

***In vitro* Antimicrobial Activity of *Camellia sinensis* L and *Erica multiflora* L used for the Treatment of Urinary Infection in West Algeria**

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Like many plants, *Camellia sinensis* L and *Erica multiflora* L were used in Algerian traditional medicine for the treatment of urinary tract infections and a number of other diseases. To provide a scientific basis to the traditional use of these plants, aqueous and organic extracts were screened for their potential antibacterial and antifungal. *In vitro* antibacterial and antifungal activity of aqueous and organic extracts were determined with using agar-well diffusion method. However, Minimum Inhibitory Concentration (MIC) of active extracts was determined by using micro-plate dilution test. Finally their antimicrobial effects were compared to some standard antibiotics. Among the tow plants screened, *Camellia sinensis* L was found to be more active than *Erica multiflora* L. It was observed that the hot water and methanolic extracts of *Camellia sinensis* L showed higher inhibitory activity against selected microbial species than the other solvents extracts. Minimum Inhibitory Concentration (MICs) of aqueous and methanolic extracts is ranged between 0.039 to 0.312 mg /L and 0.039 to 0.625 mg /L respectively. The results obtained showed a wider spectrum of activity of extracts but less strong inhibition as compared to the investigated commercials antibiotics. The antimicrobial efficacy demonstrated by these plants provides a scientific basis that validates their traditional uses as home remedies for the treatment of urinary infection.

Key words: Medicinal plants; urinary tract infections; antibacterial activity; antifungal activity; MIC.

INTRODUCTION

Nature was useful like a rich reserve in medicinal plants during thousands of years, and a number of modern impressing drug were isolated from natural sources, in particular of vegetable origin¹. The use of herbs in complementary and alternative medicine has increased dramatically over the last 20-25 years².

According to the World Health Organization (WHO), traditional medicines are used by 65-80% of World population for their needs in primary health care. In addition, the emergence of resistant strains to various drugs is linked to the indiscriminate use of antibiotics to treat infectious diseases which generates a gain interest phytotherapeutic³.

This resistance of the pathogenic micro-organisms to human was developed because of the blind use of the commercial drugs for antimicrobial effect, generally used in the treatment of the infectious diseases⁴.

However, Beneficial effects for health of many plants used for centuries as flavoring agents

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in foods and beverages have been claimed for prevention of food spoilage and as antimicrobial agents against pathogenic microorganisms. The antimicrobial potential of different medicinal plants was studied in depth over the world^{5,6,7,8}, but only a few studies have been conducted in a systematic manner. Phytochemical and pharmacological studies of many plants were followed by the isolation of some natural antimicrobials agents⁹.

In African region as around the world, traditional medicine used plants to treat acute and chronic diseases in rural and urban areas. In addition, urinary tract infections are very common and are a major concern for public health. They are more common in women of childbearing age than men, or they occur at an advance age^{10,11}.

Urinary tract infections are a frequent disease. They represent the second cause of consultation in infectious diseases, after pulmonary infections¹². For this, in our study, two medicinal plants namely; *Camellia sinensis* L and *Erica multiflora* L belonging to the families Theaceae and Asteraceae respectively was selected to assess their antimicrobial properties. Immediately to provide a scientific justification for these traditional remedies, this study was carried out in order to assess their antimicrobial potential using aqueous and organic extracts against some clinically important bacteria and yeast.

MATERIALS AND METHODS

All standard antibiotics were obtained from Pasteur Institute of Algiers, Algeria. Solvents were obtained from Merck, Germany and Sigma Chemicals, USA, respectively.

