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Antimicrobial Activity of 22 Plants Used in Urolithiasis Medicine in Western Algeria

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ABSTRACT

Objective: Our investigation is about the determination of the antibacterial efficiency of 22 medicinal plants on the four most frequent bacteria in urinary infections. These infections are responsible for more than 15% of urinary stones formation. **Methods:** We have initiated an extraction liquid/solid. In this respect, we have used water extractions according to the standard methods utilized by the local population, i.e: (i) the décoction, (ii) the infusion, (iii) the macération and (iiii) the percolation. The microorganisms used are *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The strains were isolated from patients having urinary infections. The antibiotic disks Kanamicin, Colistin, Amoxicillin, Gentamicin, Ampicillin were applied to the reference bacteria at concentrations of 30 μ g, 50 μ g, 25 μ g, 10 μ g et 10 μ g respectively. **Results:** These studies showed that decoction (d) had the higher effect with 43.3% followed by percolation (p) (28.3%) and maceration (m) (16.7%). Infusion (i) had a limited effect (11.7%). *Escherichia coli* (*E.coli*), *Proteus mirabilis*, *Staphylococcus aureus* showed an average sensitivity of 28% in each case. However, *Pseudomonas aeruginosa*, a highly pathogenic and resistant bacteria showed up to 17.5% of sensitivity. 16.3% of the plant extracts showed a high antimicrobial activity. *Pseudomonas aeruginosa* was highly resistant to Kanamycin, Amoxicillin and Ampicillin and at a lower extent to Colistin and Gentamicin. However, it was sensitive to some plant extracts such as *Allium sativum*, *Artemisia compestris*(p,m), *Citrus aurantium*(p), *Cotula cinerea*(p), *Lavandula officinalis* (d), *Globularia alypum* (d), *Juniperus phoeniceae* (m), *Olea europaea* (p), *Pistacia lentiscus* (m), *Trachyspermum ammi* (m), *Zygophyllum album* (p) and *Zingiber officinalis* (d). **Conclusion:** The present work shows that most of the studied plants are potentially a good source of antimicrobial agents and it proves the importance of such plants in urolithiasis medicine and alternative healthcare.

1. Introduction

Plants represent an extraordinary reservoir of new preventives and curatives molecules. In the world, up to 500.000 plant species are reported [1]. Few of them are used in herbal medicine. The medicinal properties of plants come from the presence of bioactive agents in their extracts. The most important elements are alkaloids, flavonoids, vitamins, tannins, essential oils, organic acids, resins, fat oils, saponins

and polysaccharides [2–4]. The literature indicates that the antibacterial activity is due to different chemical agents in the extract, including essential oils, flavonoids, triterpenoids and other natural phenolic compounds. They are recognized as active antimicrobial compounds [4–7]. Many pharmacology, chemistry and toxicology laboratories are interested in exploiting new bioactive products from plants. New extraction and identification methods are continuously developed.

The Mediterranean region is relatively rich with plants (between 15.000 and 20.000 species) [8]. Algeria, a North African country with a large variety of soils (littoral, steppe, mountains and desert) and climates, possesses a rich flora (more than 3.000 species and 1.000 genders) [9].

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The Algerian population has used plants since time immemorial to treat various types of diseases. Plant extracts are widely used as antibacterial, antidiuretic, anti-inflammatory agents, antioxidants, antiseptics, antispasmodics, fungicides, pesticides and remedies for flu and intestinal transit disorders. These practices are based on traditional and cultural beliefs. The development of herbal medicine led to the identification of many plants deemed in folk medicine as curative or preventive remedies for many diseases. This empiric knowledge could be used as a very useful source of data in order to build a solid scientific herbal medicine. During the last decade, the use of herbal medicine has increased. People have fallen back on their ancestral knowledge of everyday remedies. This is mainly due to the high cost of hospital treatment and medicines.

