: Refluxed Methanolic Extract, MME: Macerated Methanolic Extract.

Table 4: MICs (mg/mL) of the tested extracts.

Plants		<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Berberis vulgaris</i>	RME	0.4	6.4	6.4	1.6	3.2	6.4
	MME	0.4	12.8	1.6	1.6	6.4	3.2
<i>Cassia angustifolia</i>	RME	1.6	12.8	12.8	12.8	12.8	12.8
	MME	1.6	3.2	12.8	6.4	12.8	12.8
<i>Cinnamomum cassia</i>	RME	0.8	6.4	1.6	1.6	12.8	3.2
	MME	0.1	0.8	3.2	6.4	12.8	12.8
<i>Cistus monspeliensis</i>	RME	0.1	6.4	1.6	1.6	3.2	3.2
	MME	0.2	1.6	1.6	1.6	3.2	1.6
<i>Nigella sativa</i>	RME	6.4	12.8	12.8	6.4	12.8	12.8
	MME	3.2	12.8	12.8	12.8	12.8	12.8
<i>Punica granatum</i>	RME	0.1	12.8	3.2	3.2	3.2	6.4
	MME	0.2	1.6	3.2	0.8	12.8	1.6
<i>Rhus tripartita</i>	RME	0.8	6.4	12.8	3.2	1.6	12.8
	MME	0.2	6.4	3.2	6.4	1.6	12.8
<i>Withania frutescens</i>	RME	1.6	12.8	12.8	12.8	12.8	12.8
	MME	1.6	12.8	6.4	6.4	12.8	12.8
<i>Zingiber officinale</i>	RME	12.8	12.8	12.8	6.4	12.8	12.8
	MME	12.8	12.8	12.8	12.8	12.8	12.8
<i>Gentamicin</i> (µg/ml)		0.25	8	0.5	0.5	4	0.5

RME: Refluxed Methanolic Extract, MME: Macerated Methanolic Extract.

of MICs values. Finally, the extracts of *Cassia angustifolia* leaves, *Nigella sativa* seeds and *Zingiber officinale* dry rhizome showed higher values of MICs varying from 1.6 to 12.8 mg/mL.

DISCUSSION

The results of this study are a contribution to the valorization of some medicinal plants parts that are used in Algerian traditional medicine. Their antimicrobial properties to fight against various bacterial infections have been reported in several studies. According to Mezouar *et al.* methanolic extracts of root barks of *B. vulgaris* have presented a very weak antibacterial activity against all tested strains including *S. aureus*.²⁵

Comparing results found in this study with those of the literature, we notice in a previous work on antimicrobial activity of some medicinal plants from Tunisia, that methanolic extracts of *C. monspeliensis* leaves have shown an interesting activity against *P. aeruginosa*, *S. aureus*, *E. faecalis* with inhibition zones diameters of 18.0, 20.0 and 15.0 mm, respectively.²⁶ Whereas, water-methanol extracts of fruit peels of pomegranate (*P. granatum*) have demonstrated a moderate activity when they were tested on *S. aureus*, *P. aeruginosa* and *K. pneumoniae* (13.0, 18.0 and 16.0 mm, respectively).²⁷ this activity of pomegranate peels could be attributed to tannins, for which antimicrobial activity has been demonstrated.⁴

For *C. cassia*, some authors have reported the antibacterial activity of alcoholic peels extracts against different bacterial strains; the results showed no activity of these extracts in terms of inhibition zones diameters against the tested strains such as *E. faecalis*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*.²⁸ On the other hand, the results found in this study concerning the activity of *R. tripartita* aerial parts extracts are in agreement with other previous works which found significant antibacterial activity of leaves alcoholic extracts against methicillin-resistant *S. aureus*,¹⁶ and no activity against *E. coli* and *P. aeruginosa*.²⁹ For *W. frutescens*, El Bouzidi *et al.* have reported different antibacterial activities of leaves methanolic extracts against *S. aureus* (11.5 mm), *K. pneumoniae* (18.0 mm), *P. fluorescens* (14.5 mm) and no activity against *E. coli*.³⁰

It was observed in the present study that *Staphylococcus aureus* was the most sensitive compared to other strains, while *K. pneumoniae* was the most resistant strain to all tested plant parts extracts. Indeed, the difference in sensitivity between Gram positive and Gram negative bacteria can be ascribed to morphological differences between these microorganisms, above all to differences in the permeability of the cell wall.³¹

The results of MICs showed that MIC values of *B. vulgaris* root barks extracts found in this study were lower than those found in a previous work, which were on the order of 2.5 and 5 mg/mL against *E. faecalis* and *S. aureus* and 20 mg/mL against *E. coli* and *E. cloacae*, respectively.²⁵ This activity is due to berberine, an alkaloid from *Berberis* species, responsible of antimicrobial activity against Gram positive and Gram negative bacteria.³² Comparatively, the ethanolic extracts of *Cinnamomum zeylanicum* barks gave MBC values ranging from 2.5 to more than 10 mg/mL, against a variety of Gram-positive and Gram-negative bacterial strains.³³ Previous studies consider that the antibacterial effect of cinnamon was probably due to its major compound, cinnamaldehyde, whose antibacterial power has been proved.³⁴

