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Antibacterial and antifungal screening of four medicinal plants

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PEER REVIEW

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Comments
This is a valuable research work in which authors have demonstrated the antimicrobial activities of four medicinal plants extracts against some organisms responsible for the majority of the majority of nosocomial and toxi-infections. The activity was assessed based on diameter of inhibition zone and MIC by diffusion disk and solid agar dilution methods. These four medicinal plants were found to be a promising source to antimicrobial agent which can treat infections caused by the tested organisms.
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ABSTRACT

Objective: To describe the ethnopharmacology study and screening of the antimicrobial activity of hydroalcoholic and chloroform extracts of the four plants *Ceratonia siliqua* (*C. siliqua*), *Salvadora persica*, *Aloe vera* and *Anastatica hierochuntica*.
Methods: The antimicrobial activity was determined using diffusion disk and solid agar dilution methods against 12 bacteria, according to the recommendations of the Clinical and Laboratory Standards Institute.
Results: The ethnopharmacology study provided useful information about how the parts used for the preparation of extracts. The extracts obtained by maceration reveal variable yields depending on the polarity of the solvent used. The higher yields were those extracts obtained by the hydroalcoholic solvents. The *Anastatica hierochuntica* and *C. siliqua* extracts were by far the most interesting and exerted significant antibacterial activity (minimum inhibitory concentration of 0.07 to 0.13 mg/mL).
Conclusions: These results suggest that *C. siliqua* could serve as an alternative source of antibacterial agents for human protection against infectious diseases.

KEYWORDS
Antibacterial activity, Antifungal activity, Ethnopharmacology, Screening

1. Introduction

Infectious diseases are so far responsible for 43% of deaths in the poor country, even if they have lost ground in developed societies (1% of all deaths) due to hygiene and urban sanitation, anti-infectives and vaccines[1].

In Algeria, as in other developing countries, infectious diseases remain until this day a public health problem because of their frequency and severity. The situation is further more concern because of the emergence of new strains and the emergence of uncommon infections that are resistant to conventional treatment[2,3].

Given these challenges posed by the use of antibacterial agents available, it is essential to find effective new antimicrobial substances and broad spectrum of action. One of the strategies of this research is to explore the plants used in traditional medicine. Plant extracts have been traditionally used in folk medicine against various diseases[4].

Indeed, 80% of the African population still uses traditional medicine for which the majority of therapy involves the use of active ingredients of medicinal plants. These plant species also important to the health of populations and should be studied scientifically to better use[5].

However, although the flora of Algeria is rich and varied, it is still little exploited scientifically especially in the field of the fight against infectious diseases[6].

The goal is to find more plants with antimicrobial

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properties as well as to rationalize the use of medicinal plants. This work focuses on four plants used effectively in various traditional treatments of various diseases in Algeria and elsewhere. The *in vitro* antimicrobial activity of hydroalcoholic and chloroformic extracts of the plants were studied against some bacteria involved in infectious diseases.

2. Materials and methods

2.1. Plant material

The proposed material for the study (Table 1) was harvested in different area at different periods and were stored in the dark at ambient temperature in our laboratory. The plants were identified and authenticated by Dr. Nadjib Rahmoun, Département de Biologie, Section of Plant Biology, Tlemcen University.

2.2. Ethnopharmacological study

In order to know the way in which the selected plants were traditionally used, it should be started initially by ethnopharmacological investigation of these plants. This study was performed by conducting a questionnaire that contains the photo, vernacular name, traditional use, used parts and extraction method of the plant. This questionnaire was given to herbalists, nurserymen, botanists, pharmacists and traditional users.

2.3. Preparation of extracts

Plant crude extracts were prepared according to the method of Sharma (1990) that was fully described in the previous paper[7]. Extraction was carried out from the crushed dry leaves. Briefly, 25 g of powdered plant material was soaked in 100 mL of solvent. Various extractions were carried out using hydroalcoholic and chloroform solvents. Each mixture was stirred for 24 h. At the end of each extraction the extract was passed through a Whatman No. 1 filter paper (Whatman, UK). The volatile filtrates obtained were concentrated under vacuum on a rotary evaporator at low temperature 30 °C. The extracts were stored at 4 °C until further use.