The high incidence of stone urinary infection in Algeria and in developing countries made the study of plants antimicrobial activity against urinary urolithiasis germs necessary. More than 200 bacteria species are responsible for urinary infections and use urea as a nitrogen source. Many bacteria adhere easily to the urinary mucous membranes. Some of them developed resistance towards conventional antibiotics. Therefore new attempts are being made in order to search for new antimicrobial agents and thus to overcome the problems of resistance and side effects of the currently available antimicrobial agents [10]. In Algeria, our preliminary studies showed that 25.2% of phosphate ammonia magnesium (PAM) stones have an infectious origin due to bacteria adhering to the urinary mucous membranes. The most frequent species are *E. coli* (29.7%), *Proteus*, *Pseudomonas* and *Staphylococcus* [11]. The *Proteus* group is the prime cause of urinary stone formation. It is particularly sticky to the renal epithelial cells and it is resistant to many antibiotics. By several mechanisms, it participates in the genesis and the development of ammonia-magnesium phosphate stone (MAP). This formation is related to the action of the urease produced by the micro-organism. This type of stone results from a super saturation of urine with magnesium, ammonium, phosphate and carbonate. Germs not producing urease such as *E. coli* could help the phosphate calcium crystals precipitation [12]. *E. coli*, as *Proteus*, is able to deteriorate the surface of the epithelium thus generating an inflammatory reaction and the adherence of bacteria and crystals to the membranes [13,14]. These various phenomena lead to lithogenesis [15].

An ethnobotanical enquiry in the west of Algeria allowed us to select plants known for their antimicrobial activity. In the present work, we establish the antimicrobial effect of 22 medicinal plants on four bacterial strains identified during urinary infections.

2. Material and Methods

2.1. Ethnobotanical survey

The ethnobotanical survey was conducted in Mostaganem

region (littoral town) and in Tiaret (high plateaux town, 150 km from the sea). In these regions, many people with kidney problems were using medicinal plants and were followed by our laboratory. The ethnobotanical survey showed that plants were used in herbal medicine for their antiseptic, astringent and antispasmodic properties in the urinary system. All the parts were exploited: leaves, roots, seeds and flowers. Some mixtures like *Myrtus communis*, *Thymus vulgaris* and *Lavandula officinalis* were used in decoction against urinary infection.

2.2. Samples collection

We selected 22 plant species frequently used in traditional medicine in the west of Algeria (Table 1). These Mediterranean plants were collected during springs 2004 and 2005. The samples were open air dried away from sunlight. Leaves and flowers were isolated from the rest of the sample and were conserved in plastic bags for further analysis. The plant species were stored until used at the STEVA laboratory, Sciences faculty, Mostaganem University.

2.3. Aqueous Extraction method

Aqueous extracts were prepared in distilled water. The extract was prepared using a procedure similar to the popular one, with some minor modifications. The fresh material (20g) of each selected plant was air dried at room temperature. Then it was ground. Each dry powdered plant was infused, decocted, percolated and macerated in distilled water. During infusion, the powder was left submerged in boiled water for 15 to 20 minutes. The container was covered in order to keep all active elements. Decoction (d) is obtained by boiling the powder in water during 5 to 10 minutes. Maceration (m) results from putting the powder in cold water for 24 hours. Percolation (p) involves sending repeatedly water steam through the powder. It is a fast and efficient method, especially used for leaves and flowers.

Then the extract was filtered using muslin or Whatman paper no.1. The filtrate obtained was put on a sterile glass plate and covered with foil. Then it was dried at 40°C overnight. The water evaporated and the final extract powder was collected and stored in a dry cool place. The powder obtained was used to prepare solutions at 50mg/ml concentrations in distilled water.

2.4. Antibacterial activity tests.

2.4.1. Micro-organisms

The test strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 11775, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 10145 used for the bioassay were obtained from the America Type Culture Collection. The same species were isolated from patients with urinary infection

