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Short Communication: Antibacterial activity of certain Saudi Arabian medicinal plants used in folk medicine against different groups of bacteria

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Abstract. Al-Ghanayem AA, Al Sobeai SM, Alhussaini MS, Joseph B, Saadabi AM. *2017. Antibacterial activity of certain Saudi Arabian medicinal plants used in folk medicine against different groups of bacteria. Nusantara Bioscience 9: 392-395*. Medicinal plants from Saudi Arabia has been used in folk medicine for treatment of many diseases. The present research is on medicinal plants, which are locally available such as *Acacia ehrenbergiana* (Arabic: Salam) (Fabaceae), *Calotropis procera* (Arabic: Ausher) (Apocynaceae), *Haloxylon salicornicum* (Arabic: Rimth) (Amaranthaceae), *Panicum turgidum* (Arabic: Thuman) (Poaceae), *Tamarix arabica* (Arabic: Athal) (Tamaricaceae), *Rhazya stricta* (Arabic: Harmal) (Apocynaceae) *Rumex vesicarius* (Arabic: Humeid) (Polygonaceae) for antimicrobial activity by agar well diffusion method. Minimum Inhibitory Concentration (MIC) has been determined by broth micro dilution method. *A. ehrenbergiana* was active against *Klebsiella pneumoniae*. *R. vesicarius* and *T. arabica* showed antibacterial activity against *Proteus vulgaris. C. procera* showed a moderate inhibitory activity against the bacterial pathogens tested. *H. salicornicum* extract was more effective towards Gram-positive bacteria. However, other extracts such as *P. turgidum* and *R. stricta* were less active against both Gram-positive and negative bacteria tested. The results will be helpful in discovering new phytochemical components with antibacterial activity that can be used against multidrug-resistant bacterial strains.

Keywords: *Acacia ehrenbergiana*, antibacterial activity, *Calotropis procera*, medicinal plants, MIC, *Rumex vesicarius*

INTRODUCTION

Higher plants and their products have been used from ancient days for treating human diseases, and the usefulness of plant-based medicinal compounds gifted by nature is well understood. Some of these compounds have been supported for the design and development of synthetic drugs. Therefore, plants still have been a promising source of newer drugs (Veeresham 2012). There are a large number of unexplored plants and plant products with high therapeutic value. The antimicrobial activity of some medicinal plants present in the local area is not well explored. Meanwhile, some plants have been tested for their activities, but the reports are not well documented. Some of plant-based products from several geographical areas have been successfully entering the pharmaceutical industries because their phytochemical and biological studies have been conducted. There are many phytochemical components such as alkaloids, terpenes, flavonoids, glycosides, etc., showing a variety of pharmacological activities (Edeoga and Ikem 2002). On this basis, a number of locally available plants will be screened for both antibacterial and antifungal activities. A thorough investigation to evaluate the efficacy and safety of these herbal drugs will lead to development and identification of valuable compounds (Kesavan et al. 2007).

Development of antibiotic resistance has been increased worldwide. Because of the overdosage and unlimited usage of antibiotics for treatment of infections and preservation of food, bacterial and fungal pathogens have mutated and become multidrug-resistant (Nascimento et al. 2000). The growing concern of antimicrobial resistance becomes a global problem, and there is a necessary need to find out new potential antimicrobial agents which are safe and affordable to the public against multidrug-resistant strains of bacteria and fungi (Susana et al. 2007). The new antimicrobial agents can be used as synergetic drugs or in combination with conventional antimicrobial agents. There is an urgent need to develop novel molecules. Therefore, it is crucial to identify the antimicrobial potential of locally available plants and know the phytochemical composition to create a proper documentation. In the present study, some medicinal plants which are locally available in the region of Saudi Arabia such as *Acacia ehrenbergiana* (Arabic: Salam) (Fabaceae), *Calotropis procera* (Arabic: Ausher) (Apocynaceae), *Haloxylon salicornicum* (Arabic: Rimth) (Amaranthaceae), *Panicum turgidum* (Arabic: Thuman) (Poaceae), *Tamarix arabica* (Arabic: Athal) (Tamaricaceae), *Rhazya stricta* (Arabic: Harmal) (Apocynaceae) *Rumex vesicarius* (Arabic: Humeid) (Polygonaceae) were analyzed for antimicrobial activity.

MATERIALS AND METHODS

Plant materials

Plants materials were collected from Shaqra area of Saudi Arabia and were authenticated by the Department of Botany, King Saud University, Kingdom of Saudi Arabia; voucher specimens were deposited at the department.

Preparation of plant extracts

The freshly collected aerial parts of the plants especially leaves were washed with running tap water and dried under shade. The materials were chopped and ground into coarse powder and extracted with methanol for 20 h. The preparation was evaporated under vacuum to remove the solvents, and the extracts obtained were separately dissolved in Dimethyl sulfoxide (DMSO) to get a stock of 100 mg/mL concentration and kept in a refrigerator until used.

