



Experimental Study of Antibacterial Activity of Desert Medicinal Plants Against Pathogens

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ABSTRACT

The aim of the present study was to screen leaf and roots of *Leptadenia pyrotechnica* and fruit and roots of *Citrullus colocynthis* for the antimicrobial activity against bacteria. The ethanolic and acetic extract of the *Leptadenia pyrotechnica* and *Citrullus colocynthis* was studied for its antagonistic activity against some of the Gram positive and Gram negative bacteria. In vitro antimicrobial test was performed by agar well diffusion method on Nutrient agar Medium. *Pseudomonas aeruginosa* was most susceptible to these extracts except acetone root extract of *Citrullus colocynthis* among tested bacterial strains. *Leptadenia pyrotechnica* and *Citrullus colocynthis* ethanolic and acetic extracts demonstrated a dose-dependent (500 µg/ml, 250 µg/ml, and 125 µg/ml) anti-microbial activity in-vitro.

KEYWORDS

Antimicrobial activities, desert medicinal plant.

Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to relative unavailability of medicine and emergence of widespread drug resistant microorganisms (Okeke *et al.*, 2005). The World Health Organization reported that 80% of the world population relies chiefly on traditional medicines involving the use of plant extracts or their active constituents (WHO, 1993). India is also well known for Ayurveda, which is one of important traditional medicine practiced. Rajasthan Desert is a potential source of herbal plants, covers most of the north-western part of Rajasthan state.

The fruits of *Citrullus colocynthis* (L.) Schrader (Cucurbitaceae family) commonly known as bitter apple or bitter cucumber and useful against fever, intestinal parasites, hepatic and abdominal diseases. Root extract of the plant is generally used against jaundice in Sudan, Iran and India (Ivora *et al.*, 1989).

Leptadenia pyrotechnica (Forssk.) Decne, commonly known as *khimp* belonging to the family Asclepiadaceae, is widespread in Indian desert, tropical Africa, Asia and the Mediterranean region and in the sandy plains in the Western Gulf countries. It is an erect, much branched, often leafless, erect shrub (Bhandari, 1990). There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Singh and Pandey, 1998; Kuttan 1996). The plant is generally known for its significant uses for treatment of wounds and some skin diseases. The pods fruits of this plant are cooked as a vegetable (Qureshi, 2002).

The present study was undertaken to evaluate antimicrobial activity of leaf stalk, root and fruit extracts of *Citrullus colocynthis* and *Leptadenia pyrotechnica* against selected bacterial pathogens that causes various diseases in human being.

Materials and Methods

Collection of Plant materials—The Leaf stalk, fruits and Root of *Citrullus colocynthis* and *Leptadenia pyrotechnica* plant were collected from IASE (D) University, campus Sardarshahr (Rajasthan). Collected material was shade dried, made to fine powder.

Preparation of plant extract

10 grams of dried powder of leaf stalk roots, fruit was soaked in 100ml of 90% acetone and ethanol and it was placed on shaker at 120 rpm for 30 minutes. It was kept for two weeks with intermittent shaking. Filtered with Whatman no.1 filter paper. Concentration was maintained at 40 °C under reduced pressure using rotator evaporator. The extract obtained was stored in bottles and stored at 4°C.

Microorganisms used

Six different bacterial strains were used throughout the present work: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella flexneri* and *E.coli*. These strains were purchased from the Microbial Type Culture collection (MTCC) and Institute of Microbial Technology; Chandigarh (India). The bacterial isolates were maintained on nutrient agar slants and stored at 4°C with regular transfer at monthly intervals.

Preparation of bacterial inoculums

Inoculums of employed bacterial strains were prepared in autoclaved Nutrient broth and incubated at 37°C for 24 hours.

Evaluation of antibacterial susceptibility

Agar well diffusion method was used for the evaluation of antibacterial susceptibility. Nutrient agar medium was prepared, autoclaved at 121°C for 15 minutes and solidified in petriplates. Each plate contained 20ml of this medium. Each bacterial strain was uniformly spread on this medium in separate plates (Omeregic *et al.*, 2010). Wells of 6 mm diameter were bored in solidified media (Ekpo & Etim, 2009).

The same method was used to determine the inhibitory effect of both plants extracts on the tested microorganisms. Holes of 6 mm diameter were made into which the plant extracts (50 µl) was applied. Ampicillin was used as positive control (Ekpo & Etim, 2009). The culture plates were incubated at 37 °C for 24 hours. The antibacterial activity was measured from the formation of the clearing zone due to inhibition of bacterial growth around the treated area. Zones of inhibition were recorded in millimeters and the experiments were repeated thrice.

Results and discussion

As shown in table -1 and figures 1&2 antimicrobial screening of acetone and ethanol (90%) extracts of leaves, fruits and roots of *Citrullus colocynthis* and *Leptadenia pyrotechnica*

showed positive results against all six test organisms (*Staphylococcus aureus* , *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella flexneri* and *E.coli*.(Table1,Figs-1 ,2).

