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Research Article

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Phytochemical analysis and Antibacterial activity of the *Balanites roxburghii* aerial parts

Rajananda Swamy T*, Ganga Rao B

Department of Pharmacognosy and Phytochemistry, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530003.

*****Corresponding Author: E-mail: rajapharma@gmail.com; Mobile: +91-94900 52637.

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Abstract

Balanites roxburghii have been using in cure of different infections in conventional medicine. The present work carried out on the basis of its ethno medical survey and folkloric information, on phytochemical analysis and evaluation antibacterial activity of aerial parts extracts *of B. roxburghii*. The different extracts (hexane, ethyl acetate and ethanol) were tested for the presence of different bioactive metabolites using standard analytical tests and further evaluated for their antibacterial activity. The qualitative analysis of extracts of *B. roxburhii* showed presence of bioaactive compounds like steroids, flavanoids, phenols, glycosides, tannins, carbohydrates etc., The extracts gave negative results for the amino acids, oils and saponins. Rifampcin used as a standard drug in antibacterial activities. The possibility of activity may be due to the natural compounds present in the extracts either as individually or mixtures. Further research is worth full on *B. roxburghii* in evaluation of biological activities and isolation and characterization of them.

Key Words: *B. roxburghii*, Aerial parts, Phytochemical analysis and Antibacterial activity.

1. Introduction

As old as humankind the plants has been using as a source for treating different infections, disease and health disorders around the world (Thomson, 1978). Thousands of plant species are exist, and only 1 to 10% are used as food by humans and other animals and very less species are using for treating the diseases around the world in different countries, South Asian countries like India, China, Sri Lanka, Nepal etc were in the first place in medicinal plants usage (Newman *et al.,* 2000; Srivastava *et al.,* 1996). Wide variety plants with medicinal value were available in India. For pharmaceutical preparation of modern medicine and indirectly as folk remedies, these plants were used. Phytochemicals with different biological activities have great utility as biomolecules (Powers, 2004; Iwu, 2002; Singh and Barrett, 2006). Phytochemicals are in a variety of forms and different concentrations to each other in plants. Some of the main phytochemicals include: isoflavonoids, carotenoids, lignans, steroids, anthraquinones, resins and gums (Barnes *et al.,* 2007; Bohlin L and Bruhn, 1999; Feher M and Schmidt, 2003). The present study was carried out for giving the scientific evidence in the presence of antibacterial activity and phytochemicals of the known medicinal plant *Balanites roxburghii.* The fruits of it have been using in treatment of cough, antifertility and skin illness. Leaves are using for treatment of jaundice. In case of pain and swelling, traditional healers used the plant bark. The paste of bark is used to treat snake-bite and dog bite (Padmashali *et al.,* 2006; Kirikar and Basu, 1935; Varshney IP and Shamsuddin, 1962) but there was very less scientific evidences regarding biological activities of *B. roxburghii*. So, the author selected the aerial parts of *B. roxburghii* for phytochemical analysis and to evaluate antibacterial activity.

2. Materials and methods

2.1Chemicals and Drugs

The chemical used in this research were analytical grade.

2.2 Plant material collection and extracts preparation

The plant material was collected at Andhra Pradesh, India, during the month July, 2012. The plant authentication was done by Rtd. Prof. M.

Venkaih, Department of Botany, Andhra University, Visakhapatnam. The dried material under shade were made into coarse powder. The powder material was used for extraction with different solvents (hexane, ethyl acetate and hydroalcoholic) using maceration and collected extractive solvent was concentrated using rota vapor.

2.3 Phytochemical analysis

Phytochemical studies were carried out for hexane, ethyl acetate and hydro alcoholic extracts of *B. roxburghii* aerial parts to detect the presence of different phytochemical constituents like steroids, terpenoides, tannins, flavanoids, saponins, glycosides, amino acids etc by using standard procedures (Ayoola *et al.,* 2008; Prashant Tiwari *et al.,* 2011; Ganga Rao *et al.,* 2013).

2.4 Quantification of Phenolic and Alkaloidal Content

2.4.1 Phenolic content estimation

Phenolic content was estimated using method Ayoola *et al.,* 2008 i.e. basec on FC-reagent. The products of the metal oxide reduction have a blue color and maximum absorption at 765nm. At that wavelength, the light intensity is proportional to the concentration of phenols. Concentration of phenolic content in Gallic acid total equivalents was measured by using standard Gallic acid calibration curve (Singleton *et al.,* 1999).

