

Evaluation of Antibacterial Activity of Some Medicinal Plants Used in Sudanese Folk Medicine for Treatment of Gastrointestinal Tract Infections

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To cite this article:

Hatil Hashim Elkamali, Sana Eltayeb Mahjoob. Evaluation of Antibacterial Activity of Some Medicinal Plants Used in Sudanese Folk Medicine for Treatment of Gastrointestinal Tract Infections. *American Journal of Life Sciences*. Vol. 3, No. 3, 2015, pp. 230-237.

doi: 10.11648/j.ajls.20150303.25

Abstract: Punica granatum, Citrullus colocynthes, Curcuma long, Albizia lebbeck and Azadirachta indica are used in traditional medical practice in Sudan for the treatment of gastrointestinal tract infections. The methanol and aqueous extracts from different plant parts (Peel of fruits, rhizome, fruits, leaves, and Gum) respectively at a concentration of 100 mg/ml, were evaluated against 20 clinical isolates (2 were Salmonella typhi, 5 Proteus mirabilis, 4 Escherichia coli, 5 Pseudomonas aeruginosa, 3 Staphylococcus aureus, one was Salmonella para typhi B) and 5 standard bacterial strains (Staphylococcus aureus ATCC 25923), Bacillus subtilis (NCTC 8236), Escherichia coli (ATCC 25922), Salmonella typhi (ATCC 1319106) and Klebsiella pneumoniae (ATCC 35657) were tested for their antibacterial properties using the Agar Diffusion Technique in vitro. Of all plants methanolic and aqueous extracts of Punica granatum were the most active with clinical isolates and standard bacterial strains showed relatively high antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 14 and 24 mm, whereas the methanolic extract of Curcuma long showed high antibacterial activity against Proteus mirabilis clinical isolate (IZ = 20 mm). Most susceptible Gram-negative clinical Isolates bacteria were Escherichia coli and Proteus mirabilis. Most susceptible Gram negative standard bacteria were Bacillus subtilis (NCTC 8236) and Escherichia coli (ATCC 25922) and least susceptible Gram negative bacterium was Klebsiella pneumoniae (ATCC 35657). In Gram positive standard bacteria, most susceptible was S. aureus (ATCC 25923). Antibiotics were used as standards drug for antibacterial assay. The results obtained appeared to confirm the antibacterial potential of the plants investigated, and their usefulness in the treatment of gastrointestinal tract infections.

Keywords: Antibacterial activity, Medicinal Plants, Clinical Isolates and Standard Pathogenic Bacteria

1. Introduction

Microbes have existed on earth since millions of years ago, being one of the oldest creatures on this planet, infectious diseases remain the second leading cause of death worldwide (Abdallah, 2011). Increase of microbial resistance is a world health problem (WHO, 2001). Development of new antibacterial principles to substitute with inefficient ones is a major weapon to combat the problem. Many plants have been used in traditional medicine because of their antimicrobial traits, which were due to bioactive compounds normally accumulated as secondary metabolites in plant cells. These products were known by their active substances (Nascimento et al., 2000). Some of the active compounds inhibit the

growth of disease causing microbes either singly or in combination microbes by binding their surface proteins, breaking the peptide bonds, acting as chelating agents, altering their biochemical system or by preventing utilization of available nutrients to the microorganisms. Some compounds also cause lysis of microbial cells (Maji et al., 2010).

Punica granatum fruit extracts possessed strong in vitro antibacterial activity against many bacteria tested (Duman et al., 2009, Ahmad and Beg, 2001, Moorthy et al., 2013). and used in treatment of diarrhea, dysentery (Chengaiyah et al., 2010, Daset al., 1999, Mathabeet al., 2006). P. granatum delayed S. aureus growth and subsequent enterotoxin production. Gallic acid showed the highest antibacterial

activity. Aqueous and methanol extracts of peels showed maximum antibacterial activity and great potential as antibacterial compounds (Duman et al., 2009, Nazet et al., 2007, Vijayanand and Hemapriya, 2011, Fenglinet al., 2004). *P.granatum* showed great potential as antimicrobial agents in food processing in form of food preservatives and natural dyes or spices. (Chengaiyah et al., 2010, Devatkal, et al., 2013, Shanet al., 2007). Aqueous extract of *P.granatum* was highly effective against enterohaemorrhagic *E.coli* O157: H7 (Voravuthikunchai et al., 2004).