Culture of microorganisms

References bacteria and yeast namely; *E. coli* ATCC 25922, *Proteus mirabilis* ATCC 7002, *Klebsiella pneumoniae* ATCC 27736, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 33862 and *Candida albicans* ATCC 10231 were obtained from Pasteur Institute of Algiers, Algeria. The same strains were isolated from different urine samples. Patients included in the test had signs and symptoms suggestive of acute cystitis which was strongly suspected bacterial origin by the positivity of the dipstick (presence of Leukocytes and/or nitrites). A cytobacteriologic examination of urines, was

considered to be positive if bacteriuria of a single germ ($\geq 10^5$ UFC/mL for negative-Gram bacteria or $\geq 10^4$ UFC/mL for positive-Gram bacteria), associated with pyuria ($> 10\,000$ Leukocytes/ml). The microbial identification was made according to conventional methods^{13,14}.

All bacteria were maintained on nutrient agar and Sabouraud agar for yeast and kept in +4°C.

Inoculum preparation

A handle of isolated colony was inoculated in the bubble nutritive at 37°C/24h for bacteria and 25°C/48h for yeast. The activity of the microbial suspension is then followed of an adjustment with water peptone in order to obtain a turbidity visually comparable to 0,5 McFarland standard, then diluted to have an approximate concentration 10^5 UFC/mL for bacteria and 10^8 YC/mL for yeast. ¶ ¶

Plants

Erica multiflora L was collected during the flowering period, March 2014, from western Algeria (Oran, latitude 35°48' North, longitude 00°22' West with bioclimatic Semi-arid and temperate winters). In addition, tea used in our experiment is known as green tea from China (reference 0071). A voucher specimen was deposited in our laboratory for future reference. Samples were stored in the dark at +4 °C.

Extraction

Aqueous extract

25 g of each powder sample of the two plants was soaked with 250 mL of boiling distilled water for 10 min. After filtration (Whatman paper N°1), the extract obtained was concentrated and lyophilized with Rotavapor (R110).

Organic extract

Organic extracts of the plants were prepared using four different solvents with decreasing polarity¹⁵. 25 g of powder of different parts studied were extracted with 3 x 50 mL of petroleum ether and agitated for 3 x 24 hours. After filtration, with Whatman paper N°1, the marc was then mixed with 3 x 50 mL of dichloromethane for 3 x 24 hours. The same procedure was followed for methanol and ethanol. The extracts obtained after filtration was concentrated to dryness under reduced pressure at 40 °C with Rotavapor (R110).

Antibacterial and antifungal activity:

The sensitivity of different bacterial and fungal strains with different extracts was measured in terms of the inhibition zone using the agar diffusion method (ADA)¹⁶.

Plates containing Muller-Hinton agar were inoculated with 0,2 mL of inoculate. Organic and aqueous extracts were dissolved in DMSO (5%), for an initial concentration of 100 mg/mL. Disks (6 mm diameter) was dropped with 0,1 mL of extract (10mg/disc). The plates inoculated with different microorganism were incubated at 37°C/24h for bacteria and at 25°C/48h for yeast, then, the diameters of the inhibition zones were measured. The antimicrobial activity of different plant extracts was compared with some antibiotics commonly used to know; Nalidixic Acid (30µg/disc), Cefazoline (30µg/disc), Colistine (50µg/disc), Doxycycline (30µg/disc), Erythromycine (15µg/disc), Kanamycine (30µg/disc), Norfloxacin (5µg/disc), Pristinamycine (15µg/disc), Rifampicine (30µg/disc), Trimethoprim + Sulfamide (1.23/23.75µg/disc). However, Nystatine (30 ppm/disc) is used for the positive test to yeast. All tests were done in triplicate.

Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of active extracts was measured by the agar dilution method¹⁷. The plates of nutrient agar containing varying concentrations, each organic and aqueous extract of plant (10 mg/mL), was serially diluted to give an initial concentration 2,5 mg/mL in the first plate then diluted to 0,25 mg/mL. 100 µL of microbial culture in the exponential growth phase was diluted to give a final concentration 10⁵ CFU/mL, in which was added to the various extracts.

The plates were incubated at 37°C/24h for bacteria and at 25°C/48h for yeast, the lowest extract concentration completely inhibiting microbial growth is defined as the MIC. The experiments were performed in triplicate.