The extraction of *Aloe vera* (*A. vera*) was performed by the aspiration of mucilage sheets with a syringe.

The yield is calculated by the following formula:

$$\text{Rendement } (\%) = \frac{m_0}{m_1} \times 100$$

Where m_0 is evaporated extract mass, and m_1 is initial vegetal mater mass.

2.4. In vitro biological assay

2.4.1. Evaluation of the antibacterial activity

The *in vitro* antimicrobial activity of the examined extracts was assessed by the Kirby-Bauer disk diffusion and the solid agar dilution methods, according to the recommendations of the National Committee for Clinical Laboratory Standards[8,9].

A panel of 12 well-documented pathogenic bacteria was used in the study. The following bacteria, obtained from the laboratory, Antibiotiques Antifongiques: Physico-Chimie, Synthèse et Activité Biologique, Département de Biologie, Tlemcen University, were used: *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Escherichia coli* ATCC 25922 (*E. coli*), *Salmonella typhimurium* ATCC 13311 (*S. typhimurium*), *Acinetobacter baumanii* ATCC 19606 (*A. baumanii*), *Klebsiella pneumonia* ATCC 700603 (*K. pneumonia*), *Enterobacter cloacae* ATCC 13047 (*E. cloacae*), *Citrobacter freundii* ATCC 8090, *Proteus mirabilis* ATCC 35659 (*P. mirabilis*) (Gram-negative bacteria), *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus cereus* ATCC 10876 (*B. cereus*), *Enterococcus faecalis* ATCC 49452 (*E. faecalis*), *Lysteria monocytogenes* ATCC 15313(Gram-positive bacteria), and the yeasts: *Candida albicans* ATCC IP 444 (*C. albicans*), *C. albicans* ATCC 10231, *C. albicans* ATCC 26790. These are Gram-positive and Gram-negative bacteria known to be the cause of many infectious diseases such as skin diseases, respiratory, digestive and urinary systems.

3.4.2. The disk diffusion method

The disk diffusion method against bacteria and yeasts was fully described in the previous paper[10]. Positive controls were made with gentamycin and ciprofloxacin for bacteria. Amphotericin B was used as positive control for yeasts. An additional negative control disk without any sample but impregnated with the equivalent amount of dimethyl sulfoxide solvent was also used in the assay. The antimicrobial activity was considered beyond a diameter of 9 mm or more. The data were the mean of three replicates.

3.4.3. Solid agar dilution method

Mueller-Hinton agar medium was prepared in the flasks and sterilized. To 19 mL this medium, 1 mL of extract was added in order to get the fold serial dilution desirable. The Petri dish was thoroughly mixed by stirring. A negative control was also prepared in the same way using solvent dimethyl sulfoxide and water. The Petri dish was inoculated with 1 μL of bacterial suspension adjusted and diluted to

Table 1

Parts, harvest periods and regions of the used plants.

Local name	Family/Latin binomial	Voucher specimen N°	Tested part	Harvest period	Harvest area
Carob	Leguminosae/*C. siliqua*	LBPes C.S. 15.02	Fruits and seeds	April 2013	Nédroma (Algeria)
Sabar	Aloaceae/*A. vera*	010102	Leaves	May 2013	Marsa El Kbir (Algeria)
Siwak	Salvadoraceae/*S. persica*	2215	Roots	April 2013	Timimoune (Algeria)
Kaff maryam	Cruciferae/*A. hierochuntica*	14562	Whole plants	May 2013	Mecca (Saudi Arabia)

the concentration of 10^7 CFU (0.5 McFarland standard diluted to 10%), and the final inoculum required is 10^4 CFU per spot. Gentamycin and ciprofloxacin were used as positive controls for bacteria. The minimum inhibitory concentration (MIC) of *C. albicans* was performed as for bacteria but the culture medium used was supplemented with Mueller-Hinton, 2% glucose and 0.5 μg/ mL methylene blue with pH of 7.4. The fungal suspension was set at 0.12-0.15 (λ=530 nm) at final concentration of 1×10^3-5×10^3 CFU. Amphotericin B was used as positive control.

