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ANTIBACTERIAL ACTIVITY OF ROOT AND FRUIT EXTRACTS OF *LEPTADENIA PYROTECHNICA* (ASCLEPIADACEAE) FROM PAKISTAN

MEHMOODA MUNAZIR[1], RAHMATULLAH QURESHI[1*], MUHAMMAD ARSHAD[1] AND MUHAMMAD GULFRAZ[2]

[1]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[2]*Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
Corresponding author's e-mail: rahmatullahq@yahoo.com

Abstract

The *In vitro* antibacterial activity of *Leptadenia pyrotechnica* roots and fruits extracts was investigated against *Staphylococcus epidermidis* and *S. aureus* by using agar-well diffusion assay. Plant samples were collected from Thal desert of Pakistan and eight different solvents viz. n-hexane, chloroform, acetone, ethylacetate, butanol, methanol, ethanol and water were used for the preparation of extracts. *S. aureus* was found highly susceptible and inhibited by all solvent extracts. Both plant parts effectively inhibited the growth of both the pathogens; however, root extracts showed a little more supremacy in this respect. Methanolic extract of both parts generated the best results by inhibiting growth of both pathogens. The activity was strongly affected by variation in solvents and concentrations of extracts. The antibacterial activity of *L. pyrotechnica* is reported for the first time.

Introduction

Bacteria are ubiquitous and cause various types of human diseases including urinary tract infections (Bouza *et al.,* 2001; Khan & Musharraf, 2004), nosocomial bloodstream infections (Blot *et al*., 2005), wound infections (Wassilew, 1989), brain abscess (Rau *et al.,* 2002), pneumonia (Rubinstein *et al*., 2008), asthma (Chan-Yeung & Lam, 1986), community-acquired pneumonia (Tillotson & Lerner, 1967; Grant *et al*., 2000) and skin infections (Delahaye *et al*., 2009). Some of the bacterial strains cause serious infections in human beings and in many cases death may result, for example, intestinal infection is the most common cause of diarrhea worldwide and is estimated to be responsible for the deaths of 3-4 million individuals each year (Anon., 1996).

Throughout the world, antibiotics are used to treat all microbial infections; however, bacteria are gradually becoming resistant against antibiotics (Jacobs, 1998). Furthermore, during recent years, over-use of antibiotics is also creating problems. This alarming situation calls for an accelerated search for novel antibacterial drugs and therapeutic agents (Saeed & Tariq, 2005).

It has also been observed that many of the currently used anti-microbial drugs are natural products derived from plants (Poonkothai *et al*., 2005a; Poonkothai *et al*., 2005b) and have been identified from ethno-medicinal data from various regions of the globe. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last few decades intensive studies have been carried out for the development of natural therapies. This fact highlights the significance of medicinal plants in our lives since these plants and their extracts have been used since beginning of human civilization on this planet. The presence of antimicrobial agents in plants has opened new avenues for the discovery of novel natural products that can serve as substitutes for antibiotics resistant to pathogenic microbes (Delahaye *et al*., 2009). Although plant extracts have great potential in treating of infectious diseases caused by resistant microorganisms (Nasir & Chanda, 2006), but it is surprising to note that less than 5% world plant species have been analyzed so far as potential medicine, while rests of the 95% of plants are still to be analyzed (Mukherjee, 2004).

Leptadenia pyrotechnica (Forssk.) Decne. (Commonly known as Khipp) belonging to family Asclepiadaceae is one of such medicinal plants, which has not been investigated sufficiently for its antimicrobial activity especially from Pakistan. This plant belongs to family Asclepiadaceae. It is an erect, much branched, generally leafless shrub which is distributed throughout desert areas of Pakistan. This species is medicinally important and is also used for its fibers (Ali, 1983). The plant is ethnomedicinally used for the treatment of wounds and some skin diseases. Poultice of young twigs of this plant is applied externally to relieve pain and inflammation and unripe pods/fruits of this plant are cooked as a vegetable (Qureshi, 2002). The other studies reported that it has antispasmodic, anti-inflammatory, antihistaminic, antibacterial, hypoglycemic, diuretic, urolith expulsion, expectorant property and found useful in gout and rheumatism (Aquino *et al*., 1996; Abd El-Ghani & Amer, 2003; Cioffi *et al*., 2006; Panwara & Tarafdarb, 2006; Jain *et al*., 2007).

