Antibacterial Activity of *Francoeuria crispa, Pulicaria undulata, Ziziphus spina-christi* and *Cucurbita pepo* Against Seven Standard Pathogenic Bacteria

Hatil Hashim El-Kamali[1*] and Sana Al-Tayeb Mahjoub[2]

[1] Department of Botany, Faculty of Science and Technology, Omdurman Islamic University P.O. Box # 382, Omdurman, Sudan, E.Mail: htlkamali@yahoo.com

[2]Department of Biochemistry, Faculty of Medical Laboratory, Omdurman Islamic University, P.O. Box # 382, Omdurman, Sudan

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Abstract

The antibacterial potentials of the medicinal plants *Francoeuria crispa* (Forssk.) Cass., *Pulicaria undulata* (L.) Kostel, *Ziziphus spina-christi* (L.) Desf. and *Cucurbita pepo* L. Ethanol, petroleum ether, ethyl Acetate, methanol and aqueous extracts, at a concentration of 100 mg/ml, were evaluated against selected bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella para typhi* B (0650) and *Klebsiella pneumoniae* (ATCC 1312) using the Agar Diffusion Technique in vitro. Minimum Inhibitory Concentration (MIC) values were also determined for the most active plant extracts. Of all extracts the ethanolic extract of *Pulicaria undulata* was the most active, whereas, the aqueous extract of *Ziziphus spina – christi* stem bark was the most active of all aqueous extracts tested. The ethyl acetate extract of *F. crispa* showed activity against both Gram positive and Gram negative bacteria. Most susceptible Gram-negative bacteria were *E. coli* and *P. vulgaris* and least susceptible was *S. para typhi* B. In Gram –positive bacteria, most and least susceptible were *S. aureus* and *B. subtilis* respectively. The lowest MIC values were <3.125 and 6.25 µg/ml for the crude extracts of ethyl acetate of *Pulicaria undulata* and crude methanolic extract of *Ziziphus spina-christi*, respectively. These results provide promising baseline information for the potential use of these crude extracts in the treatment of bacterial infections.

Key words: Antibacterial activity, medicinal plants, standard pathogenic bacteria.

Introduction

The search for substances with high antibacterial properties has been one of the most intensive researches of this time. It is known that plant produce certain chemicals which are naturally toxic to bacteria but not to humans. It is an established fact that intensive use of antibiotics is often followed by the development of resistant strains. Search for new antibiotics continues unabated. Increase of microbial resistance is a world health problem (Gerding *et al*., 1991).

The principal objectives of the present research work were to determine the antibacterial potential of four selected medicinal plants against seven standard bacterial strains. This selection was guided by lack or incompleteness of information in literature on antibacterial activity of their extracts. Extracts of the four

medicinal plants: *Francoeuria crispa* (aerial parts), *Pulicaria undulata* (aerial parts), *Ziziphus spina –christi* (leaves, stem bark) and *Cucurbita pepo* (fruits) were tested against seven standard bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella para typhi B* (0650), and *Klebsiella pneumoniae* (ATCC 1312).

Materials and Methods

Plant Material

The aerial parts *of Francoeuria crispa* (HK 210) and *Pulicaria undulata* (HK 211), and *Ziziphus spina –christi* stem bark and leaves (HK 425) were collected from EL-FITEHAB area, West White Nile, Omdurman Province. Fresh fruits of *Cucurbita pepo* were purchased from the Omdurman market. The plants were identified in the Botany Department, Faculty of Science and Technology, Omdurman Islamic University by comparison with herbarium specimen of the Department. The plants were spread and dried in the shade for three weeks and then pulverized with a mechanical grinder.