All the plates were inoculated at 37 °C for 24 h. The MIC (of bacteria and yeasts) was considered as the weakest concentration for which there was no visual growth. The data were the mean of three replicates.

3. Results

3.1. Ethnopharmacological study

This study was carried out by performing a questionnaire which was placed at the disposal of users of various professions. The results obtained are shown in Table 2. It can be noted that the fruits are the common part of the four plants. All the plants were used traditionally in different treatment and with different extraction methods.

Table 2

Ethnopharmacological uses of the medicinal plants tested.

Plants	Traditional uses	parts used	Modes of uses
A. vera	Beauty products Healing, Moisturizing	Roots, leaves	Dry powder
A. hierochuntica	Hypoglycemic, Regulator of steroid, Hormones	Roots, leaves, seeds and fruits	Infusion, maceration and dry powder
C. siliqua	Stomach problems, Diarrheal, Food (chocolate)	Seeds and fruits	Infusion, maceration and dry powder
S. persica	Tooth brushing, Against bacteria, Mouth, Gum problems	Roots and fruits	Infusion, maceration and dry powder

3.2. Yield

The preparation of extracts from different parts of selected plants was performed using two solvents of different polarities. The yields obtained by different extracts are shown in Table 3. All hydroalcoholic extracts showed the best yield compared with the results of the chloroform extracts. *A. vera* showed a yield of about 22.00%.

Table 3

Extraction yields of the studied plants.

Plant	Part	Solvents	Concentration (g/mL)	Yield (%)
C. siliqua	Seeds	Hydroalcoholic	7.00	1.60
		Chloroforme	0.16	1.08
S. persica	Roots	Hydroalcoholic	7.00	10.00
		Chloroforme	0.06	1.12
A. hierochuntica	Whole plant	Hydroalcoholic	7.00	2.00
		Chloroforme	0.09	1.40
A. vera	Mucilage	–	5.50	22.00

The extracts were prepared by solid-liquid extraction, using solvents with different polarities. There is a great influence of the solvent polarity on yield. The latter increases with the polarity of the solvent, which has been proved by many previous works conducted on other plants[7,11]. These studies show that the evaluation of the antimicrobial activity must begin with the polar solvent.

3.3. Antibacterial activity

Evaluation of the antibacterial activity of hydroalcoholic and chloroform extracts of the studied plants was determined initially by the disk diffusion method against different bacteria. These bacterial strains are Gram-negative species frequently encountered in infectious diseases.

The results of the diameters of inhibition zones are shown in the Table 4. It can be noted that the most interesting plant is *Ceratonia siliqua* (*C. siliqua*) which showed antibacterial activity against five Gram-negative bacteria. The diameters of the zones of inhibition were between 15 mm and 30 mm against *A. baumanii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. typhimurium*. The hydroalcoholic and chloroform

Table 4

Antimicrobial activity of the tested extracts.