Screening for antimicrobial activity

The plant extracts were tested against different types of bacteria such as *Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Enterobacter cloacae, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Streptococcus pneumoniae*. The actively growing cultures of bacteria were inoculated into 4 mL of peptone water and incubated at 37^{0}C for 4 h. The turbidity of the growing cultures was adjusted to match 0.5 units of McFarland solution (Cheesbrough 2008). Agar well diffusion method was adopted to determine the antibacterial activity of extracts (Kavanagh 1972).

Minimum inhibitory concentration

Broth microdilution method was used to determine Minimum Inhibitory Concentration (MIC) for the active crude extracts against the test microorganisms. Test bacterial cultures (100 µl of bacterial culture containing 10^5 CFU/mL) were inoculated into tubes containing different concentrations of extracts (75, 37.5, 18.8, 9.4, 4.7, 2.4, 1.2, 0.6, 0.3, 0.15 mg/mL) and incubated at 37^{o}C for 24 h. The values were determined by detecting the inhibition of visible growth in the culture tubes. Minimum Bactericidal Concentration (MBC) was assayed by sub-culturing the broth onto freshly prepared solid media and further incubated at 37^{o}C for 24 h. The lowest concentration of MIC tubes which did not show any growth of bacterial colonies was regarded as MBC (CLSI 2012).

RESULTS AND DISCUSSION

The methanol extracts of all the plants were tested for antibacterial activity against *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *E. cloacae*, *K. pneumoniae*, *E. faecalis*, *E. coli*, *B. cereus*, *S. aureus* and *S. pneumoniae*. The previous study with *C. procera* has been shown that the plant has antibacterial and antiparasitic activities (Larhsini et al. 1999). In the present study, *R. vesicarius* and *T. arabica* at a concentration of 10 mg/mL showed inhibitory activity towards *P. vulgaris*. *A. ehrenbergiana* showed an antibacterial effect on *K. pneumoniae* at 10 mg/mL. These results clearly show that high extract concentration has high antibacterial activity. Recently, Rahim et al. (2016) studied the antimicrobial activity of methanol extract of *A. ehrenbergiana* which showed a wide spectrum activity against bacterial isolates but a poor inhibitory activity on fungi. *R. stricta* showed relatively weak antibacterial activities against all Gram-negative bacteria tested. A recent study showed that *R. stricta* solvent extract has antimicrobial activity against Gram-negative and Gram-positive bacteria and according to phytochemical analysis, the extract contains organic alkaloid (Raziuddin et al. 2016).

Table 2. Antibacterial activity of methanol extracts from different medicinal plants against Gram-positive bacteria *

Plant extract (mg/mL)		*Bacillus cereus*	*Staphylococcus aureus*	*Streptococcus pneumoniae*
Rimth	1	10	11	9
	10	17	18	18
Humeid	1	9	7	9
	10	12	11	10
Harmal	1	8	9	7
	10	13	14	13
Athal	1	12	8	8
	10	18	15	17
Salam	1	8	9	12
	10	13	13	17
Ausher	1	10	8	7
	10	12	9	10
Thuman	1	11	8	7
	10	13	11	9

Note: *values are means of 3 replicates (inhibition zone in mm)

Table 1. Antibacterial activity of methanol extracts from different medicinal plants against Gram-negative bacteria *

Isolates	Plant extract (mg/mL)													
	Rimth		Humeid		Harmal		Athal		Salam		Ausher		Thuman	
	1	10	1	10	1	10	1	10	1	10	1	10	1	10
Pseudomonas aeruginosa	8	10	7	11	7	9	8	10	10	12	9	11	7	11
Enterobacter cloacae	7	10	8	12	8	9	9	15	8	17	8	10	9	12
Enterococcus faecalis	9	15	9	12	8	11	10	17	11	14	8	15	7	12
Escherichia coli	7	9	8	13	7	10	8	13	7	11	7	11	8	12
Klebsiella pneumoniae	7	11	8	15	9	10	11	17	10	18	7	12	6	13
Proteus vulgaris	9	13	12	18	7	8	11	18	10	15	13	17	7	13
Salmonella typhi	6	11	8	17	9	10	7	15	9	14	8	14	9	15

Note: *values are means of 3 replicates (inhibition zone in mm)

Table 3. MIC and MBC values of methanol extracts of the selected plants against Gram-negative bacteria*

