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In Vitro: Inhibitory Effects of Three Traditional and Medicinal Plants On Some Human Pathogenic Bacteria

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Abstract: The misuse of antibiotics leads to the spread of resistant bacteria, which cause serious health problems. The search for new antibiotics is urgent. Secondary products of different plants showed inhibitory effects to different bacterial pathogens. All the tested bacteria were from the reference strains. *Abutilon pannosum, Salvadora persica* and *Matricaria chamomilla* were collected and extracted with either water or methanol. Their antimicrobial activities were determined using agar well diffusion method and compared to control. The water extracts and the methanol extracts of the tested plant materials were the most active compared to the ethanol extracts. No Activity or very weak activities were recorded for petroleum ether, diethyl ether, acetone and benzene. Water extract of *A. pannosum* showed excellent activity against *Escherichia coli* and *Enterococcus faecalis*, with MIC ranged from 20-25 mg/ml while the maximum activity of *Salvadora persica* was against *Salmonella enterica* and *Pseudomonas earuginosa*. On contrast, the maximum activity of *M. chamomilla* water extract was against *Proteus mirabilis* ATCC 43071 and *P. mirabilis* ATCC 12153 (MIC 30 mg/ml). No toxicity or antitumor activities were detected for the three active water extracts of the three tested plants. The presence of some secondary products in the three tested plant extracts was also evaluated. In conclusion, the water extracts of the three tested plants can be used to treat different bacterial pathogens with no toxicity.

Keywords: Medicinal plants; *Abutilon pannosum; Salvadora persica; Matricaria chamomilla;* Bacterial pathogens, MIC.

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I. INTRODUCTION

Since 1999s and until now, the emergences of multidrug resistant human bacterial pathogens are increased due to misuse of antibiotics (WHO, 2015, Elabd *et al*., 2015). Control and manage human infections by the extensively resistant clinical bacterial isolates is difficult (Magiorakos, *et al*., 2012). The appearance of antimicrobial resistance is dangerous and threatening human life. Moreover, the isolation rates of multidrug resistant bacteria like resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus epidermidis*
(MRSE) in addition to carbapenem resistant Gram negative are continuously increased which posed a serious risk and therapeutic problems. Until now, for treatment of multidrug resistant bacterial infections, no effective antimicrobial agents are currently available (Gomez-Flor *et al*., 2006, Upadhyay *et al*., 2010, Qureshi *et al* 2012) and search for new antibiotics must be started. Medicinal plants are a part of complementary medicine. Plant secondary metabolites are important and can be used worldwide as antioxidant or to treat many bacterial infections (Khalighi-Sigaroodi *et al*., 2012, Kone *et al*., 2004). In developing countries directly or indirectly, medicinal plants or their extracts are used to treat many diseases and help in prevention of cancer and heart disease. Due to the benefits of medicinal plants, they are used by the popular in traditional medicine to treat many diseases, maintaining good health (Silva *et al*., 2010) and in many pharmaceutical preparations (Shinwari and Khan, 1998).

Abutilon pannosum (Abutilon) considered one of the important genera of the family Malvaceae which contained 2300 species belonging to 88 genera. All genera and species were found in tropical, subtropical and temperate regions, India, Pakistan, China and Saudi Arabia (Nasir and Ali, 1979). Different species of the genus Abutilon has medical importance (Bagi *et al*., 1985; Rahuman *et al*., 2008). Some species of genus *Abutilon* recorded antibacterial activity (Muhammad *et al*., 2009; Arulsamy *et al*., 2009; Survase *et al*., 2012)

quercetin, kaempferol and flavonoids derivative (Sharma and Ahmad, 1989; Akiyama *et al*., 2001; Sammia, 2008).

