Academic Journal of Plant Sciences 2 (2): 109-112, 2009
ISSN 1995-8986

Antimicrobial Activity and Phytochemical Analysis of Stem Bark of *Balanites roxburghii* Planch.

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Abstract: The phytochemical analysis of both the aqueous and ethanolic extracts of medicinal plant *Balanites roxburghii* Planch. and their antibacterial activities against clinical isolates, *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Staphylococcus aureus* were investigated. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, gum and mucilage in varying concentration. Both the ethanolic and aqueous extracts inhibited the growth of the test organisms with *S. typhi* showing the highest susceptibility. This research supports the local use of the bark of the plant *Balanites roxburghii* for prophylactic and therapeutic purposes against bacterial infection.

Key words: *Balanites roxburghii,* Phytochemical analysis, Antimicrobial activity, Activity index, Therapeutic purpose

INTRODUCTION

Balanites roxburghii Planch. (Simarubaceae) locally known as Hingota, is one of the most common but neglected wild plant species of the dry land areas of India. Traditionally it is used as emetic, anthelmintic, anti-fungal, purgative, cathartic, colic, in whooping cough, skin diseases and dog bite. According to Ayurveda, bark is anthelmintic, spasmolytic, used in cough and skin diseases. Leaf is anthelmintic whereas root is emetic. Fruits are used in treatment of whooping cough and in skin diseases. The paste of bark is prepared and applied externally on the affected part of the body [1]. The whole plant is used in treatment of snake-bite. Seeds are used as expectorant (given in the treatment of cough) and colic [2]. Kernel is used in skin diseases and burns [3]. Roots and fruits contain 0.2-2.2 % and 0.3-3.8 % diosgenin (used in contraceptives), respectively. The steroids (sapogenin) are employed in the synthesis of drug including sex hormones and oral contraceptives. In case of pain and swelling, the bark of plants is used by traditional healers. The plant *Balanites roxburghii* having antifertility efficacy [4] and anti-inflammatory activity [5].

Balanites roxburghii which contains steroidal saponins has spermicidal, cardiovascular, molluscidal properties [6]. *Balanites roxburghii* pericarp extract show contraceptive efficacy in male mice [7].

Exhaustive literature survey indicated that systematic pharmacological work has not been done so far on this plant. Hence, this plant was selected to find its antimicrobial activity.

MATERIALS AND METHODS

Collection of Plant Materials: The stem bark of *Balanites roxburghii* was collected from the roadside location of the village- Doniapura, Gormi, Bhind (M.P.) and was identified by Prof. J. R. Patel, Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind (M.P.). Plant material was collected in the month of June-2007 and preserved in herbarium of institution (voucher specimen no. J-52). The stem bark of the plant was separated, dried in shade and coarsely powdered with mechanical grinder.

Preparation of Extracts: Dried bark of *Balanites roxburghii* were powdered and added to distilled water and boiled on slow heat for 2 hr. It was then filtered through 8 layers of musilin cloth and centrifuged at 5000 rpm for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121°C and at 15 lbs pressure and stored at

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4°C. For solvent extraction, the air-dried powder was taken in ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hours. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume and stored at 4°C in airtight bottle.

Microorganisms: The species of bacterial organisms were *S. aureus, E. coli, K. pneumoniae* and *S. typhi*. They were clinical isolates obtained from institute of Microbial technology, Chandigarh, India. The cultures of bacteria were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests and sub-cultured on to nutrient broth for 24 h prior to testing [8, 9].

Phytochemical Screening: To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1 ml of the filterate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. Exact 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, to test for presence of tannins, about 0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins. Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone. The presence of cardiac glycosides was confirmed by Liberman's test, Salkowski test and Keller-Killani test [10, 11].

Antimicrobial Susceptibity Test: The spreading method was used. Twenty four hours old cultures of the organisms to be tested were used. A loopful of the cultures was uniformly spread over the surface of a sterile Muller-Hilton agar with a sterile bent rod. The extract was diluted to obtain different concentration of, 62.5, 125, 250 and 500 mg/ml using sterile peptone water. Various concentrations of the prepared extracts were used to fill hole bored by 5 mm cork borer in the inoculated agar. The plates were made in triplicate with one for the test organism- gentamycine, standard drug.

All plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition in the triplicate plates was measured by calculating the difference between core borer (5 mm) and the diameters of inhibition [12] and their mean designated as ZI. The activity indices, designated as AI, were calculated as the division of zone of inhibition of the extract by that of the standard drug i.e. Gentamycin [13].

Tube Dilution Method: The extracts were diluted into different concentrations of 62.5, 125, 250 and 500 mg/ml with sterile peptone water in test tubes. Ethanol and water were used as the control. To each of the dilution was added 0.2 ml broth culture of the test organism. The tubes were incubated at 37°C for 24 h after which turbidity reading was taken using turbidiometer. Extracts added with peptone water served as control.

RESULTS

Phytochemistry of the Plant Extracts: Phytochemical analysis of both the extract revealed that carbohydrate, alkaloid, flavonoids, saponins, Tannins & phenolic compound are generally present in both the extracts. Glycosides, phytosterols, gum and mucilage were found only in aqueous extract and protein, fat and oils were found in ethanolic extract. Table 1 shows the phytochemical screening results of aqueous and ethanolic extracts of the plant *Balanites roxburghii* used in this study.

Antimicrobial Activity: Both crude ethanolic and aqueous forms of the extracts of *Balanites roxburghii* exhibited varying degree of antimicrobial activities against the test organisms. On a general note, ethanolic extracts exhibited higher degree of antibacterial activities than the aqueous extracts. At 62.5 mg/ml, crude ethanolic extract

Table 1: Qualitative analysis of aqueous and ethanolic extracts of stem bark of *Balanites roxburghii*

S. No.	Chemical constituents	Aqueous extract	Ethanolic extract
1.	Alkaloids	+ve	+ve
2.	Carbohydrates	+ve	+ve
3.	Glycosides	+ve	-ve
4.	Saponins	+ve	+ve
5.	Phytosterols	+ve	-ve
6.	Proteins	-ve	+ve
7.	Flavonoids	+ve	+ve
8.	Fat & oils	-ve	+ve
9.	Tannins & phenolic compound	+ve	+ve
10.	Gum and Mucilage	+ve	-ve

+ve indicates presence of chemical constituents and –ve indicates absence of chemical constituents

Table 2: Antibacterial activity of *Balanites roxburghii* extracts using the agar diffusion technique (mm)

		62.5 mg/ml				125 mg/ml				250 mg/ml				500 mg/ml			
		A		B		A		B		A		B		A		B	
Isolate	Gentamycin	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I
E. coli	7.5±1.0	3.8±0.1	0.5	3.0±0.3	0.5	5.0±1.0	0.7	4.0±0.2	0.5	6.0+0.5	0.8	4.8±0.3	0.6	9.5±10	1.3	6.0±0.5	0.8
Staph. aureus	9.5±1.0	4.0±0.5	0.4	3.5±0.6	0.4	6.4±1.0	0.7	4.0±1.0	0.4	6.5+1.0	1.0	5.5±0.6	0.6	8.0±11	0.8	6.5±1.0	0.7
Sal. typhi	7.8±1.1	4.0±0.3	0.5	4.4±1.0	0.5	6.8±0.5	0.9	5.8±0.5	0.5	8.5+1.0	1.1	6.6±1.0	0.9	9.0±10	1.2	7.5±1.2	1.0
K. pneumonia	6.1±1.1	3.0±0.6	0.5	4.0±0.6	0.5	6.0±0.2	1.0	5.0±0.6	0.8	6.6+0.7	1.1	5.2±0.3	0.9	7.0±10	1.2	6.6±0.5	1.1

A = ethanolic extract, B = aqueous extract, Z.I = mean zone of inhibition in mm?1SD, A.I = activity index with respect to Gentamycin

Table 3: Antibacterial activity of *Balanites roxburghii* extracts using the tube dilution method

	62.5 mg/ml		125 mg/ml		250 mg/ml		500 mg/ml	
Isolate	A	B	A	B	A	B	A	B
E. coli	4.60	5.60	3.70	4.22	2.80	3.10	1.50	2.21
Staph. aureus	5.90	5.90	4.60	4.40	3.85	3.60	2.30	2.90
Sal. typhi	4.82	4.76	3.70	3.60	2.40	2.80	1.20	1.90
K. pneumonia	6.60	6.20	5.62	5.82	4.20	4.35	3.10	3.85
Control	5.10	5.20	4.94	5.14	4.76	4.92	4.00	4.12