Bacteria		*C. siliqua*		*S. persica*		*A. hierochuntica*		*A. vera*	Gentamycin	Ciprofloxacin	Amphotericin B
		Chloroforme	Hydroalcohol	Chloroforme	Hydroalcohol	Chloroforme	Hydroalcohol				
Gram- negative	*A. baumanii*	26	15	8	20	18	7	8	14.50	24.5	–
	C. freundi	7	9	6	10	6	6	6	26.00	32.5	–
	E. cloacae	10	6	7	8	6	6	10	18.00	20.0	–
	E. coli	16	18	7	6	7	8	6	21.50	36.0	–
	K. pneumoniae	7	24	7	8	6	8	6	14.00	24.0	–
	P. aeruginosa	30	24	15	13	25	19	10	21.33	33.0	–
	P. mirabilis	6	7	6	6	6	6	7	23.00	33.5	–
	S. typhimurium	24	22	7	6	9	8	10	25.00	35.5	–
Gram- positive	*B. cereus*	30	24	20	23	10	9	6	23.00	33.5	–
	E. faecalis	22	16	7	8	7	7	7	26.00	32.5	–
	L. monocytogene	23	18	8	6	6	7	6	18.00	20.0	–
	S. aureus	8	26	7	16	6	10	6	21.33	33.0	–
Yeasts	*C. albicans* ATCC IP 444	30	11	17	11	25	21	10	–	–	20.0
	C. albicans ATCC 10231	10	11	18	10	9	12	7	–	–	21.0
	C. albicans ATCC 26790	9	9	13	8	8	17	11	–	–	21.6

–: no MIC observed.

Table 5

MICs (mg/ mL) of the tested extracts against bacteria.

Bacteria		*C. siliqua*		*S. persica*		*A. hierochuntica*		*A. vera*	Gentamycin	Ciprofloxacin	Amphotericin B
		Chloroforme	Hydroalcohol	Chloroforme	Hydroalcohol	Chloroforme	Hydroalcohol		(MIC in μg/ mL)	(MIC in μg/ mL)	(MIC in μg/ mL)
Gram-	*A. baumanii*	0.250	_	_	43.75	11.25	_	_	8.00	0.50	–
negative	*C. freundi*	2.000	21.87	0.75	0.35	0.07	5.47	_	0.50	0.16	–
	E. cloacae	0.130	_	1.50	_	_	_	4.29	0.50	0.25	–
	E. coli	0.130	5.46	_	43.75	_	_	_	0.50	0.08	–
	K. pneumoniae	_	_	0.05	0.35	_	_	34.38	8.00	0.25	–
	P. aeruginosa	0.130	175.00	0.05	5.47	0.07	87.50	34.38	0.50	0.25	–
	Proteus mirabilis	_	_	_	_	_	_	_	0.50	0.08	–
	S. typhimurium	_	_	_	_	_	87.50	137.50	0.25	0.08	–
Gram-	*B. cereus*	1.000	0.70	43.75	43.75	_	_	_	0.50	0.64	–
positive	*E. faecalis*	0.130	5.46	_	_	_	_	_	16.00	0.25	–
	L. monocytogene	5.000	87.50	_	–	–	_	_	8.00	0.25	–
	S. aureus	–	0.13	_	43.75	_	_	–	0.50	0.25	–
Yeasts	*C. albicans* ATCC IP 444	0.125	175.00	0.47	175.00	2.25	43.75	_	–	–	2
	C. albicans ATCC 10231	0.125	43.75	_	87.50	_	_	34.38	–	–	4
	C. albicans ATCC 26790	2.000	43.75	_	87.50	0.07	5.47	–	–	–	8

–: no MIC observed.

extracts of this plant showed diameters were close. The plants *Salvadora persica* (*S. persica*) and *Anastatica hierochuntica* (*A. hierochuntica*) showed good activities against *A. baumanii* and *P. aeruginosa*. The diameters of the inhibition zones were between 13 and 25 mm.

Finally, the *A. vera* showed no interesting activity against all Gram-negative bacteria. This can be explained by the inadequate method used in the extraction. *P. aeruginosa* has been the most sensitive against the whole plants extracts of *C. siliqua*, *S. persica* and *A. hierochuntica*.

The strain *B. cereus* was very sensitive to the whole extracts except that of *A. vera*. All Gram-positive strains were susceptible to both extracts *C. siliqua*, except *S. aureus* that was resistant to chloroform extract. The diameters of the zones of inhibition were between 16 mm and 30 mm. *S. persica* has shown inhibition zones whose diameter were between 16 mm and 23 mm against of *B. cereus* and *S. aureus* strains. *A. vera* and *A. hierochuntica* showed no interesting activity against all positive bacteria grams.