Protocol for preparation of plant extracts

Different extracts were prepared by a modification of the method according to Robinson (1963), the protocol is prescribed below: (a) freshly dried and healthy plant material was ground into fine powder in an electric grinder. (b) two hundred grams of plant powder was macerated with ethanol (EtOH) in a conical flask for 24 hours. Mother liquor (crude EtOH extract) was filtered out and the dried residual plant material (marc) was again macerated with petroleum ether (60-80^{o}C) for 24 hours, filtered and was evaporated to dryness at room temperature (petroleum ether extract). (c) the residual plant material after maceration with petroleum ether was again macerated with ethyl acetate for 24 hours, filtered and was evaporated to dryness (ethyl acetate extract). (d) the residual plant material obtained in step (c) was macerated with methanol for 24 hours, filtered and was evaporated to obtain methanolic extract, while plant material residue was discarded.

Preparation of aqueous extract

Air- dried plant material (100 g) was ground to a fine powder. It was poured with distilled water (1litre), and left for 24 hours at room temperature. The mother liquor was filtered. The filtrate, thus obtained was evaporated to complete dryness at room temperature. The residue thus obtained was the aqueous plant extract. All extracts were stored dry in sterilized containers at room temperature until used for antibacterial testing. At the time of testing, the extracts were reconstituted to a concentration of 100mg/ml in Dimethyl Sulphoxide (DMSO).

Antibacterial Testing

The antibacterial activity was tested by well-agar diffusion method (Cruickshank, 1975; Cheesbrough, 1984). 250 ml of sterilized nutrient agar was used for testing. The inoculum size of each test organism was adjusted to suspension of 10^{6} cells. 2 ml of 24 hours old culture of bacteria were added to 250 ml of melted cooled test agar and after through mixing, approximately 20 ml of this seeded agar were poured into 10 cm diameter presterilized Petri dishes and allowed to solidify. Three wells (10 mm in diameter) were bored in the agar using a sterile cork borer and the agar discs were removed. 0.1 ml aliquots of the reconstituted extract were placed into a well with a pipette and the plate was held for 2 hours at room temperature for diffusion of extract into agar. Subsequently,

the plate was incubated at 37 ^{oC} for 24 hours. After incubation, the diameter of the zones of inhibition was measured to the nearest mm.

The minimum inhibitory concentration (MIC) was determined by a modification of the agar diffusion method according to Cruickshank (1975). A two-fold serial dilution of each extract was prepared in DMSO (diluent) to achieve a decreasing range of extract concentrations from 100 mg/ml to approximately 3.125mg/ml. A 0.1 ml sample of each dilution was introduced into duplicate wells in a nutrient agar plate already seeded with bacterial cells as described above. Incubation was at 37 ^{oC} for 24 hours. The lowest concentration of extract showing a zone of inhibition was taken as the MIC.

Gentamicin, Tetracycline, and Ampicillin were used at concentrations ranging from 40 mg/ml to 5 mg/ml, as positive control and DMSO as a negative control.

Results and Discussion

The antibacterial potential of the medicinal plants: *Francoeuria crispa* (Forssk.) Cass. , *Pulicaria undulata* (L.) Kostel, *Ziziphus spina-christi* (L.) Desf. and *Cucurbita pepo* L. of the ethanol, petroleum ether, ethyl Acetate , methanol and aqueous extracts were evaluated against selected bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella para typhi* B (0650) and *Klebsiella pneumoniae* (ATCC 1312). The results of diameters of the zones of inhibitions of extracts and antibiotics and Minimum Inhibitory Inhibition are presented in Tables 1 - 4 , respectively and were interpreted as sensitive, (18 mm), intermediate (14-17 mm) and resistant (<14mm) (Barry *et al*., 1970; Cruickshank *et al*., 1975).