Statistical analysis

All values were expressed as; value ± standard deviation and the comparison of antibacterial and antifungal activity of samples with standards antibiotics were evaluated by applying t-test. Values P ≤ 0,05 were considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION**Yields**

Extraction of the aerial parts with different solvents showed that the highest efficiency is found with *Camellia sinensis* L aqueous extracts¹⁸. however the lowest yield is observed with dichloromethanolic extracts from the species of *Erica multiflora* L (Table 1).

These variations of the extractive values of various solvents used could be due to the differential solubility of the components in these solvents¹⁹.

Antimicrobial activity of aqueous and organic extracts

As shown in Table 2, aqueous and organic extracts from different plant species studied showed a very interesting antimicrobial activity with the diameters of zone of inhibition ranging between 10,6–32,3 mm, against some/or all urinary infection causative microorganism tested.

However, the organic extracts of *Camellia sinensis* L showed similar results in zone of inhibition to those observed in aqueous extracts with some variations. The extract prepared with methanol gave the best inhibition zones ranging from 10,6–33,6 mm (Table 2).

Sensitivity of *E. coli*, *Pseudomonas aerogenosa* and *Candida albicans* to aqueous extract is comparable to those found by²⁰.

All Gram-negative bacteria tested were manifested total resistance to all organic extracts of *E. multiflora* L while resistance and sensitivity were moderate to the Gram-positive bacteria and yeast. The differences observed could be due to the filtration of the extract, which could lead to the removal of key components responsible for the antimicrobial activity. The variations observed in the present study and previous versions could be attributed to environmental and climatic conditions, stress differences, extraction protocol and methods used to evaluate the antimicrobial activity.

Other studies on the antimicrobial activity of plants reveal that the extracts of these last one are more active on positive-Gram bacteria than negative-Gram bacteria²¹⁻²⁸.

The greatest sensitivity of Gram-positive bacteria (*S. aureus*) could be explained by chemical

Table 1. Yield results

Plant	Part of plant	yield (%) w/w				
		AE	PEE	DE	ME	EE
<i>C. sinensis</i> L	L	34.6	16.1	10.2	31.1	28.4
<i>E. multiflora</i> L	F+L	26.6	13.5	9.7	18.6	15.1

F: Flower - L: Leaf - AE: Aqueous extract - PEE: Petroleum ether extract -
DE: Dichloromethanolic extract - ME: methanolic extract - EE: Ethanolic extract

components which have a antibacterial capacity present in the extracts rough of these lasts one are more active on Gram-positive bacteria than Gram-negative bacteria^{29, 30} and allotted to their layer external of peptidoglycane which is not an effective barrier against the permeability³¹⁻³².

Gram-negative bacteria have an external phospholipidic envelope carrying the components lipopolysaccharides structural which make the cellular wall impermeable to lipophilic aqueous solutions and limit the diffusion of the active component³³. Contrary to Gram-negative bacteria, Gram-positive bacteria allow the direct contact of the components of extract with the membrane, which increase the permeability of the ions, which by turn cause the cellular explosion, or the weakening of their enzymatic systems³⁴

Minimum inhibitory concentration (MIC)

Strains which showed a good sensitivity considerably to the extracts were selected after determining the minimum inhibitory concentration (MIC). MIC's values depended to strains and plants. Strong ability of methanol extraction could generate a number of active constituents responsible for antimicrobial activity. The efficiency of methanolic extracts was confirmed by MIC (Table 3). The minimum inhibitory concentration of the aqueous and methanolic extracts is ranged between 0,039–0,312 mg/mL and 0,039–0,0625 mg/mL, respectively. This activity can be allotted to the presence of a significant concentration of the active component by the extraction with these solvents^{35, 36}.

Various conditions of extraction for *Camellia sinensis* L as well as the effectiveness of various solvents were used in former studies, which result a variety of compounds measured as phenolic compounds and flavonoïdes total contents³⁷.