Some studies reported phytochmical constituents of the plant without mentioning their antimicrobial activity (Noor *et al*., 1993; Abd EL-Ghani & Amer, 2003; El-Hassan *et al*., 2003; Cioffi *et al*., 2006; Heneidak *et al*., 2006; Panwara & Tarafdarb, 2006; Moustafa *et al*., 2007; Moustafa *et al*., 2009). The present study was undertaken to evaluate antibacterial activity of root and fruit extracts of this plant against selected bacterial pathogens that cause a multitude of diseases in human beings.

Materials and Methods

Collection and identification of plant samples: Plant materials of *L. pyrotechnica* were collected from Thal desert, Punjab Pakistan. Plant specimens were prepared, identified and authenticated with the help of "Flora of Pakistan" (Ali, 1983) and voucher specimens deposited in the Herbarium of Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi.

Preparation of extracts: All plant parts were separated, washed in running tap water for some time so as to remove dust particles, dried in open air at room temperature which are then ground to fine powder and stored in air tight containers in dark at 4°C for further use. Extracts were prepared using different organic solvents (ranging from non-polar to polar solvents i.e., n-hexane, chloroform, acetone, ethylactate, butanol, ethanol, methanol and water). For this purpose, 5g of each powdered plant sample was extracted with 50ml of each solvent. All extractions were carried out by shaking each sample for 24 hours at 28°C and then centrifuged at 10,000 rpm for 10-15 minutes and filtered. These were allowed to dry at 40°C. The dried extracts were dissolved in 30% dimethylsulfoxide (DMSO) for antibacterial assays (Al-Bakri *et al*., 2007). Different concentrations of each extract (i.e. 50mg/ml, 40mg/ml, 30mg/ml, 20mg/ml and 10mg/ml) were prepared and kept in refrigerator for further use.

Preparation of bacterial inoculums: Inoculums of bacterial isolates (i.e. *Staphylococcus aureus* and *S. epidermidis*) were prepared in autoclaved Lauria-Bertini liquid media and incubated at 37°C for 24 hours (Omoregie *et al*., 2010).

Antibacterial susceptibility test: Antibacterial activity of all individual extracts was assessed by using agar well diffusion method. For this purpose, L. B. solid media was prepared, autoclaved at 121°C for 15 minutes and solidified in petri plates. Each plate contained 20ml of this media. An inoculum (turbidity adjusted to approximately 10^8 CFU/ml of bacterium, compared with 0.5 Mc Farland standards) (Mahida & Mohan, 2007) of respective bacterial strain was uniformly spread on this media in separate plates (Omoregie *et al*., 2010). Wells of 6 mm diameter were bored in solidified media (Ekpo & Etim, 2009). Plant extracts, standard antibiotic and DMSO were injected in respective wells. Gentamycin used as positive (Ekpo & Etim, 2009) and DMSO as negative control. Each extract was replicated thrice. The plates were incubated at 37°C for 24 hours.

Measurement of zone of inhibition: The zone of inhibition for each extract was observed, measured and expressed in mm (Omoregie *et al*., 2010). Later on the activity index (A.I.) and Percent Inhibition (P.I.) were calculated for all solvent extracts obtained at a concentration of 50mg/ml using the following formula:

$$\text{A.I} = \frac{\text{Mean zone of inhibition of each solvent extract}}{\text{Zone of inhibition obtained for standard antibiotic}}$$

$$\text{P.I.} = \text{Activity index} \times 100$$

Results and Discussion

The genus *Staphylococcus* represents gram positive bacteria and many members cause a variety of in diseases human and animals. *S. epidermidis* causes acne vulgaris, a severe skin infection characterized by inflammatory to non-inflammatory papules along with pus formation. It infects skin areas with heavy sebaceous follicles (Harisaranraj *et al*., 2010). *S. aureus* is another skin pathogen which causes infection on face and nose. It is a nosocomial pathogen that causes localized, diffused and highly contagious skin infections (impetigo). This bacterial species can grow on dry surfaces, forming biofilms, thus increasing chances of contamination and transmission. It may cause life threatening infections including staphylococcal scalded skin syndrom and pyaemia. The species also infects dairy cows causing bovine mastitis, resulting into severe losses to dairy industory in Pakistan (Bachaya *et al*., 2005). There exists a strong need to control such species so as to protect health from losses. But, due to increasing resistance in such pathogenic species has proved a serious threat for health. Plant-based drugs are supposed to be suitable and reliable alternate in the prevailing situation.