It can be noted that MICs of methanolic extracts of *C. monspeliensis* aerial parts found in this study were lower than those found by Bouamama *et al.*, which were 25 mg/mL against *S. aureus* and *E. coli* and 50 mg/mL against *P. aeruginosa*.³⁵ However, MICs of methanolic extracts of *P. granatum* fruit peels were substantially in accordance with those previously obtained, which were of 2 and 0.5 mg/mL against *Staphylococcus aureus* and *Bacillus subtilis* and of 2 and 1 mg/mL *K. pneumoniae* and *E. coli*, respectively.²⁷ For *R. tripartita*, Habibi *et al.* have reported MICs values of leaves ethanolic extracts higher than obtained in the present study that were of 50 mg/mL against *E. coli* and *P. aeruginosa*.²⁹

In addition, it has been reported according to the literature, that MICs of leaves methanolic extracts of *W. frutescens* were 0.8 mg/mL against *S. aureus* and 0.2 mg/mL against *E. coli* and *K. pneumoniae*,³⁰ which were lower than that found in this study.

In the present study, extracts of *Cassia angustifolia* leaves, *Nigella sativa* seeds and *Zingiber officinale* dry rhizome have shown practically low

activities. The observed activities of these extracts were relatively different from those found in other works. Thus, in the study of Gnanavel *et al.*, the n-butanol leaves extracts of *Cassia angustifolia* exhibited maximum zone of inhibition against *Staphylococcus aureus* (17.0 mm), *Salmonella typhi* (12.0 mm) and *Klebsiella pneumoniae* (10.0 mm); while, methanol extracts have not shown any activity against both the isolates.³⁶ Bameri *et al.* also showed that MICs values of leaf methanol extract of *C. angustifolia* exhibited stronger activity against *K. pneumonia* and *E. coli* (0.62 and 1.25 mg/mL, respectively).¹¹

For *Nigella sativa*, it was cited that seeds oil showed pronounced antibacterial activity against *S. aureus* and *P. aeruginosa* (24.0 and 20.0 mm) and no activity against *E. coli*, at 1:10 oil dilution.³⁷ On the other hand, in earlier study of Ekwenye and Elegalam, *E. coli* and *S. typhi* were weakly sensitive to ethanolic extracts of ginger (*Z. officinale*) dry rhizome where inhibition zones diameters were of 09.0 and 10.0 mm, respectively.³⁸ Whereas, MICs of methanolic extracts of *Z. officinale* dry rhizome were of 3.50, 1.75, 1.75, and 3.50 mg/mL against *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis*, respectively.²⁰

It has been reported that the relationship between zone of inhibition and MIC values may be greatly affected by the composition of crude extracts that are mixture of phytoconstituents which may influence the diffusion power of the active constituents, and the different level of intrinsic tolerance of test strains to antimicrobials which can differ MIC values from one isolate to another.³⁹

CONCLUSION

In this study, hydromethanolic extracts of nine medicinal plant organs used in Algerian traditional medicine were assessed for their antibacterial activities. The results indicated that extracts of *Berberis vulgaris* root barks, *Cinnamomum cassia* peels, *Cistus monspeliensis* aerial parts, *Punica granatum* fruit peels, *Rhus tripartita* aerial parts and *Withania frutescens* leaves have potential antibacterial effects on bacterial strains tested, especially *S. aureus*. This was confirmed by determination of both diameters of inhibition zones and minimal inhibitory concentrations. This indicated that these plants have potentially antibacterial properties and could be used in the development of novel antibacterial agents.

Other investigations are necessary to be done on a wide range of bacteria and fungi to assess the spectrum of such plants parts extracts. Moreover, other parts of the examined plants are also needed to be assessed for their antibacterial activity. Further studies on isolation and chemical structure determination of active compounds from these extracts are necessary for their utilization to treat infections caused by pathogenic and often multi-drug resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS USED

MIC: Minimal inhibitory concentration; **DMSO:** Dimethylsulfoxid; **CFU:** Colony forming units; **ATCC:** American type culture collection; **RME:** Refluxed methanolic extract; **MME:** Macerated methanolic extract.

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SUMMARY

- This paper reported the antibacterial activity of hydromethanolic extracts of nine medicinal plants that are used in Algerian traditional medicine, against different Gram-positive and Gram-negative bacterial strains.
- The Refluxed and Macerated extracts of *B. vulgaris*, *C. monspeliensis* and *P. granatum* have shown relatively high activity, mainly against *S. aureus*, *E. faecalis* and *E. cloacae*.
- This activity supports the use of these plants extracts in the treatment of infectious diseases caused by multi-drug resistant bacteria.

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