Arak tree, also named Miswak or the toothbrush tree, *Salvadora persica* L, is from the Salvadoraceae family which contained 182 species, out of them Miswak is the most important species. Small Miswak sticks is mainly used as chewing parts as toothpicks for maintaining oral hygiene in Arab, Asian and, African countries (Sher *et al*., 2011, Goyal *et al*., 2011). The different parts of this plant have medical importance for oral hygiene. Both water and organic extracts of Miswak showed inhibitory activities for bacteria that developed dental plaque and periodontitis (Sofrata *et al*., 2008). The extract Miswak showed antagonistic activities on different cariogenic and periodontal bacteria, in vitro. The tested bacteria were *Staphylococcus aureus, Streptococcus mutans, S. faecalis, S. pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa, Porphyromonas gingivalis* and *Haemophilus influenzae*, in addition to one yeast, *Candida albicans* (Sofrata *et al*., 2008, Naseem *et al*., 2014). Miswak extract was effective as antimicrobial agent in the endodontic treatment of teeth with necrotic pulps (Chelli-Chentouf *et al*., 2012, Sofrata *et al*., 2011).

Chamomile or *Matricaria chamomilla* is one of the most famous medicinal plants. It is a herbal plant, 15-50 cm high, gives beautiful flowers quickly and mainly in Europe and Arab Countries beaches (Svab, 1979). Haslam (1989) reported that Chamomile flowers contained the phenolic compound, flavonoides (a glycogen, apigenin, flavon glycoside and lutoline) and glycosides (anthemic acid, anthamedine and matricarin). Chamomile is used as anti-inflammatory agent, and was used to remove pain, calm headaches and tooth aches (Owlia *et al*., 2007). In India, chamomile is commercial and medicinal crop, with high international market price. Thus, it was cultivated as industrial, valuable and commercial crop and its oil is usually used in India (Owlia *et al*., 2007). The aim of the present study was carried out to determine the antibacterial activity of different extracts of three selected plats on Gram positive and negative bacteria against Ampicillin (20 µg/ ml).

II. MATERIALS AND METHODS

2.1. Plant material collection

The plant materials, *Abutilon pannosum* (Abutilon) leaves, *Salvadora persica* (Arak) roots and *Matricaria chamomilla* (Chamomile) flowers were collected from Jazan, Saudi Arabia. At room temperature (25–27°C), the collected plant materials were shade dried under aeration for 10-15 days. After plant identification, complete sample of each plant was preserved at the herbarium of Biology Department, Umm Al Qurah University, Faculty of Applied Science, Makkah Al Mukkaramah; Saudi Arabia.

2.2. Tested microorganisms

The tested bacteria were obtained from the culture collection of King Fahad General Hospital, Jeddah, Saudi Arabia. The used tested bacteria were either Gram negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *K. pneumoniae* ATCC 13883, *Salmonella enterica* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 43071, *P. mirabilis* ATCC 12153 or Gram positive (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and MR *S. aureus* ATCC 33591).

2.3. Preparation of soluble plant extracts

Abutilon pannosum (Abutilon) leaves; *Salvadora persica* (Arak) roots and *Matricaria chamomilla* (Chamomile) flowers were collected and dried. Each sample was cut into small pieces, using an electrical blender, ground into a fine powder, which was extracted using boiling water or organic solvents, methanol, ethanol, petroleum ether, diethyl ether, acetone and benzene. All solvents were obtained from Sigma-Aldrich Company. The mixture (50 g of each powder + 100 ml of the extractor) was agitated for 8 hr. at room temperature, and then maintained for more 4 hr to settle. The obtained extract was filtered through Whatman No.1 filter paper and the solvent was removed using a lyophilizer in case of using water or rotary evaporator at 40°C in case of using methanol. The obtained residue was weighed and dissolved in 2 ml DMSO and used for the next experiments.