Aqueous and diluted acetone extracts of *Citrullus colocynthis* showed antibacterial activity (Khatibi and Teymorri, 2011). Ethanol extract showed inhibitory activity against *S. aureus* more than aqueous extract and had a similar inhibitory effect with Novobiocin (Najafi, et al., 2010). Crude ethanol extract of *C. colocynthis* were found to be active against Gram positive and Gram negative bacilli (Memon et al., 2003, Khatibi, 2011). All extracts of *C.colocynthis* exhibited considerably less activity against *K. pneumoniae* and *S.typhi* (Paul, 2008). *Citrullus colocynthis* showed antibacterial and anticandidal properties (Ramanathan et al., 2011).

Curcuma longa revealed antibacterial and antimicrobial activity (Chandarana et al., 2005, Sunilson et al., 2009). Natural preservatives of *C.longa* as phenolic curcuminoids, essential oil, used as an antimicrobial for preserving food and antifungal activity against *Aspergillus* and aflatoxins (Singh et al., 2010). The methanol extracts of *A.lebbeck* were effective against *E.coli* and *Salmonella* strains associated with infectious diarrhea (Uma et al., 2009). Methanolic, ethyl acetate and chloroform extracts of *A.lebbeck* bark showed significant activity against some pathogenic bacteria (Yogisha and Raveesha, 2009). Also aqueous extracts of *A.lebbeck* seeds showed antimicrobial activity (Nwozo et al., 2010). The total glycosides, cardenolide glycosides, anthraquinone glycosides isolated from the stem bark revealed antimicrobial activity against *S.aureus*, *P.aeruginosa*, *Candida albicans* and plant fungi *Helminthosporium sativum* (Mishra et al., 2010). Natural preservatives of *A.lebbeck* leaves and stem bark were *Albizia* saponins A, B, C, epicatechin and procyanidins, used as an antimicrobial for preserving food and antifungal activity against *Aspergillus*, aflatoxin and possessed antibacterial action (Singh et al., 2010).

Azadirachta indica extract had significant antibacterial activity against the multi-drug-resistant *V.cholera* (Thakurta et al., 2007, Solanki, 2010).

Different Neem extracts and their corresponding sulfates had various inhibition activities against the *S.aureus*, *E.coli*, *C.albicans*, *A. niger* and *Penicillium citrinum* (Helmy et al., 2007). Crude extracts of seeds of *A.indica* against pathogenic *S.aureus*, *S.pyogenes*, *E.coli* and *P.aeruginosa* associated with ear and eye infections, established scientific rationale for its uses as antimicrobial agents (El-Mahmood et al., 2010). Alcohol extracts of *A. Indica* bark demonstrated moderate antibacterial activity against *S. aureus* and *E.coli* (Ahmad and Beg, 2001). Natural preservatives of *A. indica* leaves and

seeds showed antimicrobial activity against *S.aureus*, *Salmonella typhosa*, *Trichophyton* spp. *Microsporum* spp, and *Epidermaphyton floccosum* (Singh et al., 2010). An antimicrobial effect of neem extract has been demonstrated against streptococcus mutans and *S. faecalis* (Okunomo and Egbo, 2010). Ethanol extract of neem showed the maximum zone of inhibition against *Streptococcus Mutans* and *Enterococcus faecalis* (Kumar and Sidhu, 2011). Petroleum ether and chloroform extract of *A.indica* leaves showed strong antimicrobial activity against *S.mutans*, *Streptococcus salivarius*. *Fusobacterium nucleatum* was highly sensitive to both ethanol and aqueous extract of neem (Lekshmi et al., 2012). The formulation of *A.indica* help in preventing dental caries and gingivitis through plaque control (Phatak et al., 2011). The extracts of *A.indica* chewing sticks were effective against *Streptococcus mutans* and *Streptococcus faecalis*. Chewing sticks were recommended as oral hygiene tools for health promotion in developing countries (Almas, 1999).