Isolates	Plant extract (mg/mL)													
	Rimth		Humeid		Harmal		Athal		Salam		Ausher		Thuman	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter cloacae	18.8	37.5	18.8	37.5	37.5	75	37.5	75	37.5	75	9.4	37.5	9.4	18.8
Enterococcus faecalis	9.4	18.8	9.4	18.8	9.4	37.5	9.4	37.5	9.4	18.8	9.4	18.8	37.5	75
Escherichia coli	18.8	37.5	9.4	37.5	9.4	18.8	9.4	18.8	9.4	18.8	9.4	37.5	9.4	37.5
Klebsiella pneumoniae	18.8	37.5	18.8	37.5	18.8	37.5	18.8	37.5	9.4	37.5	9.4	18.8	18.8	37.5
Proteus vulgaris	18.8	37.5	4.7	9.4	9.4	18.8	9.4	18.8	37.5	18.8	37.5	18.8	9.4	37.5
Pseudomonas aeruginosa	18.8	37.5	18.8	37.5	18.8	37.5	18.8	37.5	18.8	37.5	18.8	9.4	37.5	18.8
Salmonella typhi	37.5	75	9.4	37.5	18.8	37.5	18.8	37.5	18.8	37.5	37.5	75	37.5	75

Note: *values are in mg/mL

Table 4. MIC and MBC values of methanol extracts of the selected plants against Gram-positive bacteria *

Isolates	Plant extract (mg/mL)													
	Rimth		Humeid		Harmal		Athal		Salam		Ausher		Thuman	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus cereus	9.4	18.8	18.8	37.5	18.8	37.5	9.4	18.8	18.8	37.5	37.5	75	18.8	37.5
Staphylococcus aureus	9.4	18.8	18.8	37.5	18.8	37.5	9.4	18.8	18.8	37.5	18.8	37.5	18.8	37.5
Streptococcus pneumoniae	9.4	18.8	37.5	75	37.5	75	9.4	18.8	9.4	18.8	37.5	75	37.5	75

Note: *values are in mg/mL

Table 5. Antibiotic sensitivity pattern shown by the test isolates *

Bacterial isolates	Tetra-cycline (30)	Erythro-mycin (15)	Chlor-amphenicol (30)	Cipro-floxacin (5)	Genta-micin (10)
B. cereus	19	15	17	18	21
E. cloacae	14	17	18	16	19
E. coli	18	19	18	21	17
E. faecalis	20	21	22	19	20
K. pneumoniae	15	17	19	20	20
P. aeruginosa	15	14	12	11	17
P. vulgaris	20	24	21	24	20
S. aureus	20	24	20	18	17
S. pneumoniae	14	16	21	16	15
S. typhi	15	18	17	19	17

Note: *values are inhibition zone in mm

Calotropis procera showed moderate inhibitory activity. *C. procera* has been used to treat skin disorders, intestinal worms, etc. (Parrotta 2001). Anti-mycobacterial and antibacterial activities of *C. procera* against Treponema have been reported (Kew 1985). Furthermore, it has been used for the treatment of water at water purification plants to reduce the total viable count of microorganisms to an extent (Shittu et al. 2004). Antimicrobial activity of ethanolic extracts of *C. procera* has been shown to be effective against bacterial such as E. coli and fungal pathogens like *Candida albicans* (Kareem et al. 2008). Plant secondary metabolites were reported to play an essential role in plant physiology; have potential effect as antioxidants, anti-allergic anti-inflammatory anti-cancer and antihypertensive agents on a human body; and show antimicrobial activities as well (Daglia 2012). *P. turgidum* has been studied for its antimicrobial activity and found to be effective against *C. albicans* and *Streptococcus pyogenes*. Phytochemical analysis revealed that hot and cold extracts of *P. turgidum* consist of alkaloids, flavonoids, tannins and saponin compounds (El-Desoukey 2017). In the present study, however, only a moderate antibacterial activity was shown by *P. turgidum* against all Gram-negative bacteria tested. Minimum inhibitory concentration and minimum bactericidal concentration of the extracts was tested against Gram-negative bacteria, and the results are shown in Table 3.

Haloxylon salicornicum showed an antibacterial effect on all Gram-positive bacteria tested. The activity increases with the dose of the extracts. *H. salicornicum* has been reported for antidiabetic and antiseptic activity. Several alkaloids have been extracted from *H. salicornicum* (Benkrief et al. 1990). *T. arabica* also had considerable antimicrobial activity against Gram-positive strains. The antioxidant and antimicrobial activity of *Tamarix* sp. has been reported (Sultanova et al. 2001). However, both *H. salicornicum* and *T. arabica* did not inhibit the Gram-negative bacteria as strongly as they inhibited the Gram-positive bacteria. Minimum inhibitory concentration was 9.4 mg/mL for *H. salicornicum* and *T. arabica* whereas, the minimum bactericidal concentration was 18.8 mg/mL (Table 4). Antibacterial activities of the most active plant extract were compared with that of the commercial antibiotics (Table 5). The results show that the selected plants have antimicrobial activities and further extended studies are needed pharmacological aspects and its phytochemical analysis.

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