2.4. Antibacterial Activities

Disc diffusion agar method described by Irobi and Daramola (1993) was used for detection of the antibacterial activity of the different plant extracts. Inoculated plates containing 10 ml of the Mueller Hinton agar and inoculated with 0.1 ml of bacterial suspension ($2x10^6$ cfu/ml) were allowed to solidify. Paper discs (5 mm) soaked in each of the crude plant extracts were put on the surface of each inoculated plate. Ampicillin (20 µg/ml) and DMSO were used as positive and negative controls, respectively (Aly and Gumgumjii, 2011). After plate incubation at 37°C for 24 hr, the mean diameter of the inhibition zones were measured. For the active extracts, the minimal inhibitory concentrations were determined using modified fluorescein diacetate method as described by Chand *et al*. (1994).

2.5. Toxicity and Antitumor Activity of the Plant Extracts

Artemia salina larva was used to detect the toxic effect of each plant and LD_{50} for each extract was determined (Chelkowski, 1989, Aly and Gumgumjee, 2011). Furthermore, antitumor activity of the three tested extracts against the tumor cell line MCF-7 was determined at 37°C, under a humidified atmosphere, 90% air and 10% CO_2, in RPMI 1640 medium, containing 10% fetal calf serum. After 48 hr, percentage of cell viability was determined and LD_{50} was calculated.

2.6. Phytochemical Analysis

The presence of the active constituent, anthocyanins, betacyanins, flavonoides, saponins, steroids and Phytobutanins were detected. Studies were carried out on the chemical analysis of those extracts using methods described by Fadeyi *et al*. (1987), Varadharajan *et al*. (2012).

2.7. Statistical analysis

Differences between the test and control groups were analyzed with chi-square test using a statistical package program (Sigma Plot version 11.0). *P*-values less than 0.01 were considered to be significant.

III. RESULTS AND DISCUSSION

In different countries, hundreds of medicinal plants are used as antimicrobial agents and they are vital sources of numerous powerful medicines (Srivastava *et al*., 1980). *Abutilon pannosum, Salvadora persica* and *Matricaria chamomilla* were from the families Malvaceae, Salvadoraceae and Composite, respectively (Table 1 and Figure 1). The three plant samples were extracted and tested for their antimicrobial activities. The results of the study were shown in Table (2) and Figure (2). The water extracts and the methanol extracts of the tested plant materials were the most active compared to the ethanol extracts. No Activity or very weak activity were recorded for petroleum ether, diethyl ether, acetone and benzene, thus their results were neglected and didn't shown. The water extract of *A. pannosum* leaves showed a strong inhibitory effect (18-19 mm) for the growth of *E. coli* and *E. faecalis*, moderate inhibitory effect (15 mm) for the growth of *S. aureus* and *P. aeruginosa.* A weak inhibitory effect (7 mm) for the growth of MRSA *S. aureus*, *P. mirabilis* ATCC 12153 and *P. mirabilis* ATCC 43071 was recorded. No activity was recorded for *A. pannosum* water extract against *S. enterica*, *K. pneumoniae* ATCC 13883 and *K. pneumonia* ATCC 700603. For the methanol extracts of *A. pannosum* leaves, lower activities were recorded compared to the water extracts. It showed maximum activities against *P. mirabilis* ATCC 12153 (17 mm) and mediated effect (10-15 mm) for the growth of *E. coli* and *P. aeruginosa* while no activities was receded against *K. pneumoniae* ATCC 700603, *S. enterica* and MRSA *S. aureus*.

Table (1). The tested plants, their families and extracted parts.

Plant used	Common name	Family	Extracted part
Abutilon pannosum	Abutilon	Malvaceae	Leaves
Salvadora persica	Arak, Miswak	Salvadoraceae	Roots
Matricaria chamomilla	Chamomile	Composite	Flowers