The use of traditional medicines in primary health care system is well recognized throughout the world (Miller & Morris, 2004). A number of studies on antibacterial activity of medicinal plants extracts have appeared in the literature (Taniguchi *et al*., 1978; Balandrin *et al*., 1985; Ali *et al*., 2001; Mothana & Lindequist, 2005). However a large number of species are yet to be analyzed. *L. pyrotechnica* is one of such species, which has not been scientifically evaluated for the establishment of medicinal properties.

The present study investigated *In vitro* antibacterial activity of eight different solvent extracts i.e., n-hexane (A), chloroform (B), acetone (C), ethyl acetate (D), butanol (E), ethanol (F), methanol (G) and water (H) of roots and fruits of *Leptadenia pyrotechnica*. The results revealed that both parts extract showed promising antibacterial activity. There was a huge difference in inhibitory activity of all solvent extracts in case of both plant parts (Tables 1 & 2). Furthermore, it has been observed that the activity was found as dose dependent. N-hexane and butanol based roots and fruit extracts failed to express activity against both bacterial strains, however, ethanol, methanol, water and ethyl acetate exhibited good activity against them.

Results showed that *S. aureus* was highly susceptible and inhibited by all solvent extracts. On comparing both parts extracts, it has been observed that root extracts had strongest inhibitory activity against *S. aureus* (Fig. 1). Contrary, fruit extracts were found more effective than root extracts in checking the growth of *S. epidermidis*, however, methanolic and ethanolic extracts showed little supremacy from root base (Fig. 1).

Among solvents, methanolic extracts exhibited remarkable/strongest activity. The activity of solvents were in the order of methanol> ethanol> ethyl acetate>chloroform>acetone>water>butanol >n-hexane. With reference to organisms, *S. aureus* was inhibited best by methanolic root extract with 81% growth inhibition, followed by methanolic fruit extract (76%). The same sequence observed for controlling *S. epidermidis* as it was inhibited best by methanolic root extract with 100% P.I., followed by methanolic fruit extract (82% P.I.).

The results of present study are in agreement with the findings of Lopes-Lutz *et al*., (2008) who reported that plant extracts are effective in inhibiting growth of *S. aureus.* The present study revealed that methanolic plant extracts exhibited highly significant antibacterial activity against the tested organisms.

Table 1. Antibacterial activity of fruit extracts of *L. pyrotechnica* measured at a concentration of 50mg/ml.

Bacterial Strains	Solvents	Zone of inhibition expressed in mm	Zone of inhibition of standard drug expressed in mm	Activity Index	% Inhibition
S. aureus	A	-	16	0	0
	B	14	25	0.56	56
	C	9	17	0.53	53
	D	10	18	0.55	55
	E	-	20	0	0
	F	16	26	0.61	61
	G	19	25	0.76	76
	H	-	18	0	0
S. epidermidis	A	-	21	0	0
	B	8	17	0.47	47
	C	7	16	0.44	44
	D	10	18	0.55	55
	E	-	19	0	0
	F	14	19	0.74	74
	G	14	17	0.82	82
	H	10	18	0.55	55

Table 2. Antibacterial activity of root extracts of *L. pyrotechnica* measured at a concentration of 50mg/ml.

Bacterial strains	Solvents	Zone of inhibition expressed in mm	Zone of inhibition of standard drug expressed in mm	Activity Index	% Inhibition
S. aureus	A	-	13	0	0
	B	10	15	0.67	67
	C	11	15	0.73	73
	D	12	17	0.71	71
	E	-	14	0	0
	F	12	15	0.80	80
	G	13	16	0.81	81
	H	-	13	0	0
S. epidermidis	A	-	14	0	0
	B	8	13	0.62	62
	C	9	17	0.53	53
	D	10	17	0.59	59
	E	9	13	0.69	69
	F	8	14	0.57	57
	G	15	15	1	100
	H	8	14	0.57	57