Table1

Ethnobotanical uses of the medicinal plants tested

N°	Latin binomial/Family	Local name	Tested part	Pharmaceutical utility
1	<i>Allium sativum</i> (Liliaceae)	Thoum	seeds	L: facilitates the digestion, hypertensive, anti-hair loss, anti-cholesterol. R: anti-fermentation and intestinal gases, vermifuge, bactericide, Immuno-stimulant, antibiotic, lung affection; diuretic; stomachic and hypertensive. In large quantities irritate the urinary and digestive systems. hypoglycaemic, antiseptic urinary, cholagogue, antidote poison
2	<i>Ajuga iva</i> . (Lamiaceae)	Chendgoura	leaves	L: antispasmodic, headaches, kidneys and bladder, Anti-diabetes, antifungal activity, antimicrobial, disinfectant, healing stomach, anti-hypertensive, diuretic, febrifuge. R: rheumatism; hypoglycemic; anti-inflammatory, antifungal, antimicrobial, hypothermic, anthelmintic; used against the headaches, kidney stones and bladder, disinfectant; used against gastro-intestinal, anthelmintic
3	<i>Artemisia compestris</i> (Asteraceae)	Dgoufet	leaves	L: desintoxicating, calm the ventral pains, vermifuge, antibacterial, anti-inflammatory. R: emmenagogue, digestive, vermifuge; hypoglycaemic anti-epileptic, vulnerary, vermifuge; used against the constipation, mycosis
4	<i>Cotula cinerea</i> (Asteraceae)	Gartoufa	leaves	L: antispasmodic, anti diarrhoeal, desintoxicating. R: anti colic, aromatize the tea, cough, anti diarrhoea, bronchial, laryngeal and pulmonary and scent milk
5	<i>Citrus aurantium</i> (Rutaceae)	Bourtoukal	leaves, zest	L: antispasmodic, sedative, calming R: sedative, antispasmodic; calms epilepsy; nervous system, migraine
6	<i>Erica multiflora</i> . (Ericaceae)	Khalnadj	aerial	L: diseases of the kidneys and the urinary bladder lithiasis, stain-resisting of skin. R: astringent, urinary and diuretic disinfectant
7	<i>Foeniculum vulgare</i> (Apiaceae)	Besbass	leaves	L: problem of colon, urinary lithiasis, expectorant, emmenagogue, antispasmodic. R: stimulative, carminative, galactagogue, emmenagogue, diuretic; used against the sluggishness of the bowels, cystitis, bronchitis, gastritis, enteritis
8	<i>Globularia alypum</i> .L (Globulariaceae)	Tesselgha	leaves	L: anti cholesterol, constipation R: purgative, cholagogue, purgative, cholagogue, stimulative, disinfectant, mycosis; hypoglycaemic, laxative, cholagogue, sedative, stomachic; against the gastric and pulmonary haemorrhages; laxative,
9	<i>Juniperus phoeniceae</i> (Cupressaceae)	Arar phoeniki	fruits	L: pesticide, detergent (external use), urinary infection, Stomach pain, leaves decocted (diabetes, diarrhoea and rheumatism) the fruits (ulcerations of the skin and the abscesses); antidiabetes, used against the pulmonary affections, diuretic.
10	<i>Lavandula officinalis</i> (Lamiaceae)	khezama	flowers	L: problem of colon, urinary infection, draft skin affections and wounds, burns, rheumatic. R: antispasmodic, tonic, disinfectant, diuretic, sedative, stomachic, cholagogue, migraine; relieve, nervous anxiety, cramps, tonic, disinfectant, diuretic, sedative, rheumatism; hypoglycaemia; diuretic, disinfecting, sudorific
11	<i>Myrtus communis</i> (Myrtaceae)	Rayhan	leaves	L: urinary infection. R: disinfectant, astringent, diuretic, anti-haemorrhoid, antispasmodic, haemostatic, antibacterial activity; for respiratory diseases, urinary, disinfectant, astringent, anti-diarrhoeal, disorder gastro- intestine.
12	<i>Mespilus germanica</i> (Rosaceae)	Zaarour	leaves	L: anti lithiasis. R: hypoglycaemia
13	<i>Mentha viridis</i> (Lamiaceae)	Naânaâ	leaves	L: abdominal pain, distension, vomiting, facilitates digestion, constipation, slows hair loss, hypo tensor. R: stomachic, carminative and disinfectants; giddiness (
14	<i>Olea europaea</i> .L (Oleaceae)	Zitoun	leaves	L: hypo tensor, diuretic, antidiabetic, facilitate the hepatic functions, febrifuge, antioxidant R: Anti-malaria; lithiasis, colic or nephritic; hypoglycaemia, hypotensive, cholagogue, anthelmintic
15	<i>Pallenis spinosa</i> (Asteraceae)	Nougð	leaves	L: eczema R: plant anti-Rheumatism, muscular contraction, tire, vomiting for the new one born, diabetes, headaches, disinfecting