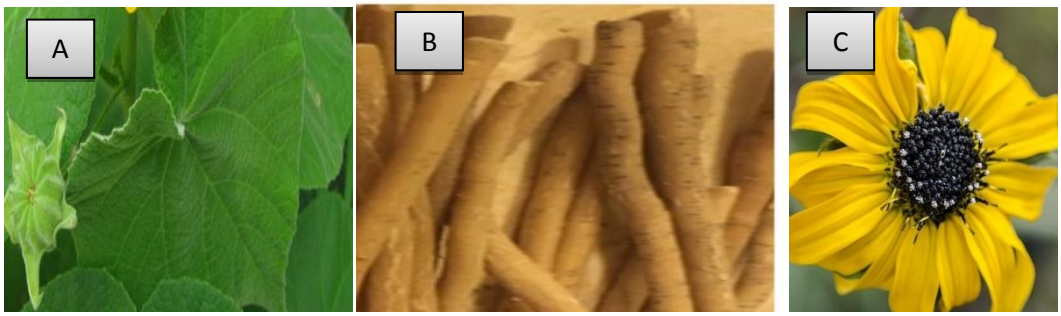

Figure (1). The collected plant parts, A: *Abutilon pannosum* leaves, B: *Salvadora persica* roots and C: *Matricaria chamomilla* flowers

It is noted that the extracts of leaves of *Abutilon* using distilled water or methanol have inhibiting effect on the growth of the most studied bacterial strains using paper disk method on both Gram positive and negative bacteria, *E. faecalis* and *E. coli*. This is consistent with the results of Poonkothai (2006) which showed that the methanol extract of the leaves of *Abutilon* plant was a significant inhibitor for the growth of *E. coli*. The results of Mohamed *et al*. (2010) showed that out of 23 different plants, from19 families in Sudan, *Abutilon* plant was the most active against some pathogenic bacteria such as *E. coli; S. aureus* and *K. pneumoniae*, thus can be used

in the treatment of various diseases and the chemical survey revealed the presence of tannins and steroid and terpenoid. They added that the methanolic extract has the excellent ability to inhibit bacteria due to the active substances in the methanolic extract which act as an inhibitor of bacterial growth.

Table (2): The antimicrobial activity (diameter of inhibition zone, mm) of water and methanol extracts of the three tested plants

Tested plant / Tested bacterium	*Abutilon pannosum*		*Salvadora persica*		*Matricaria chamomilla*		Ampicillin (control)
	Water	Methanol	Water	Methanol	Water	Methanol	
E. coli ATCC 25922	18.0	13.0	10.0	12.0	12.0	7.0	19
Klebsiella pneumoniae ATCC 700603	ND	ND	12.0	9.0	10.0	ND	22
Klebsiella pneumoniae ATCC 13883	ND	10.0	10.0	9.0	10.0	ND	22
Salmonella enterica ATCC 14028	ND	ND	15.0	9.0	10.0	10.0	20
Pseudomonas aeruginosa ATCC 27853	15.0	13.0	15.0	ND	10.0	10.0	22
Proteus mirabilis ATCC 43071	10.0	10.0	13.0	9.0	19.0	16.0	29
Proteus mirabilis ATCC 12153	10.0	17.0	10.0	ND	15.0	13.0	19
Enterococcus faecalis ATCC 29212	19.0	10.0	13.0	ND	10.0	10.0	27
Staphylococcus aureus ATCC 25923	15.0	10.0	10.0	9.0	10.0	ND	22
MR *S. aureus* ATCC 33591	7.0	ND	13.0	9.0	10.0	ND	13

ND: not detected

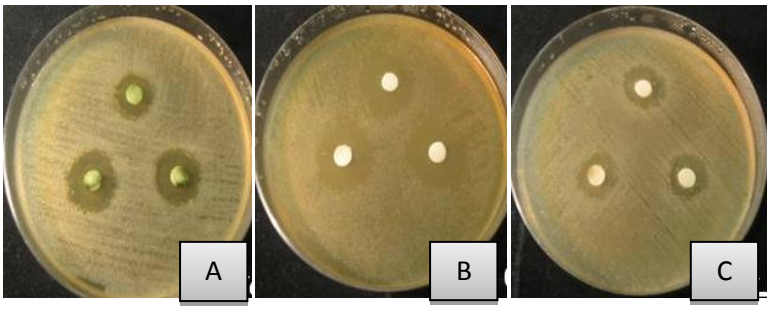

Figure (2): Effect of the water extract of the three tested plant extracts on *E. faecalis*, (A): *Abutilon pannosum* leave extract, (B): *Salvadora persica* root extract and (C): *Matricaria chamomilla* flower extract.