The principal objectives of the present research work were to determine the antibacterial potential of five selected medicinal plants against bacterial strains associated with gastrointestinal tract infections

2. Materials and Methods

Five medicinal plants *Punica granatum* fruits peel, *Citrullus colocynthis* fruits, *Curcuma longa* rhizome, *Albizia lebeck* leaves, and *Azadirachta indica* Gum were purchased from Omdurman market on the basis of undocumented reports for antibacterial and antioxidant activity. The plants were identified in the Botany Department, Faculty of Science and Technology, Omdurman Islamic University by supervisor Prof. Hatil Elkamali and by comparison with herbarium of the Department. The plants were spread and dried in the shade for three weeks and then pulverized with a mechanical grinder.

Two hundred grams of all plants was macerated separately with 50% methanol (MeOH) in a conical flask for 24 hours. Mother liquor (crude MeOH extract) was filtered and evaporated to dryness. The dry crude extract was sterilized. 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 methanol.

Air-dried plant material (100 g) was ground to a fine powder. It was poured with distilled water (1 litre), 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 aqueous plant extract.

The antibacterial activity was tested by well-agar diffusion method (Cruickshank et al., 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 thorough mixing, approximately 20 ml of this seeded agar

were poured into 10 cm diameter presterilized petri dishes and allowed to solidify. Four 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 was 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 °C for 24 hours. After incubation, the diameter of the zones of inhibition were measured to the nearest mm.

Multidisc for antimicrobial susceptibility testing from Axiom laboratories, New Delhi 1100055 for Gram negative and Gram positive isolates were used as positive control and methanol as a negative control.

3. Results and Discussion

Methanolic extract of *P.granatum* fruit peel was found more effective against most tested bacteria (1Z = 20mm) as in *Salmonella typhi* isolate No.(1), *P.mirabilis* isolates No. (4), *E.coli* isolate No.(5), *S.aureus* isolate No.(12), *S. typhi* isolate No.(18) and *Paeruginosa* isolate No.(7). Moderately active to *P.mirabilis* isolates No.(2, 3,16), *Paeruginosa* isolate No.(7, 15), and *S.aureus* isolates No (11). (1Z = 14-18 mm). *B.subtilis* (NCTC 8236) and *S.aureus* (ATCC 25923) showed promising result (1Z = 24-20 mm). Bacterial species: *K.pneumoniae* (ATCC 35657), *E.coli* (ATCC 25922) and *S.typhi* (ATCC1319106) were found to be resistant. (Table 1). *Curcuma longa* roots showed high antibacterial activity

against *P.mirabilis* isolate No.(3) (1Z = 20 mm) (Table 1). Methanol extract of *C.colocynthes*-fruits, *A.lebbeck* leaves and *A.indica* gum, were found ineffective against all tested Gram-positive and Gram-negative bacteria.

All bacterial species were found to be resistant against aqueous extracts of all plants except *P.granatum* fruit peel. Aqueous extract of *P.granatum* peel showed promising result against *P.mirabilis* isolate No.(4), *P. aeruginosa* isolate No.(8) and *E.coli* isolate No. (9) (1Z = 20 mm) but moderate activity was observed against *S.typhi* isolate No.(1), *S. aureus* isolate No.(14) and *P. aeruginosa* isolate No.(20) (1Z = 14 mm) (Table 1). Aqueous extract of *C.colocynthes*-fruits, *A.lebbeck* leaves, were found ineffective against all tested bacteria.

The antibacterial activity related to known antibiotics was calculated. The results are shown in Table- 2.

Ceftizoxime (CI) was found effective against *P.mirabilis* isolate No. (2) and *Paeruginosa* isolate No.(13) at concentration 30 mcg, and moderate effectively was observed against *Paeruginosa* isolate No.(15) and *E.coli* (ATCC 25922). Cefotaxime (CF) showed promising result against *P.mirabilis* isolate No.(4) at concentration 30 mcg (Table 2). It was showed no antibacterial activity against *S.aureus* (ATCC 25923) and *S. typhi* (ATCC1319106). Ciprofloxacin (CP) showed a fairly high degree of sensitivity to *E.coli* isolate No.(6), *Paeruginosa* isolate No.(7), *S. typhi* B isolate No. (17) and *B.subtilis* (NCTC 8236) at concentration 5 mcg, and demonstrated antibacterial activity against *E.coli* (ATCC 25922), *K. pneumoniae* (ATCC 35657) (Table 3).