Maximum antimicrobial activity was exhibited by the acetone fruit extract and acetone root extracts of *Citrullus colocynthis* against *E.coli* 31mm,31mm, respectively. Leaf stalk acetone extract and acetone root extracts of *Leptadenia pyrotechnica* also showed maximum antibacterial activity against *E.coli* it was 30mm, 30mm respectively among all the tested strains.

Pseudomonas aeruginosa showed resistance against acetone root extracts of *Citrullus colocynthis* as well the standard antibiotic ampicillin(Table 1 & fig.2) . The antimicrobial activity of plant extracts was compared to standard antibiotic ampicillin 500mg/ml(Table 1 and Figures 1&2).Among solvents, acetonic extracts of both the plants exhibited remarkable activity (Table 1 and Figures 1&2).The extracts of both the plants were applied with three doses 500mg/ml,250mg/ml,125mg/ml and the inhibition zone were observed in order 500mg/ml>250mg/ml>125mg/ml (Table 1 and Figures 1&2).*E.coli* was most susceptible to all the extracts among all strains followed by *Salmonella typhi*, *Shigella flexneri*,*Klebsiella pneumonia*,*Staphylococcus aureus* , *Pseudomonas aeruginosa* (Table 1 and Figures 1&2).

The above antimicrobial study shows that the fruit and root extracts of *Citrullus colocynthis* inhibit the growth of Gram positive(*Staphylococcus aureus*) and Gram negative bacteria (*Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella flexneri* and *E.coli*.Table-1& Fig -2) except *Pseudomonas aeruginosa*. Similar results observed by Memon *et al.*, (2003) that the ethanolic extract of *Citrullus colocynthis* which is active against gram positive bacteria i.e. *Bacillus pumilus* and *Staphylococcus aureus* whereas it is inactive against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. However present study

reveals that acetonic extracts show strong activity against *E.coli* whereas inactive against *Pseudomonas aeruginosa* (Table-1& Fig -2).

Rasool Khatibi *et al.*. (2011) screened *C. colocynthis* aqueous and diluted acetone extracts (roots, stems, leaves,fruit and seeds)for antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) and various *Candida* spp. (*Candida glabrata*, *Candida albicans*, *Candida parapsilosis* and *Candida kreusei*). They reported positive antibacterial activity of extracts of *C. colocynthis* against above mentioned bacteria.

Sandhya and Biradar(2013) reported that root, stem and leaves extracts of *Citrullus colocynthis* prepared with solvents like acetone , ethanol , petroleum ether , methanol and water were found effective against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella shigella*, *Candida albicans*. Ilham *et al.*, (2013) also reported the antibacterial efficiency mature fruits of *Citrullus colocynthis*, similar to present findings.

As shown in Table 1 &figure 1 leaf stalk and root extract of *Leptadenia pyrotechnica* inhibit the growth of gram positive and Gram negative bacteria. Shaw *et al.*,(2013)also suggested that varying concentrations of the ethanolic extracts of root and aerial part of *Leptadenia pyrotechnica* show antibacterial activity against the selected strains (25 to 1000 µg / ml) *S. aureus* and *B.subtilis* and ethanolic extract of the aerial part of the plant showed moderate activity against *Candida albicans*.Rather several workers like Al Fatmi *et al.*, (2007)and Praveen Upadhayay *et al.*,(2007) reported that the whole plant of *Leptadenia pyrotechnica* proved to have antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

Table -1.Antimicrobial activities of leaf stalk,root and fruit extracts of *L. pyrotechnica* and *C. colocynthis* against Gram positive and Gram positive Bacteria

Plant Extract Name of the Bacteria		Zone of inhibition in mm																								
		<i>Leptadenia pyrotechnica</i> Leaf stalk						<i>Leptadenia pyrotechnica</i> Root						<i>Citrullus colocynthis</i> Fruit						<i>Citrullus colocynthis</i> Root						Control Ampicillin
		Acetone Leaf stalk			Ethanol Leaf stalk			Acetone Root			Ethanol Root			Acetone Fruit			Ethanol Fruit			Acetone Root			Ethanol Root			-
		Concentration of extracts																								
		a	b	c	a	b	c	A	b	c	a	b	c	a	b	c	a	b	C	a	b	c	a	b	c	
Staphylococcus aureus		16	12	10	17	14	11	20	15	12	18	13	11	19	16	13	19	14	13	18	16	12	15	13	11	12
Pseudomonas aeruginosa		18	14	12	18	16	13	20	16	14	18	16	13	18	16	14	18	14	11	0	0	0	20	17	12	0
Klebsiella pneumonia		25	16	12	22	17	12	22	19	12	20	16	12	19	17	13	21	19	13	21	18	13	22	19	16	25
Salmonella typhi		26	18	13	16	16	12	24	18	14	22	16	14	24	18	13	25	20	12	24	19	12	22	18	14	27
Shigella flexneri		22	18	14	14	14	12	23	19	16	24	16	12	22	17	12	21	19	13	21	18	10	21	19	13	25
E. coli		30	20	14	21	18	15	30	20	16	20	15	12	31	25	14	30	21	14	31	20	12	25	16	16	30