The results of the use of aqueous extract of the roots of the *S. persica* plant showed a good reduction (10-15 mm) for the growth of most bacterial strains as follows *P. mirabilis* ATCC 43071, *P. mirabilis* ATCC 12153, *E. faecalis*, MRSA *S. aureus*, *P. aeruginosa*, *K. pneumoniae* ATCC 700603 and *S. enterica*. Moreover from the results of the study, it was noted that the water extract of *S. persica* roots has a weak inhibitory effect (7-10 mm) for the growth of three bacterial strains, *S. aureus*, *E. coli* and *K. pneumoniae* ATCC 13883. The effect of methanolic extract on the roots of the *S. persica* showed a moderating (10-15 mm) inhibitory effect on the growth of *E. coli* and a weak inhibitory effect (7-10 mm) for the growth of *P. miraiblis* ATCC 43071, MRSA *S. aureus*, *K. pneumoniae* ATCC 700603; *K. pneumoniae* ATCC 13883, *S. enterica* and *S. aureus*.

The effect of *M. chamomilla* flowers extract on different bacterial growth was determined. The results in table 1 showed the effect of *M. chamomilla* flowers extracts, obtained by either distilled water or methanol. The two extracts inhibited the growth of the tested bacterial strains. The water extract of *M. chamomilla* flowers showed a strong inhibitory effect (15-20 mm) for the growth of both tested strains *P. mirabilis* and moderate inhibitory effect of 10-15 mm for the growth of *E. coli*. A weak inhibitory effect (7-10 mm) was for the growth of the other tested bacterial strains, including three Gram positive bacterial strains, *E. faecalis*, MR *S. aureus* and *S. aureus* and four Gram negative bacteria, *P. aeruginosa; K. pneumoniae* ATCC 700603, *K. pneumonia*e ATCC 13883 and *S. enterica*. From the results shown, the two plant extracts of *M. chamomilla* (distilled water or methanol) have a strong inhibitory effect for the growth of most of the bacterial strains studied. The results showed that the methanolic extract of *M. chamomilla* flowers had a strong inhibitory effect (15-20 mm) for the growth of *P. mirabilis* ATCC 43071 and moderate inhibitory effect (10-15 mm) for the growth of *P. mirabilis* ATCC 12153. A weak inhibitor (7-10 mm) for the growth of each of the following bacterial strains, *E. faecalis, P. aerginosa, E. coli* and *S. enterica* was recorded. The resistance of two gram-positive bacterial strains MRSA and *S. aureus* in addition to two Gram-negative bacteria, *K. pneumoniae* ATCC 13883 and *K. pneumoniae* ATCC 700603 to the effect of methanolic extract of *M. chamomilla* was recorded.

In the world market, the demand for chamomile was increased due to the medicinal values and herbal medicines are healthy and free from side effects, thus there is still a wide scope for detect different uses of chamomile (Singh *et al*., 2011). The results of the survey conducted by Dulger and Gonuz (2004) for a number of extracts revealed that chamomile is a magic medicinal plant, used in the treatment of various microbial diseases, caused by *E. coli; S. arueus; K. pneumoniae; P. aeruginosa*, *Proteus vulgaris*, *Bacillus cereus* and *Mycobacterium smegmatis*. The study by Essawi and Srour (2000) showed a significant difference in the activity of the effect of organic and aquatic extracts of chamomile flowers, and this is observed with the current study. Aqueous or organic extract of chamomile flowers have many therapeutics values and used to cure mouth injuries, respiratory and digestive infection (Ribereau-Gayon, 1979).