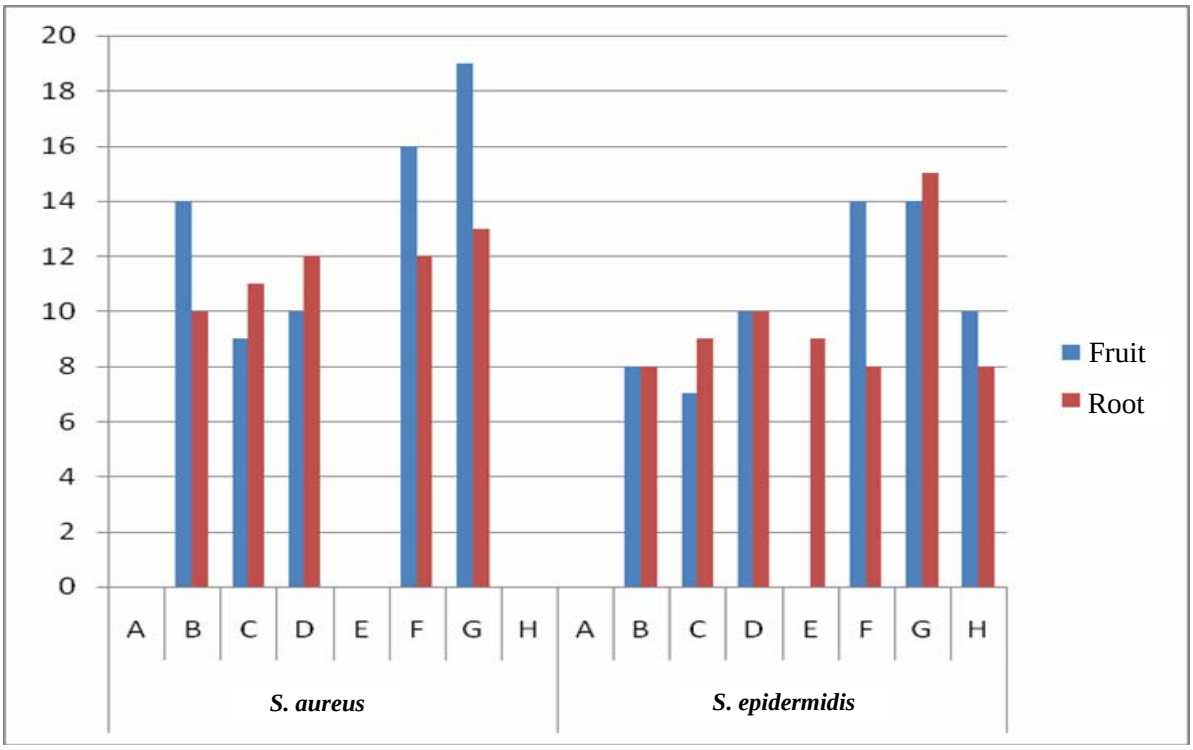

Fig. 1. Comparison of zone inhibition (mm) of Root and fruit extracts.

Minimum inhibitory concentration (MIC): The methanolic fruit extract exhibited profound inhibition (7 mm) against *S. aureus* at the lowest dose of 10 mg/ml, whereas, the root extract was also found effective (9mm) in checking growth of *S. epidermidis* at the same concentration (Fig. 1). This extract if investigated further may lead to discover some novel compounds responsible for antibacterial activity that can be harvested by pharmaceutical industry. This study provided a good insight into extraction efficiency of different solvents and established relationship between extraction efficiency and bioactivity of solvents.

Antibacterial activity of various plant species has been highlighted from across the world and there are numerous studies that documented strong activity in this respect. Such curing properties of plants are due to the presence of various phytochemicals in them (Blot *et al*., 2005). Since past two decades antimicrobial activities of plants and plant parts like root, stem, leaf and flowers have been documented. It is hypothesized that present findings will be used as benchmark for searching new compounds from the plant extracts.

Conclusion

The antibacterial activity of *L. pyrotechnica* is reported for the first time. Both plant parts of *L. pyrotechnica* showed good antibacterial activity. The methanolic root extracts exhibited promising antibacterial activity and inhibited the growth of *S. epidermidis* at par with the standard antibiotic (i.e. Gentamycin). Moreover, performance of methanolic extracts was the best amongst all solvents. The present results are stressing the need for in-depth analysis of methanol extracts in order to obtain potential compound responsible for antibacterial activity. Furthermore, the antibacterial activity of various parts of the plant validated scientifically the traditional use of the plant for treating skin diseases by the natives of desert habitats.

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