Table (1). Antibacterial Activity of Methanolic and Aqueous Extracts of Studied Plants against Clinical Isolates and Standard Bacteria.

No	Clinical isolates bacteria	Extract	<i>Punica granatum</i>	<i>Curcuma longa</i>	<i>Citrullus colocynthes</i>	<i>Albizia lebbeck</i>	<i>Azardirachta Indica</i>
1	<i>Salmonella typhi</i>	Me OH	20	4	2	2	4
		H ₂ O	14	-	-	-	-
2	<i>Proteus mirabilis</i>	Me OH	16	4	2	2	4
		H ₂ O	10	-	-	-	-
3	<i>Proteus mirabilis</i>	MeOH	16	20	10	2	4
		H ₂ O	8	-	-	-	-
4	<i>Proteus mirabilis</i>	MeOH	22	4	-	2	6
		H ₂ O	20	-	-	-	-
5	<i>Escherichia coli</i>	MeOH	20	4	-	-	4
		H ₂ O	6	-	-	-	-
6	<i>Escherichia coli</i>	MeOH	9	4	-	-	4
		H ₂ O	12	-	-	-	-
7	<i>Pseudomonas aeruginosa</i>	MeOH	18	2	-	-	4
		H ₂ O	2	-	-	-	-
8	<i>Pseudomonas aeruginosa</i>	MeOH	8	2	-	2	4
		H ₂ O	20	-	-	-	-
9	<i>Escherichia coli</i>	MeOH	6	2	-	-	4
		H ₂ O	20	-	-	-	-
10	<i>Escherichia coli</i>	MeOH	2	4	-	-	6
		H ₂ O	10	-	-	-	-
11	<i>Staphylococcus aureus</i>	MeOH	18	8	6	2	4
		H ₂ O	12	-	-	-	-
12	<i>Staphylococcus aureus</i>	MeOH	20	2	-	2	2
		H ₂ O	-	-	-	-	-
13	<i>Pseudomonas aeruginosa</i>	MeOH	10	10	10	2	10
		H ₂ O	-	-	-	-	-
14	<i>Staphylococcus aureus</i>	MeOH	12	2	-	2	4
		H ₂ O	14	-	-	-	-
15	<i>Pseudomonas aeruginosa</i>	MeOH	14	2	-	2	4

No	Clinical isolates bacteria	Extract	Punica granatum	Curcuma longa	Citrullus colocynthes	Albizia lebbeck	Azardirachta Indica
16	Proteus mirabilis	H ₂ O	10	-	-	-	-
		MeOH	18	-	-	-	4
		H ₂ O	10	-	-	-	-
17	Salmonella paratyphi B	MeOH	4	8	8	2	4
		H ₂ O	12	-	-	-	-
18	Salmonella typhi	MeOH	20	2	-	-	4
		H ₂ O	8	-	-	-	-
19	Proteus mirabilis	MeOH	12	2	-	6	4
		H ₂ O	8	-	-	-	-
20	Pseudomonas aeruginosa	MeOH	8	2	-	2	4
		H ₂ O	14	-	-	-	-
Standard bacteria							
1	Staphylococcus aureus (ATCC 25923)	MeOH	20	6	6	8	8
		H ₂ O	8	-	-	-	7
2	Bacillus subtilis (NCTC 8236)	MeOH	24	6	6	6	-
		H ₂ O	10	-	-	-	-
3	Escherichia coli (ATCC 25922)	MeOH	6	6	6	4	4
		H ₂ O	-	-	-	-	-
4	Klebsiella pneumonia (ATCC 35657)	MeOH	-	4	2	-	-
		H ₂ O	10	-	-	-	-
5	Salmonella typhi (ATCC1319106)	MeOH	6	2	2	2	4
		H ₂ O	10	-	-	-	4

Values are the mean of four replicates; - no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well).Methanol did not show any inhibitory activity. Sensitive :> 18, intermediate: 14-18mm, Resistant :<14mm, -:No inhibition zone.ATCC: American Type Collection Culture-NCTC: National Collection Type Culture

Table (2). Antibacterial Activity ofAntibiotics- Gram (-ve) against Clinical isolates.