Moreover, the phenolic compounds can also be associated to other components of structure such as the glucides and proteins. ¶Consequently, there is not a universal procedure of extraction adapted to the extraction of the whole of phenolic compounds for plants. ¶Solvents such as methanol, ethanol and their combinations were used for the extraction of the phenolic compounds starting from vegetable matters, often with different proportions of water³⁸.

The flavonoïdes among the most diversified and extended groups of the natural compounds are probably the most significant natural phenolic compounds. ¶These compounds have a broad spectrum of chemical and biological activities, including properties of trapping of radicals³⁹. ¶

Comparison of the activity of extracts with the standard antibiotics

Different cultures have responded to standard antibiotics and led to a variables inhibition zones 7 to 39,3 mm (Table 4). Methanolic and aqueous extracts of *Camellia sinensis* L have marked the best efficiencies against nearly all microorganisms compared to standard antibiotics. The student test T showed statistically a significant difference in the antimicrobial activity of extracts from *Camellia sinensis* L and antibiotics ($P < 0,05$) whereas marked resistance of some strains to some antibiotics (Table 4). This characteristic of resistance developed by these microbial stocks with time due to the exposure repeated to drug or the mutation⁴⁰.

Statistically, a non significant difference was observed for the inhibitory activity of methanolic and aqueous extracts. However, if we compare the antimicrobial potential of methanolic and aqueous extract of each plant, the aqueous extract of *Camellia sinensis* L activity has shown

Table 2. Antimicrobial activity results of various extracts from *Camellia sinensis* L and *Erica multiflora* L

Microorganisms	Disc Diffusion (inhibition zone, mm)													
	<i>C. sinensis</i> L						<i>E. multiflora</i> L							
	AE	PEE	DE	ME	EE	AE	PEE	DE	ME	EE				
Gram negative bacteria														
<i>E. coli</i> ATCC 25922	22 ± 0.6	8.3 ± 0.4	8 ± 0.0	19.3 ± 0.9	9 ± 0.8	14.6 ± 0.4	-	-	-	-	-	-	-	-
<i>E. coli</i> (CI)	12.3 ± 0.4	8.3 ± 0.4	9 ± 0.0	12.6 ± 0.4	8.3 ± 0.4	10.6 ± 0.4	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> ATCC 7002	16.3 ± 0.3	13.3 ± 1.2	12.3 ± 1.2	18.3 ± 0.6	11 ± 0.8	13.6 ± 0.4	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> (CI)	18.3 ± 0.3	10.3 ± 0.4	9.6 ± 1.6	15 ± 0.8	8 ± 0.0	16.6 ± 0.9	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ATCC 27736	15.6 ± 0.6	14.3 ± 0.4	12 ± 0.0	10.6 ± 0.9	11 ± 1.6	11.6 ± 0.9	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> (CI)	14.6 ± 0.4	11.6 ± 1.6	10 ± 0.0	14 ± 0.8	8.3 ± 0.4	13 ± 0.8	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	17 ± 0.8	19.3 ± 0.9	15.3 ± 0.4	17 ± 0.8	19 ± 1.6	14.3 ± 0.4	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> (CI)	15.3 ± 0.3	12.6 ± 0.4	11.6 ± 0.4	13.3 ± 0.9	8 ± 0.0	11 ± 0.8	-	-	-	-	-	-	-	-
Gram positive bacteria														
<i>Staphylococcus aureus</i> ATCC 33862	32.3 ± 0.4	30.3 ± 1.2	31 ± 0.8	32.6 ± 0.3	24.6 ± 0.4	25 ± 0.8	-	-	15 ± 0.8	-	-	-	-	-
<i>Staphylococcus aureus</i> (CI)	31 ± 0.8	29.3 ± 0.4	25 ± 0.8	33.6 ± 0.3	15 ± 0.8	18 ± 0.8	-	-	21 ± 0.8	-	-	-	-	-
Yeast														
<i>Conidida albicans</i> ATCC 10231	28.6 ± 0.3	15.6 ± 0.9	24.3 ± 0.9	30.3 ± 0.4	19 ± 0.8	22 ± 0.0	-	-	11.6 ± 0.4	11.3 ± 0.4	-	-	-	-
<i>Conidida albicans</i> (CI)	27 ± 0.4	20 ± 0.0	20.3 ± 0.9	26.3 ± 0.3	9.6 ± 0.4	22 ± 0.4	-	-	14.6 ± 0.4	16.6 ± 1.2	-	-	-	-