A = ethanolic extract and B = aqueous extract

had higher antibacterial activity with mean zones of inhibition 3.8±0.1 mm (A.I = 0.5) and 4.0±0.5 mm (A.I = 0.4) than crude aqueous extract with mean zones of inhibition 3.0±10.3 mm (A.I = 0.4) and 3.5?10.6 mm (A.I = 0.4) against *E. coli* and *S. aureus,* respectively. Besides that, aqueous extract had higher antibacterial activities [mean zone of inhibition 4.4±1.0 mm (A.I = 0.6) and 4.0±0.6 mm (A.I = 0.6) and 4.0±0.6 mm (A.I = 0.7)] than ethanolic extract [4.0±0.3 mm (A.I = 0.5) and 3.0 ±0.6 mm (A.I = 0.5)] against *S. typhi* and *K. pneumoniae*, respectively.

Equal or sometimes higher activities were observed at concentration of 250, 500 mg/ml by the crude ethanolic extracts than the standard drug, gentamycin. Hence, the activity index, A.I = 1 against *E. coli, S. typhi* and *K.pneumoniae*. Consistently high activity indices were observed against the etiology of pneumonia at crude concentration of 125 and 250 mg/ml (Table 2).

The high activity indices were enduring with decrease in concentration from 62.5 to 500 mg/ml. Just low reduction in activities were observed as the crude extract concentration were reduced gradually from 62.5 to 500 mg/ml in both the agar diffusion set up (Table 2) and tube dilution method (Table 3). The same trend of activity in agar dilution was equally observed in tube dilution method. Ethanolic extract inhibited the growth of the four bacteria with lower turbidity than the aqueous extract. For instance at 500 mg/ml, the turbidity readings were 1.50, 2.30, 1.20, 3.10 and 4.00 NTU for crude ethanolic extract, while the reading for crude aqueous extracts were 2.21, 2.90, 1.90, 3.85 and 4.12 NTU against *S. aureus, E. coli, S. typhi* and *K. pneumoniae,* respectively. The slight higher potency observed in ethanol (control) than water was expected due to antimicrobial activity of alcohol in general.

Like the agar diffusion set up, the trend of antimicrobial activity continues until the crude extract concentration of 62.5 mg/ml where both ethanolic and aqueous extracts had equal turbidity of 5.90 against *E. coli*. Meanwhile at this same concentration of 62.5 mg/ml, higher turbidity was observed in ethanolic extract tube i.e. 4.82 and 6.60 than in aqueous extract tube i.e. 4.76 and 6.20 against *S. typhi* and *K. pneumoniae* in that order.

DISCUSSION

The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants. These agents are alkaloids, saponins, flavonoids, tannins. Research work revealed that tannins from the barks, roots etc of many plants especially simarubaceae are used to treat cells that have gone neoplastic [14]. It is obviously interesting to observe the result of high antibacterial effects of both ethanolic and aqueous extracts the four potential

pathogens of public health importance. *S. aureus,* no doubt, is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis, furuncle, etc. It is also frequently involved in both nosocomial and community acquired infections. The successful inhibition of this bacteria and its contemporary aetiology of gastroenteritis (*E. coli)* is a good development, especially when we consider the records of resistance to various conventional antibiotics by them over the years. This extract could therefore be of use in management of opportunistic infection in HIV/AIDS involving these two isolates. Similarly, the extract showed appreciable level of potency against the commonest aetiology of enteric fever. Records have it that the enteric fever had mortality rate of 10 - 15% in developing countries [15]. Both the ethanolic and aqueous extracts could be put into fixed dosage combination therapy for treating the salmonella infection. This extracts is already in use by the traditional medicine practitioners. By virtue of high activity indices above unitary value even in crude forms, the extracts have more promising therapeutic advantages than the likes of gentamycin and its amino glycoside relations when refined to produce antibiotics. In conclusion, this finding justifies the traditional use of this plant, *Balanites roxburghii*, for prophylactic and therapeutic purposes. The findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

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