	Antibiotics Clinical isolates	AS 20mcg	BA 25mcg	CF 30mcg	TZP 100/10 mcg	CH 30mcg	CP 5mcg	CI 30mcg	TE 30mcg	OF 5mcg	GM 10mcg	AK 30mcg	GF 5mcg
1	Salmonella typhi	2	-	14	10	8	10	14	2	10	10	10	10
2	Proteus mirabilis	8	10	2	12	8	10	20	2	10	10	10	10
3	Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-
4	Proteus mirabilis	-	-	20	-	-	10	-	-	-	-	-	-
5	Escherichia coli	6	-	12	12	10	-	-	2	8	14	14	14
6	Escherichia coli	-	6	8	16	-	20	-	20	10	12	10	8
7	Pseudomonas aeruginosa	-	10	4	12	-	24	-	4	18	10	14	18
8	Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
9	Escherichia coli	4	16	6	12	14	6	12	-	6	-	-	12
10	Escherichia coli	-	-	-	12	8	14	-	10	14	18	20	14
11	Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-
12	Staphylococcus aureus	2	-	-	6	-	-	-	-	6	-	-	16
13	Pseudomonas aeruginosa	10	10	10	10	10	12	20	6	10	8	8	10
14	Staphylococcus aureus	-	14	2	-	16	8	12	-	4	-	-	10
15	Pseudomonas aeruginosa	-	14	4	-	16	10	16	-	6	16	8	14
16	Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-
17	Salmonella paratyphi B	6	26	8	12	4	30	-	12	18	14	8	16
18	Salmonella typhi	-	-	-	-	-	-	6	-	-	10	6	-
19	Proteus mirabilis	2	12	2	12	16	4	10	-	4	-	-	10
20	Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-

Values are the mean of four replicates; - no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well)

Methanol did not show any inhibitory activity.

Sensitive :> 18, intermediate: 14-18mm, Resistant :<14mm, -:No inhibition zone

Multidisk for Antimicrobial Susceptibility Testing For Gram Negative Isolates:-

Ampicillin/sulbactamAS 20mcg,Co-TrimoxazoleBA 25 mcg,CefotaximeCF 30 mcg

Piperacillin/TazobactamTZP 100/10 mcg, Chloramphenicol CH 30 mcg,CiprofloxacinCP 5 mcg,CeftizoximeCI 30 mcg,TetracyclineTE 30 mcg,OfloxacinOF 5 mcg,GentamicinGM 10 mcg,AmikacinAK 30 mcg,GatifloxacinGF 5 mcg

Table (3). Antibacterial Activity ofAntibiotics-Gram (-ve) against Standard Bacteria.

Antibiotics Standard bacteria	AS 20mcg	BA 25mcg	CF 30mcg	TZP 100/10 mcg	CH 30mcg	CP 5mcg	CI 30mcg	TE 30mcg	OF 5mcg	GM 10mcg	AK 30mcg	GF 5mcg
Salmonella typhi (ATCC1319106)	4	-	-	-	6	-	-	10	-	-	-	-
Klebsiella pneumonia (ATCC 35657)	4	10	4	10	10	16	-	10	10	10	10	10
Bacillus subtilis (NCTC 8236)	6	-	8	20	20	20	-	10	10	10	10	10
Escherichia coli (ATCC 25922)	-	-	6	-	-	14	16	18	12	14	16	14
Staphylococcus aureus (ATCC 25923)	-	-	-	-	-	-	-	-	-	-	-	-

ATCC: American Type Collection Culture. NCTC: National Collection Type Culture

Values are the mean of four replicates; - no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well)

Methanol did not show any inhibitory activity.