Ethanol extracts of *Francoeuria crispa* and *Pulicaria undulata* aerial parts showed high antibacterial activity against all tested bacteria except *Salmonella para typhi B* (0650). Ethanol extract of *Ziziphus spina – christi* stem bark was found effective against all tested bacteria except *Escherichia coli* (ATCC 25922). *Ziziphus spina – christi* leaves ethanolic extract showed no antibacterial activity against four tested organisms: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), and *Salmonella para typhi B* (0650) whereas bacterial species : *Bacillus subtilis* (NCTC 8236) , *Pseudomonas aeruginosa* (ATCC 27853),and *Klebsiella pneumoniae* (ATCC 1312) were found

to be resistant (1Z = 12-13 mm). Extract of *Cucurbita pepo* fruits did not show any activity against all tested Gram-positive and Gram-negative bacteria.

Petroleum ether extract of *Pulicaria undulata* aerial parts showed promising result against *Bacillus subtilis* (NCTC 8236) (1Z = 30 mm). Petroleum ether extract of *Ziziphus spina – christi* stem bark was found effective against *Bacillus subtilis* (NCTC 8236) (1Z = 15 mm), *Escherichia coli* (ATCC 25922)(1Z = 20 mm), and *Proteus vulgaris* (ATCC 6380) (1Z =20 mm). Ethyl acetate extract of *Francoeuria crispa* aerial parts showed high antibacterial activity against all tested Gram-positive and Gram-negative bacteria (1Z = range between 17 - 21 mm). Ethyl acetate extract of *Pulicaria undulata* aerial parts showed promising result against all tested bacteria (1Z = range between 16-20 mm)

except *Klebsiella pneumoniae* (ATCC 1312) was found to be resistant. Fruit extract of *Cucurbita pepo* demonstrated antibacterial activity against *Klebsiella pneumoniae* (ATCC 1312) (1Z = 16 mm).

Most of the bacterial species showed a fairly high degree of sensitivity to the methanolic extracts of *Francoeuria crispa* aerial parts (1Z = range between 17-25 mm), and *Pulicaria undulata* aerial parts (1Z = range between 15-20 mm). Crude methanol extract of *Ziziphus spina – christi* stem bark was found effective against all tested Gram- positive and Gram- negative bacteria (1Z = range between 20-30 mm).

All bacterial species were found to be resistant against aqueous extracts of *Francoeuria crispa, Pulicaria undulata* aerial parts, and *Ziziphus spina –christi* leaves. Aqueous extract of *Ziziphus spina – christi* stem bark was moderately active against most of the bacteria (1Z = range between 15-17 mm). Fruit extract of *Cucurbita pepo* showed antibacterial activity against *Escherichia coli* (ATCC 25922) (1Z =23 mm).

The ethanol, methanol, and ethyl acetate extracts of *Francoeuria crispa, Pulicaria undulata,* and *Ziziphus spina – christi* stem bark were active both against Gram-negative and Gram- positive bacteria, even the particularly Ampicillin-resistant *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris* were inhibited. This finding is interesting because conventional antibiotics are often more active against Gram- positive than Gram-negative bacteria.

The pattern of chemical selectivity towards Gram- positive bacteria is not restricted to compounds from plants, but is a general phenomenon observed among most antibiotics. However, the antibacterial activity of plant extracts against Gram- negative bacteria has been shown in other studies: extracts from *Ziziphus spina – christi* stem bark and leaves showed activity mainly against antibiotic-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa.*

The antibacterial activities of different extractives from investigated plant species were higher than those of tested standard (synthetic) antibiotics. Compared to gentamicin, ampicillin, and tetracycline (20µg/ml), the ethanol, methanol, and ethyl acetate extracts of *Francoeuria crispa,* and *Pulicaria undulata* exhibited a broader spectrum against *Pseudomonas aeruginosa, Proteus vulgaris, Salmonella para typhi B, Escherichia coli,* and *Staphylococcus aureus* and in some cases better level of antibacterial activity.

The polar extracts of *Francoeuria crispa* and *Pulicaria undulata* exhibited promising antibacterial activity against all tested bacterial species and the ethanol extract showed the highest activity. The antibacterial activity of *Francoeuria crispa* and *Pulicaria undulata* did not come as a surprise, since sesquiteprene lactones, flavonoids and essential oils in general have been associated several times with antibacterial effect (Abdel Mogib *et al.* 1990 and Elkamali *et al*., 1998).