The results of MICs of the Gram-negative and Gram-positive bacteria are shown in Table 5. It can be noted that the best results were obtained with chloroform extract of all plants. The lowest MICs obtained by *C. siliqua* were 0.130 mg/mL against *P. aeruginosa*, *E. coli* and *E. cloacae*. MICs against *C. freundi* and *A. baumanii* were 0.200 and 0.250 mg/ mL respectively. In addition, the lowest MICs obtained by *S. persica* were 0.05 mg/mL against *K. pneumoniae* and *P. aeruginosa*. MICs against *K. pneumoniae* and *C. freundi* were 0.35 mg/mL. Other results were between 0.35 and 43.75 mg/ mL. The plant *A. hierochuntica* showed MICs of 0.07 mg/mL against *C. freundi* and *P. aeruginosa*. Contrary to what was expected, the lowest MIC of *A. vera* was 4.29 mg/mL against *E. cloacae*. The lowest MIC of *C. siliqua* was 0.130 mg/mL against *E. faecalis* for the chloroform extract and against *S. aureus* for the hydroalcohol extract. However, *S. persica* showed a rather high MIC (43.75 mg/mL) against *B. cereus* and *S. aureus*.

Against *C. albicans*, chloroform extracts was more active than hydroalcohol extracts of the all plants (Table 5). The lowest MIC was recorded for *A. hierochuntica* (0.07 mg/mL) and *C. siliqua* (0.125 mg/mL).

4. Discussion

The antimicrobial properties and the use of four plants to fight against infectious diseases have been reported in several studies. Methanolic extracts of *A. hierochuntica*, *C. siliqua* have antioxidant and antimicrobial properties[12-14]. Miswak acts as antibacterial agent. While studying the effect of miswak pieces on bacteria in periodontitis and dental caries, Chaurasia *et al*. concluded that the antibacterial effect was most pronounced on *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Haemophilus influenzae*, less than on *Streptococcus mutans*, and least on *Lactobacillus acidophilus*[15]. They also reported that the antibacterial effect of miswak suggests the presence of volatile active antibacterial compounds. Whereas, in our investigation, *A. vera* extract does not show any interesting activity. These findings have also been mentioned in literatures, especially that this plant is well known for its polysaccharides and anthraquinone derivatives[16].

Finally, antimicrobial extracts from tested plants can be assumed to be useful to the producing plant in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens. The screening assays justify the use of the investigated plants in the Algerian ethnomedicine.

The results of these screening investigations confirm the great potential of plants of the Algerian ethnomedicine for production of bioactive compounds and are useful for rationalizing the use of medicinal plants in primary health care. The results complement a major research endeavor in establishing a relationship between the use of plants by scientific communities and especially clinical knowledge of the plant. The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

In Algeria, as in other developing countries, the situation of the development of multiple resistances in human pathogenic microorganisms, the undesirable side effects of certain antibiotic, and the emergence of previously uncommon infections have forced scientists into looking for new antimicrobial substances from various sources like medicinal plants.

Research frontiers

The current investigation evaluates the ethnopharmacology and *in vitro* antimicrobial activity of hydroalcoholic and chloroform extracts of four medicinal plants against twelve bacteria and three yeast.

Related reports

The four tested plants were used effectively in various traditional treatments of various diseases in Algeria and elsewhere.

Innovations and breakthroughs

The present work studied the antimicrobial activities of four medicinal plants extracts; three were cultivated in Algeria for the first time. The study revealed the promising antimicrobial activities of the different extracts with variable degrees.

Applications

Extracts of these four medicinal plants are good alternative antiseptics which can be used in the treatment of infections caused by the tested organisms.

Peer review

This is a valuable research work in which authors have demonstrated the antimicrobial activities of four medicinal plants extracts against some organisms responsible for the majority of the majority of nosocomial and toxi-infections. The activity was assessed based on diameter of inhibition zone and MIC by diffusion disk and solid agar dilution methods. These four medicinal plants were found to be a promising source to antimicrobial agent which can treat infections caused by the tested organisms.

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