Sensitive > 18, intermediate: 14-18mm, Resistant <14mm, -:No inhibition zone

Multidisk for Antimicrobial Susceptibility Testing For Gram Negative Isolates:-

Ampicillin/sulbactam AS 20mcg-Co-Trimoxazole BA 25 mcg-Cephaexin PR 30 mcg-Piperacillin/Tazobactam TZP 100/10 mcg-Chloramphenicol CH 30 mcg-

Ciprofloxacin CP 5 mcg-Ceftizoxime CI 30 mcg- Tetracycline TE 30 mcg-Ofloxacin OF 5 mcg-Gentamicin GM 10 mcg-Amikacin AK 30 mcg-

Garitofloxacin GF 5 mcg

Tetracycline (TE) showed good results against *E. coli* isolate No.(6) and *E. coli* (ATCC 25922) at concentration 30 mcg. Amikacin (AK) showed high antibacterial activity against *E. coli* isolate No.(10) at concentration 30 mcg, and moderate effectively was observed against *E. coli* isolate No.(5), *P. aeruginosa* isolate No.(7) and *E. coli* (ATCC 25922). It was showed no antibacterial activity against *S. aureus* (ATCC 25923) and *S. typhi* (ATCC1319106). Co-Trimoxazole (BA). *S. aureus* (ATCC 25923), *B. subtilis* (NCTC 8236), *E. coli* (ATCC 25922), *S. typhi* (ATCC1319106) and *K. pneumoniae* (ATCC 35657) was found to be Co-Trimoxazole resistant. Co-Trimoxazole was found effective against *S. typhi* B isolate No.(17) at concentration 25 mcg and showed good results against *E. coli* isolate No.(9), *S. aureus* (14) and *P. aeruginosa* isolate No.(15).

Piperacillin/Tazobactam (TZP) showed good results against *E. coli* isolate No.(6) at concentration 100/10 mcg, and found to be ineffective against all standard bacteria except *B. subtilis* (NCTC 8236). Chloramphenicol (CH) showed promising result against *B. subtilis* (NCTC 8236) at concentration 30 mcg, and moderate effectively was observed against *E. coli* isolate No.(9) *S. aureus* isolate No.(14), *P. aeruginosa* isolate No.(15) and *P. mirabilis* isolate No.(19), whereas *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. typhi* (ATCC1319106) and *K. pneumoniae* (ATCC 35657) was found to be Chloramphenicol resistant.

Punica granatum showed the most promising antimicrobial properties. The methanol extract was active against both Gram-negative and Gram- positive bacteria, even the particularly antibiotic-resistant *S. aureus* (ATCC 25923) and *B. subtilis* (NCTC 8236), being inhibited. Secondary metabolites of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens. The most important bioactive compounds of plants were alkaloids, flavonoids, tannins and phenolic compounds. This antibacterial activity may be due to the presence of secondary metabolites (Venkataswamy et al., 2010).

Methanolic extracts *P. granatum* exhibited promising antibacterial activity against clinical isolates *S. typhi* and *P. mirabilis*. The inhibitory effect could be due to the presence of some of the secondary metabolites like phenolic compounds, flavonoids, terpenoids, phytosterols, glycosides and tannins detected in the extract of *P. granatum* (Moorthy et al., 2013). The ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall synthesis, thereby inhibiting the microbial growth (Doss et al., 2009).

Aqueous extracts of *P. granatum* exhibits antibacterial activity against bacterial strains *P. mirabilis*, *P. aeruginosa*, *E. coli*. The inhibitory effect of tannine and phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation. These results confirmed the antibacterial potential of pomegranate and its use in traditional medicine (Duman et al., 2009).

Staphylococcus aureus clinical isolates and standard *S. aureus* (ATCC 25923) showed high sensitivity to methanolic extracts of *P. granatum* and resistant to aqueous extract, whereas exhibited antibiotic-resistant toward all Gram negative and Gram positive antibiotics. *S. aureus* was of considerable importance because it was considered to be one of the major causative agents for numerous hospital and community acquired infections (Vijayasanthi et al., 2012). Plants were able to develop new, faster and natural antimicrobials and then man-made remedies, and that is explaining why plants succeed in its fighting against microbes since millions of years while human failed (Abdallah, 2011).