(-) : inactive - CI: Clinic isolated - AE: Aqueous extract - PEE: Petroleum ether extract - DE: Dichloromethanolic extract - EM: methanolic extract - EE: Ethanolic extract

Table 3. CMI results of various extracts from *Camellia sinensis L* and *Erica multiflora L*

Microorganisms	CMI (mg.ml ⁻¹)												
	<i>C. sinensis L</i>						<i>E. multiflora L</i>						
	AE	PEE	DE	ME	EE	AE	PEE	DE	ME	EE			
Gram negative bacteria													
<i>E. coli</i> ATCC 25922	0.039	1.250	1.250	0.039	1.250	0.156	-	-	-	-	-	-	-
<i>E. coli</i> (CI)	0.312	1.250	1.250	0.312	1.250	0.625	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> ATCC 7002	0.078	0.312	0.312	0.039	0.625	0.312	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> (CI)	0.039	0.625	1.250	0.156	1.250	0.078	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> ATCC 27736	0.156	0.156	0.312	0.625	0.625	0.625	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i> (CI)	0.156	0.625	0.625	0.156	1.250	0.312	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.078	0.039	0.156	0.039	0.039	0.156	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i> (CI)	0.156	0.312	0.625	0.312	1.250	0.625	-	-	-	-	-	-	-
Gram positive bacteria													
<i>Staphylococcus aureus</i> ATCC 33862	0.078	0.078	0.078	0.078	0.156	0.156	-	-	0.625	-	-	-	-
<i>Staphylococcus aureus</i> (CI)	0.078	0.078	0.156	0.039	0.625	0.312	-	-	0.312	-	-	-	-
Yeast													
<i>Candida albicans</i> ATCC 10231	0.078	0.625	0.156	0.078	0.312	0.312	-	-	1.250	-	-	1.250	1.250
<i>Candida albicans</i> (CI)	0.156	0.312	0.312	0.156	0.312	0.312	-	-	0.625	-	-	0.625	0.625

(-): inactive - CI: Clinic isolated - AE: Aqueous extract - PEE: Petroleum ether extract - DE: Dichloromethanolic extract - EM: methanolic extract - EE: Ethanolic extract