16	<i>Pistacia lentiscus</i> (Anacardiaceae)	Dharou	leaves	L: facilitate digestion, astringent, diuretic, cholesterol. R: against eczema, paralysis, astringent, diarrhoea anti-inflammatory, against renal gallstones, antispasmodic stimulative
17	<i>Rhamnus alaternus</i> (Rhamnaceae)	Meliless	leaves	L: icterus R: purgative, diuretic, depurative
18	<i>Teucrium polium</i> .L. (Lamiaceae)	Jaâda	leaves	L: anti-inflammatory drug, anti-diarrhoea, anti-bacterial, anti-inflammatory drug, facilitates digestion, disinfectants. R: anti-diabetes, anti-eczema, tonic, for skin diseases
19	<i>Thymus vulgaris</i> .L. (Lamiaceae)	Zaâter	leaves	L: general infectious diseases. R: stimulative, disinfectant, bactericide, stomachic, choleric, expectorant, antispasmodic and vermifuge
20	<i>Trachyspermum ammi</i> .L. (Apiaceae)	Noukha	leaves	L: Plants very much used against several diseases, giddiness, antilithic, hair loss.
21	<i>Zygophyllum album</i> (Zygophyllaceae)	Agaya	leaves	L: antibacterial, anti-diarrhoeal, anti urinary lithiasis. R: hypoglycemia, antispasmodic, anti eczema; anti-rheumatic, gastric pain, liver diseases; dermatitis
22	<i>Zingiber officinalis</i> (Zingiberiaceae)	Skenjebir	root	L: anti-rheumatic, anti-bronchitis, Vomiting related to the disorder of voyage. R: anti-hepatic, antispasmodic, diseases of the kidneys, and the bladder

R: anti-hepatic, antispasmodic, diseases of the kidneys, and the bladder

L: Phytotherapy used in Western Algeria; R: References

Table 2

Antibiotics against Gram- and Gram+ bacteria

Antibiotics	Inhibition Zone (mm)			
	Gram –			Gram +
	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Staphylococcus aureus</i>
Kanamycin K (30 μ g)	25	Resisting	25	30
Colistin CL (10 μ g)	27	16	16	20
Amoxicillin AMX (25 μ g)	30	Resisting	15	15
Gentamicin GM (10 μ g)	30	14	25	45
Ampicillin AM (10 μ g)	22	Resisting	30	06

in western Algeria and were identified using standard methods [16].

Two culture media: King B and Hektoen were used for *Escherichia coli* ATCC 11775, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 10145. Chapman medium was used for *Staphylococcus aureus* ATCC 25923. The antibiotic discs of Kanamycin (30 μ g per disc), Colistin (10 μ g per disc), Amoxicillin (25 μ g per disc), Gentamicin (10 μ g per disc), Ampicillin (10 μ g per disc), were applied to the bacteria of reference. For most species, the medium selected for bacterial sensitivity was Mueller–Hinton [17,18]. This medium was inoculated with the studied strain, and incubated at 37°C for 24 hours. A bacterium is sensitive if its growth is inhibited by the antibiotic or the plant extract disc.

2.4.2. Effect of plant extracts on bacterial growth

Sterile filter paper discs (diameter: 5 mm) were impregnated with selected concentrations of medicinal plants extracts. Each disc was deposited on the surface of a solid Mueller – Hinton medium already inoculated with a pure culture of the tested bacteria [15] (Owlia, Qorban, Effat & Horieh, 2001). The extract diffused, in a uniform way, around the disc. The concentration is inversely proportional to the diameter of the disc. Petri dishes were incubated for 24 hours at 37°C

. The discs were surrounded by circular zones of inhibition and could be analyzed. The sensitivity of the bacteria was estimated by measurement of the inhibition zone diameter in mm. An average zone of inhibition was calculated for the three replicates.

3. Results

The therapeutic quality of plants is related to the growth environment, the technique of harvesting, the vegetative cycle and the parts used. Active ingredients of medicinal plants are usually complex and work synergistically. According to Rojas [3] the antimicrobial components are mainly essential oils, flavonoids, triterpenoids and others phenol compounds.