The aqueous extracts of *Francoeuria crispa* and *Pulicaria undulata* did not possess significant antibacterial activity against standard strains. These results account for why the Sudanese people do not frequently use these plants as a remedy. *Ziziphus spina –christi* stem bark extracts are active against some human pathogens such as *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella para typhi B, and Klebsiella pneumoniae*. This activity could be attributed to the presence of tannins and leucocyanidin (Rizk *et al*., 1993).

It is concluded that plant extract with low MIC value may serve as sources for compounds with therapeutic potency. *Pulicaria undulata* and *Francoeuria crispa* aerial parts showed high antibacterial activity against most

of tested bacteria. These plants are also a good candidate for phytochemical and pharmacological investigations to discover new broad spectrum bioactive compounds. *Ziziphus spina –christi* stem bark was also proved to be effective against both Gram- positive and Gram- negative bacteria. This plant also need further work to discover new broad spectrum bioactive compounds. The antibacterial activity of the medicinal plants studied was satisfactory, especially that of *Francoeuria crispa* and *Pulicaria undulata.* An adequate toxicological study must be carried out to verify the possibility of using these plants for fighting microorganisms. Further studies on other bacteria and some pathogenic fungi frequently isolated from burn wound specimens such as *Enterobacter cloacae, Enterococcus faecalis, Aspergillus* species and *Candida albicans* would be interesting.

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Table 1. Antibacterial Activity of Extracts from Investigated Different Plant Species against Standard Bacterial Strains.

Extracts	**Plant species**	**Plant parts**	**Test organisms***						
			S.a	**B.s**	**E.c**	**P.v**	**P.a**	**S.ptB**	**K.n**
Ethanol	*Francoeuria crispa*	Aerial parts	23	20	21	20	22	17	22
		Aerial parts							
	Pulicaria undulata	Stem bark	26	23	25	25	23	16	25
	*Ziziphus spina –christ*i	Leaves	21	22	-	24	21	20	20
	*Ziziphus Spina – christ*i	Fruits	-	12	-	-	13	-	12
	Cucurbita pepo		-	-	-	-	-	-	-
Petroleum ether	*Francoeuria crispa*	Aerial parts	-	15	-	-	-	-	-
		Aerial parts							-
	Pulicaria undulata	Stem bark	-	30	-	-	-	-	
	*Ziziphus spina –christ*i	Leaves	14	15	20	20	-	-	
	*Ziziphus Spina – christ*i	Fruits	-	-	-	-	-	-	
	Cucurbita pepo		-	-	-	-	-	-	-
									-
									-
Ethyl acetate	*Francoeuria crispa*	Aerial parts	20	17	20	20	21	19	19
	Pulicaria undulata	Aerial parts	19	17	20	20	20	16	-
	*Ziziphus spina –christ*i	Stem bark	-	14	12	12	11	-	12
	*Ziziphus Spina –christ*i	Leaves	-	-	-	-	-	-	-
	Cucurbita pepo	Fruits	-	12	-	-	-	-	16

Methanol	*Francoeuria crispa*	Aerial parts	25	17	22	20	20	18	19
	Pulicaria undulata	Aerial parts	18	15	20	20	18	15	15
	*Ziziphus spina –christ*i	Stem bark	16	14	13	12	12	14	15
	*Ziziphus Spina – christ*i	Leaves	13	-	-	-	-	-	-
	Cucurbita pepo	Fruits	-	-	-	-	-	-	-
Water	*Francoeuria crispa*	Aerial parts	-	-	-	-	-	-	-
	Pulicaria undulata	Aerial parts	-	-	-	-	-	-	-
	*Ziziphus spina –christ*i	Stem bark	15	16	17	15	15	13	13
	*Ziziphus Spina – christ*i	Leaves	-	-	-	-	-	-	-
	Cucurbita pepo	Fruits	-	-	23	-	13	-	-