A-500mg/ml,b-250mg/ml,c-125mg/ml

Fig.1

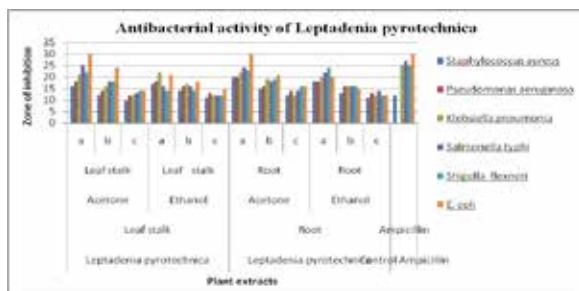
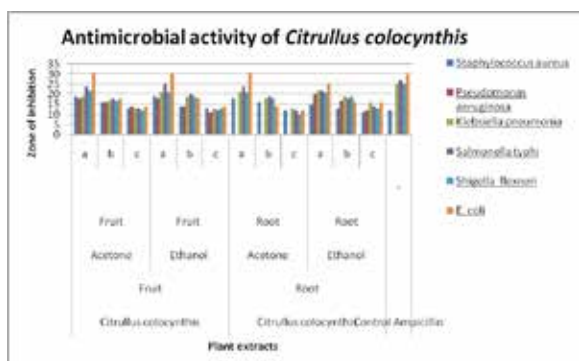


Fig.2



Conclusion

Extracts of different parts of *Citrullus colocynthis* and *Leptadenia pyrotechnica* demonstrated a dose dependent antimicrobial activity against pathogenic Gram positive and Gram negative bacteria. The acetonic fruit and root extracts exhibited promising antibacterial activity and inhibited the growth of all tested strains as compared with the standard antibiotic ampicillin.

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References:

- World Health Organization. (1993). Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram*. 28:13-14.
- Epko MA and Etim P. C(2009).Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. *J Med. Plants Res*.3(9):621-624.
- Omoregie E H, Ibrahim I, Nineka I, Sabo AM ,Koma O S and Ibumeh OJ (2010).Broad spectrum Antimicrobial Activity of Psidium guajava Linn. Leaf. *Nature and Science*. 8(12):43-50.
- Okeke IN, Laximanarayan R, Bhutta ZA, Duse AG, Jenkins P, O Brien TF, Pablos-Mendez A, Klugman KP (2005). Antimicrobial resistance in developing countries. Part-1: recent trends and current status. *Lancet Infectious diseases* 5 481-493.
- Ivorra D, Paya M, Villar A, Review of natural products and plants as potential antidiabetic drugs. *J. Ethanopharmacol*.1989; 27:243-275.
- Kuttan G. Use of *Leptadenia pyrotechnica* dunal as an adjuvant during radiation therapy. *Indian Journal Exp. Bio*. 1996; 34(9): 854-856.
- Rasool K and Jahanbakhsh T (2011). Anticandidal screening and antibacterial of *Citrullus colocynthis* in South East of Iran. *Journal of Horticulture and Forestry* .3(13):392–398.
- Sandhya V. Rodge ,S.D. Biradar(2013).Preliminary phytochemical screening and antimicrobial activity of *Citrullus colocynthis*.(Linn.) Schared. *Indian Journal of Plant Sciences*. 2 (1):19-23.
- Ilham Brynan , Hamid Hasan ,Mufeed Ewadh (2013).Antibacterial Activity of Citrullus Colocynthis against different types of bacteria. *Advances in Life Science andTechnology*. 7:48-51.
- Mahida Y and Mohan JSS (2007).Sreening of plants for their potential antibacterial activity against Staphylococcus and salmonella spp. *Nature product radiance*.6(4):301-305.
- Shaw N. H. , Desai. P,Singh G.K. (2013).Antimicrobial Activity of the Arial and Root extracts of *Leptadenia Pyrotechnica*.*International Journal of in-*

- ventions in *Pharmaceutical Sciences*.1(6):532-536.
- Qureshi, R. 92002). Ethnobotany of Rohri Hills Sindh Pakistan.*Hamdard Medicus*, 1(XLV): 86-92.
- Memon Usman, Brohi Hakeem Abdul, Syed Waseemuddin Ahmed, Iqbal Azhar and Husan Bano (2003). Antibacterial screening of *Citrullus colocynthis*. *Pakistan Journal of Pharmaceutical Sciences* 16 (1) 1-6.
- Al-Fatimi M, Wurster M, Schroder G, Lindequist U(2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethno pharmacology*.111: 657-666.
- Praveen Upadhayay B Roy S, Kumar A(2007). Traditional use of medicinal plants among rural communities of churu district in the Thar desert. *Indian Journal of Ethnopharmacology*.113:387-399.