3.1 Antibiotics activity

On each plate an appropriate reference antibiotic disc was applied depending on the tested microorganisms [19]. Kanamycin and Gentamicin served as positive controls for *E. coli*, *S. aureus* and *P. mirabilis* whereas Colistin and Gentamicin served as a positive control for *P. aeruginosa*. The antibiotics: Kanamycin (30 μ g per disc), Colistin (10 μ g

Table 3
In vitro antibacterial activity of selected plants from Algeria

Botanical name	Inhibition zones (mm)* against															
	Pseudomonas aeruginosa				Escherichia coli				Staphylococcus aureus				Proteus mirabilis			
	i	d	m	p	i	d	m	p	i	d	m	p	i	d	m	p
1–Allium sativum L	–	–	13	–	10	12	07	10	10	13	14	10	10	12	10	12
2–Ajuga iva L	–	07	–	–	12	18	11.5	12	10	17	09	12	13	13	07	15
3–Artemesia campestris L	–	–	13	12	07	13	13	12	–	12	10	13	10	13	13	13
4–Cotula cinerea L	07	–	07	14	13	20	17	15	15	14	17	13	14	11	14	13
5a– Citrus aurantium L(zeste)	08	08	–	–	15	14	15	12	10	12	10	11	14	09	14	12
5b–Citrus aurantium L (leave)	–	–	–	12	11	12	09	12	08	10	10	–	12	11	11	10
6–Erica multiflora L	08	–	08	09	12	11	13	13	13	09	12	09	12	10.5	12	14
7–Foeniculum vulgare	00	09	–	09	13	17	12	12	11	15	13	10	12	18	13	07
8– Globularia alypum L	07	13	10	10	13	10	12	10	15	10	12	14	12	10.5	10.5	10
9–Juniperus phoeniceae	–	10	13	10	–	16	8.5	17	10	16	07	14	10	14	11	15
10–Lavandula officinalis	–	13	10	07	08	15	13	15	08	13	16	15	08	10	14	16
11–Myrtus communis	11	09	11	07	10.5	09	13	15	–	10	14	13	12.5	09	11	15
12–Mespilus germanica	07	–	07	09	16	15	13	08	15	16	14	13	15	17	12	12
13–Mentha viridis L	–	–	07	–	13	12	12	14	10	12	10	11	10	10	10	10
14–Olea europaea L	08	07	10	12	10	15	10	12	11	15	11	12	09	15	10	10
15–Pallenis spinosa L	08	07	08	11	15	15	15	12	12	15	15	15	10	14	12	13
16–Pistacia lentiscus L	–	–	13	–	12	15	14	13	10	17	13	14	10	18	12	20
17–Rhamnus alaternus L	–	09	10	–	14	15	13	12	13	15	10	13	10	20	07	14
18–Teucrium polium L	–	–	–	–	12	10	11	15	11	10	08	13	10	10	09	15
19–Thymus vulgaris L	07	08	10	10	10	10	14	09	11	12	–	14	13	16	10	–
20– Trachyspermum ammi L	–	–	12	13	10	12	12	13	00	14	15	14	10	11	17	15
21–Zingiber officinalis L	–	12	08	10	08	14	14	15	10	13.5	12	16.5	11	13.5	13	16
22–Zygophyllum album L	07	08	08	12	13	12	13	14	13	15	15	15	18	15	15	14

*Disc diameter, 5mm; concentration 50mg/ml; 10 µ l/disc

i: infusion; d: decoction; m: maceration; p: percolation; – : no inhibition

per disc), Amoxicillin (25 µ g per disc), Gentamicin (10 µ g per disc) and Ampicillin (10 µ g per disc) were applied to the Gram negative and Gram positive bacteria. The results given in table 2 show the inhibition zones in mm. the bacterium Pseudomonas aeruginosa had a resistance for three antibiotics: Ampicillin, Kanamycin, Amoxicillin and a low sensitivity for Colistin and Gentamicin. The antibiogram helps for the choice of an antibiotic treatment adapted for each patient

3.2 The antibacterial effect of Plants extracts

The search for antibacterial activity was carried out on a series of 22 plant species, belonging to 15 Families, traditionally used by the Algerian Western population (table 1). The bacterial strains tested were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. If the inhibition zone exceeds 15mm in diameter, we consider the antimicrobial activity as very good. If the diameter is between 15mm and 8mm, the antibacterial activity is average. For diameters below 8mm, the antibacterial activity is weak. On the 368 tests done, 61 (16.6%) showed an inhibition zone below 8mm, 247 (67.1%) showed an inhibition zone between 8mm and 15 mm and 60 (16.3%) showed an inhibition zone above 15 mm. Our results are summarized in table 3 and table 4.