Table 4. Antibiotics activity results against bacteria and yeasts tested

Microorganisms	Antibiotics										Antifungal	
	Sxt (1.23/23.75)µg	Ra (30µg)	Pt (15µg)	Nor (5µg)	K (30µg)	E (15µg)	Do (30µg)	Cs (50µg)	Cz (30µg)	Na (30µg)	Nystatine (100 µg)	
Gram negative bacteria												
<i>E. coli</i> ATCC 25922	-	14.3 ± 0.9	10.6 ± 0.9	17 ± 0.8	-	10 ± 0.0	13.3 ± 0.9	19 ± 0.8	20 ± 1.6	-	-	-
<i>E. col</i> (CI)	24.3 ± 0.4	13.3 ± 0.9	13.3 ± 1.2	26.3 ± 1.2	15 ± 0.8	9.6 ± 0.4	9 ± 0.8	10.3 ± 0.4	12 ± 0.0	20.6 ± 0.4	-	-
<i>Proteus mirabilis</i> ATCC 7002	-	13.3 ± 0.9	-	15 ± 0.8	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i> (CI)	-	11 ± 0.8	-	14 ± 0.0	-	-	-	-	12.3 ± 0.4	7 ± 0.8	-	-
<i>Klebsiella pneumoniae</i>	11.6 ± 0.4	10.6 ± 0.4	-	20 ± 0.8	10.6 ± 0.9	-	7 ± 0.8	13.6 ± 0.9	8 ± 0.0	11 ± 0.8	-	-
ATCC 27736												
<i>Klebsiella pneumoniae</i> (CI)	22.3 ± 0.4	12 ± 0.0	-	26.6 ± 0.9	19.3 ± 0.9	10 ± 0.0	6.6 ± 0.9	12 ± 0.0	12 ± 0.8	18 ± 0.0	-	-
<i>Pseudomonas aerogenosa</i>	-	9.6 ± 0.4	-	16 ± 0.8	-	-	-	12.6 ± 0.4	-	-	-	-
ATCC 27853												
<i>Pseudomonas aerogenosa</i> (CI)	-	9.3 ± 0.4	-	21 ± 0.8	-	-	-	14.3 ± 0.9	-	-	-	-
Gram positive bacteria												
<i>Staphylococcus aureus</i>	16 ± 0.8	27.3 ± 0.9	26.3 ± 1.2	22 ± 0.0	18.3 ± 0.4	22 ± 0.8	20.3 ± 0.4	7.3 ± 0.9	27.3 ± 0.9	14 ± 0.8	-	-
ATCC 33862												
<i>Staphylococcus aureus</i> (CI)	20 ± 0.0	39.3 ± 0.4	28.6 ± 0.9	21.3 ± 0.4	7.6 ± 1.2	24.6 ± 0.9	-	12.3 ± 0.4	16.3 ± 1.2	12 ± 0.0	-	-
Yeast												
<i>Candida albicans</i> ATCC 10231	-	-	-	-	-	-	-	-	-	-	24,6 ± 0.3	-
<i>Candida albicans</i> (CI)	-	-	-	-	-	-	-	-	-	-	19,6 ± 0.4	-

(-) : inactive - 'IC': Isolé Chinique - 'Na': Nalidixic Acid - 'Cz': Cefazoline - 'Cs': Colistine - 'Do': Doxycycline - 'E': Erythromycine - 'K': Kanamycine - 'Nor': Norfloxacin - 'Pt': Pristinamycine - 'Ra': Rifampicine - 'Sxt': Trimethoprim + Sulfamide

statistically a significant activity compared to the methanol extract ($P < 0,05$) whereas there was no significant difference in *Erica multiflora L.*

CONCLUSION

In conclusion, the discovery of antimicrobial drugs based on *in vitro* studies to treat the infectious diseases and the pathogenic microbes resistant to antibiotics need several and various tests.

From that, the air part of *Camellia sinensis L* had a good inhibiting activity against the bacteria and yeast tested responsible to induce a urinary tract infection. ¶¶ The results of preliminary study demonstrated that among the various prepared extracts, *Camellia sinensis L* had good inhibitory activity against selected tested microorganisms responsible for inducing a urinary tract infection. Aqueous and methanolic extracts have showed almost an equal antimicrobial activity, which supports their use in the ethnomedicine against the infectious diseases and further, these plants can be exploited for new effective antimicrobial agents. The present study is significant because the search for new compounds pharmacologically active of the extracts led to discovered of many useful drugs in medicine. ¶ Moreover, a hope for the development of many chemiotherapeutic agents or the new models starting from these plants, which in the future can be used to improve the production of the synthesized therapeutic agents. ¶ Nevertheless, the effectiveness of these extracts must be validated *in vivo*. ¶ However these extracts contain many made up which can cause side or toxic effects. Consequently, a future studies should be concentrated on the isolation and the identification of the active compounds with an antimicrobial activity rather than to examine the extracts simply rough. ¶¶¶

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