Curcuma longa methanolic extract revealed high antibacterial activity against *P. mirabilis* (3) and showed antibiotic-resistant. (IZ = 20mm). Curcumin was a non-toxic, highly promising natural antioxidant compound with a wide spectrum of biological functions. It was expected that curcumin may find application as a novel drug in the near

future to control various diseases, including inflammatory disorders, carcinogenesis and oxidative stress-induced pathogenesis (Chattopadhyay et al., 2004).

The results revealed that the importance of Pomegranate peel extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. *P.granatum* extracts has great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes (Nascimento et al., 2000).

Bacillus subtilis (NCTC 8236) showed high sensitivity toward methanolic extract and resistant to aqueous extract and all Gram- negative and Gram-positive antibiotics, except Ciprofloxacin, Chloramphenicol, Levofloxacin and Piperacillin/Tazobactam. *E. coli* (ATCC 25922) and 75% of clinical isolates exhibited resistant to methanolic and aqueous extracts of Pomegranate peel and low sensitivity toward all Gram- negative and Gram-positive antibiotics, except Ciprofloxacin, Tetracycline and Amikacin. Diarrhoea diseases caused by Enteropathogenic *E.coli* (EPEC) were the major reasons of morbidity and mortality among children in the developing countries (Patel et al., 2008).

75% of *P. aeruginosa* showed high sensitivity toward methanolic extract, *Escherichia coli* which showed antibiotic-resistant to most antibiotic and similarly to Amikacin. In the last decades, prevalence and outbreaks of the multi-drug resistant bacterial strains has been increasingly documented throughout the world. At present most clinical isolates of *E.coli* are considered as highly resistant to most commercially known antibiotics (Abdallah, 2011). Out of the 20 clinical isolates from the infected stool, 2 were *S.typhi*, 5 *P.mirabilis*, 4 *E. coli*, 5 *P.aeruginosa*, 3 *S.aureus*, one was *S typhi* B. This is in line with fact that *P.mirabilis*, *E.coli* and *P.aeruginosa* are the most commonly taxa encountered contaminants of stool in foods.

Study on antibacterial activity of five plants brought to light some very interesting results. *Punica granatum* appeared to be the most active against tested bacteria. Methanolic extracts of *Curcuma longa* showed relatively high activity to act on *Proteus mirabilis*. The antibacterial activity of the methanolic extracts of different plants are arranged in a decreasing order as follows: *Punica granatum* > *Curcuma longa* > *Azadirachta indica* > *Albizia lebbek* > *Citrullus colocynthis*.

All bacterial species were found to be resistant against aqueous extracts of all studied plant species (except *Punica granatum*).

Compared to the most reference antibiotics, the spectrum of antibacterial activity of *P. granatum* was found to be clearly superior.

4. Conclusion

Of all extracts the methanolic extracts, *P.granatum* was the most active, whereas, the aqueous extract of *P.granatum* was the most active of all aqueous extracts tested.

Punica granatum showed the most promising antimicrobial properties. The methanol extract was active against both

Gram-negative and Gram- positive bacteria, even the particularly antibiotic -resistant *S.aureus* (ATCC 25923) and *B.subtilis* (NCTC 8236), being inhibited. Methanol extracts of *P. granatum* and *C. longa* showed high antibacterial activity against *P.mirabilis*, *E.coli*, *S.aureus* and *S.typhi* clinical isolates, whereas the aqueous extracts of all plants were found to be ineffective against all tested bacteria Gram-positive and Gram-negative except *P. granatum*, which showed high antibacterial activity against *P.mirabilis*, *E.coli*, *P.aeruginosa* clinical isolates.

Some standard bacterial species showed a fairly high degree of sensitivity to the methanolic extracts of *P. granatum*, against *B.subtilis* (NCTC 8236) and *S.aureus* (ATCC 25923), whereas the aqueous extracts of all plants were found to be ineffective against all tested bacteria

The antibacterial screening of the different extracts (methanol and water) was performed against standard and clinically isolated bacterial strains. The high antibacterial activity was found in methanolic extracts, the lowest one was found in aqueous extract.

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