The minimal inhibitory concentrations (MICs) of the water plant extracts of the three tested plants were calculated and compared with that of Ampicillin. The MIC was ranged from 20-40 mg/ml for *A. pannosum* leave extract and 30-40 mg/ml for *S. persica* and *M. chamomilla* extracts (Table 3). Up to 400µg/ml, no toxicity was found for all tested extracts. No toxicity was recorded for water extracts of the three tested plants against *Artemia salina* larva or antitumor activities against MCF-7 cells. In contrast, Aly and Gumgumjee (2011) showed that *Curcuma longa* exhibited excellent antitumor activity against Ehrlish ascites carcinoma cell line and no toxicity was found using *Artemia salina* as test organism. The antimicrobial activities of the plant extract may due to the phytochemical materials, tannin and flavonoids which at low concentration inhibit the growth of the human pathogens (Dixit *et al*., 1980, Natarajan and Lalithakumar, 1987, Ficker *et al*., 2003).

Table (3): The minimal inhibitory concentrations of water extracts of the three tested plants, Abutilon pannosum, Salvadora persica and Matricaria chamomilla.

Tested bacteria	MIC (mg/ml)			Ampicillin (µg/ml)
	A. pannosum	*S. persica*	*M. chamomilla*	
E. coli ATCC 25922	25	40	40	10.0
Klebsiella pneumoniae ATCC 700603	ND	40	40	12.5
Klebsiella pneumoniae ATCC 13883	ND	40	40	7.5
Salmonella enterica ATCC 14028	ND	30	40	7.5
Pseudomonas aeruginosa ATCC 27853	30	30	40	5.0
Proteus mirabilis ATCC 43071	40	30	30	5.0
Proteus mirabilis ATCC 12153	40	40	30	5.0
Enterococcus faecalis ATCC 29212	20	30	40	5.0
Staphylococcus aureus ATCC 25923	30	30	40	2.5
MR *S. aureus* ATCC 33591	40	30	40	>12.5

ND: Not Detected

Table (4): Toxicity and antitumor activity of the three tested extracts and compared to bleomycin**.**

Used extract	**Toxicity (LD_{50}, µg/ml)**	**Antitumor activity (LD_{50}, µg/ml)**
	Artemia salina	**MCF-7 cells**
Abutilon pannosum	**> 400**	**> 400**
Salvadora persica	**> 400**	**> 400**
Matricaria chamomilla	**> 400**	**> 400**
Bleomycin	**≥ 002**	**0.02 ± 0.001**

Table (5): The active photochemical compounds detected in the different plant extracts of the three plants

Medicinal plant	**Photochemical compounds**					
	Anthocyanin	**Betacyanins**	**Phytobutanin**	**Flavonoides**	**Steroids**	**Saponins**
Abutilon pannosum	+	-	-	+	+	-
Salvadora persica	+	-	-	-	+	-
Matricaria chamomilla	+	-	-	-	+	-

(+): The material present (-): The material absent

The water extracts of the three tested plants were analyzed for deferent materials. Anthocyanin and Stetoids were detected in all tested plants while Flavonoides were only detected in *A. pannosum*. Saponins, Phytobutanin and Betacyanins were not detected in the tested extracts. Medicinal plants have variety of

secondary metabolites such as alkaloids, flavonoids, saponins and sterol superior in their antimicrobial potential (Cowan, 1999). Alkaloids are nitrogenous compounds that contain heterocyclic ring and they have the therapeutics importance because of their great antimicrobial properties and their ability to bind with the nucleic acids (Jayasurriya *et al*., 1991). In occlusion, the water extract of *Abutilon pannosum* leaves, *Salvadora persica* roots and *Matricaria chamomilla* flower showed excellent antimicrobial activities with on toxicity, thus they can be used safely in traditional medicine to tr.

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