Table 4.

Good antibacterial activity (the inhibition zone diameter is equal or above 15mm).

Plants	<i>E. coli</i>	<i>S. aureus</i>	<i>P. mirabilis</i>
Ajuga iva L	(d)	(d)	(p)
Cotula cinerea L	(d,m,p)	(i,m)	
Foeniculum vulgare L	(d)	(d)	(d)
Juniperus phoeniceae L	(d,p)	(d)	(p)
Lavandula officinalis L	(d,p)	(m,p)	(p)
Myrtus communis	(p)		(p)
Mespilus germanica	(i,d)	(i,d)	(i,d)
Olea europaea L	(d)	(d)	(d)
Pistacia lentiscus L	(d)	(d)	(d,p)
Pallenis spinosa L	(i,d,m)	(d,m,p)	
Rhamnus alaternus L	(d)	(d)	(d)
Teucrium polium L	(p)		(p)
Trachyspermum ammi L		(m)	(m,p)
Thymus vulgaris L			(d)
Zingiber officinalis L	(p)	(p)	(p)
Zygophyllum album L		(d,m,p)	(i,d,m)

(i) infusion; (d) decoction ; (m) maceration ; (p) percolation.

4. Discussion

For *E. coli* and *S. aureus*, decoction gave the best results, whereas both percolation and decoction suited *P. mirabilis*. No plant had a good activity on *Pseudomonas aeruginosa*, known to be a resistant and very pathogenic bacterium [20]. This bacterium was slightly sensitive to the following medicinal plants: *Allium sativum*(m), *Artemesia campestris*(p,m), *citrus*

aurantium (p), *Cotula cinerea* L (p), *Lavandula officinalis* (d), *Globularia alypum*(d), *Juniperus phoeniceae* (m), *Olea europaea* L (p), *Pistacia lentiscus* L (m), *Trachyspermum ammi* L (m), *Zygophyllum album*L (p), *Zingiber officinalis*L (d).

From these results, we can conclude that some plants such as *Foeniculum vulgare* (d), *Juniperus phoeniceae* (d,p), *Lavandula officinalis* (p), *Mespilus germanica* (i,d), *Olea europaea* L (d), *Rhamnus alaternus*(d) and *Zingiber officinalis*L (p) have a very good activity on bacteria involved in urinary urolithiasis.

If we compare our results to those given in traditional use (table 1), we notice that of the 16 plants with very good activity, eight plants (*Cotula cinerea* L, *Pallenis spinosa* L, *Pistacia lentiscus* L, *Rhamnus alaternus* L, *Teucrium polium* L, *Thymus vulgaris* L, *Trachyspermum ammi* and *Zygophyllum album* L) were not commonly used for urinary urolithiasis. And the remaining nine plants were used for urolithiasis but not specifically for urinary infection. Our work shows that these plants are much more useful than traditionally believed.

The mode of extraction effect on the antimicrobial activity showed that the decoction had the highest effect with 43.3%, followed by percolation with 28.3%, then maceration with 16.7% and finally infusion with only 11.7%.

These results show once more that the mode of extraction plays a major role in the release of the active antimicrobial ingredients. This is in accordance with the wide use of decoction in traditional medicine.

4. Conclusion

The present work shows that most of the studied plants are potentially a good source of antimicrobial agents and demonstrates the importance of such plants in urolithiasis medicine and alternative healthcare. The results obtained show the influence of the technique of extraction on the antimicrobial activity of 22 plants used in folkloric medicine in Western Algeria. It validates their traditional uses from a scientific point of view. It is important to find new plants and to obtain documentation on traditional remedies before they disappear. Further studies should be brought to bear the active agents in the therapeutic process.

Conflict of interest statement

We declare that we have no conflict of interest.

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