*S.a: *Staphylococcus aureus* (ATCC 25923), B.s: *Bacillus subtilis* (NCTC 8236), E.c: *Escherichia coli* (ATCC 25922), P.a: *Pseudomonas aeruginosa* (ATCC 27853), P.v: *Proteus vulgaris* (ATCC 6380), S.ptB: *Salmonella para typhi B* (0650) , K.n: *Klebsiella pneumoniae* (ATCC 1312) Values are the mean of three replicates; -, no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well); DMSO did not show any inhibitory activity. ATCC: American Type Collection Culture ; NCTC: National Collection Type Culture

Table 2. Antibacterial activity of antibiotics against different standard group of bacteria

Test Organisms							**Concentration µg/ml**	**Antibiotics***
K.N	**S.Pt B**	**P.A**	**P.V**	**E.C**	**B.S**	**S.A**		
20	20	15	18	22	19	16	40	**Gen**
18	18	13	15	18	15	12	20	
13	16	-	-	15	12	-	10	
-	15	-	-	12	-	-	5	
20	17	16	-	16	22	15	40	**Tet**
18	12	13	-	15	20	12	20	
16	-	-	-	14	18	-	10	
15	-	-	-	12	16	-	5	

26	31	18	-	15	-	-	40	**Amp**
23	26	15	-	-	-	-	20	
20	22	-	-	-	-	-	10	
19	15	-	-	-	-	-	5	

***Gen**: Gentamicin, **Tet**: Tetracycline, **Amp**: Ampicillin

Table 3. Minimum Inhibitory Concentration (MIC), µg/ml of extracts from investigated plant species.

Extracts	**Plant species**	**Plant parts**	**Test organisms**						
			S.a	**B.s**	**E.c**	**P.v**	**P.a**	**S.ptB**	**K.n**
Ethanol	*Francoeuria crispa*	Aerial parts	50	100	›100	100	100	100	100
	Pulicaria undulata	Aerial parts	25	50	100	100	100	100	100
	*Ziziphus spina –christ*i	Stem bark	50	‹3.125	50	50	50	‹3.125	‹3.125
	*Ziziphus Spina –christ*i	Leaves	›100	›100	›100	›100	›100	›100	›100
	Cucurbita pepo	Fruits	50	100	›100	100	100	100	100
Ethyl acetate	*Francoeuria crispa*	Aerial parts	100	100	100	100	100	100	100
	Pulicaria undulata	Aerial parts	50	‹3.125	100	100	100	‹3.125	6.25
	*Ziziphus spina –christ*i	Stem bark	›100	100	›100	›100	›100	›100	›100
	*Ziziphus Spina –christ*i	Leaves	›100	›100	›100	›100	›100	›100	›100
	Cucurbita pepo	Fruits	100	100	100	100	100	100	100
Methanol	*Francoeuria crispa*	Aerial parts	50	100	›100	100	100	100	100
	Pulicaria undulata	Aerial parts	25	50	100	100	100	100	100
	*Ziziphus spina –christ*i	Stem bark	100	12.5	100	100	100	6.25	12.5
	*Ziziphus Spina –christ*i	Leaves	50	100	100	100	100	100	100
	Cucurbita pepo	Fruits	100	100	100	100	100	100	100

Table 4. Minimum Inhibition Zone (MIC) and antibacterial activity of crude methanolic extracts from *Ziziphus spins christi* (stem bark).

Bacterial Standard Strains	**S.a**	**B.s**	**E.c**	**P.v**	**P.a**	**S.pt B**	**K.n**
Diameter of Inhibition zone (mm)	20	23	21	22	20	20	21
MIC µg/ml	6.25	6.25	50	25	50	6.25	6.25