2.4.2 Alkaloid content estimation

The alkaloid content was estimated using Bromocresol green (BCG) reagent. The extracts were dissolved in 2N HCl and after filtration, 1mL was mixed with BCG reagent along with 5mL phosphate buffer. After vigorous shaking, the formed color complex was extracted with chloroform. The absorbance was measured at 470nm. The experiment was repeated for 3 times and results were given in mean±SEM. (Fazel Shamsa *et al.,* 2008; Rao Ganga *et al.,* 2011).

2.5 Evaluation of antibacterial activity by Cup-Plate Method

The cup-plate method was used to measure the antimicrobial activity of extracts at various dilutions. The Nutrient agar media was inoculated with the test organism at a temperature of 45ºC and transferred to sterile Petri-dishes. After solidification, prepare wells of 4mm diameter in the agar plates with metal borer. Then the test samples (100µl) and the standards (50µl) were added into the wells, plates were incubated at 28 ºC for 48-72hr. The existence of zone of inhibitions around the wells on Petri-dishes, indicates the presence of antibacterial activity. The diameters of the zones were measured and recorded (Indian Pharmacopoeia, 1996; Mallikarjuna Rao *et al.,* 2012).

2.5.1 Selected bacterial species for antibacterial activity

Gram positive organisms: *Staphylococcus epidermidis (S. e), Bacillus megaterium (B. m), Lactobacillus acedophillus (L. a).*

Gram negative organisms: *Escherichia coli (E. c), Slmonella typhi (S. t), Klebsiella pneumonia (K. p).*

3. Results and Discussion

The development of drug resistance in human pathogens and then they are causing potentially serious public health problems against commonly using antibiotics is resulting from the excessive and inappropriate usage of medicines (mainly antibiotics). The current environment requires a new active metabolites from different natural resources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens and therefore considered as a potential source for different classes of antimicrobial substances (Alamagboul, *et al.,* 1985; Alkofani *et al.,* 1990; Prescott *et al.,* 1996; Iwu *et al.,* 1999; Olukoya and Idugbmi, 1993; Grayer and Harborne 1994). The substances that can either inhibit the growth of micro-organisms or kill them are considered as candidates for developing new drugs for treatment of various infectious diseases (Haila *et al.,* 1999; Catalano *et al.,* 1998; Martinez *et al.,* 1996; Sunder, 1996; Taylor *et al.,* 1996). On availability of literature, selected the *B. roxuburhii* for the current study.

The phytochemical analysis gave the positive results for different chemical compounds like

alkaloids, steroids, flavanoids, terpenoids, glycosides, tannins, phenols and carbohydrates. and negative results for amino acids, oils and saponins. The hyd. alc extract showed more phenolic (31.22±2.40 mg/gm) and alkaloid (27.42±1.16 mg/gm) contents compared to other extracts. The results were given in Tables 1 and 2.

Table 1. Nature of phytoconstituents in different extracts of *B. roxburghii*.

Phytochemical constituents	Name of the *B. roxburghii* extract		
	Hexane	Ethyl acetate	Hydro alcoholic
Phytosterols	+	++	+
Terpenoids	+	+	++
Glycosides	+	+	++
Saponins	-	-	-
Flavonoids	+	++	+
Tannins	+	+	+
Carbohydrates	+	+	+
Alkaloids	+	++	++
Amino acids	-	-	-
Oils	-	-	-
Phenols	+	+	++

+, ++ = Present, – = Absent

The *B. roxburghii* extracts showed antibacterial activity on different pathogentc bacterial strains, but at lower concentrations they do not showed effective inhibition. The activity was more as concentration of extracts was increased (Table 3). Among three extracts hyd. alc extract showed better activity and more on Gram -ve strains campared to Gram +ve strains.

Table 2. Total phenolic and alkaloid contents of *B. roxburghii* extracts.

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	14.40±2.88	16.28±3.52
Ethyl acetate	22.52±1.44	24.62±0.48
Hydro alcoholic	31.22±2.40	27.42±1.16

The *B. roxburghii* extracts' antibacterial activity was comparable with standard drug rifampicin. The possibility of activity may be due to the natural compounds present in the extracts either as individually or mixtures of natural compounds. Therefore, isolation of active metabolites and evaluation of different biological activities on *B. roxburghii* was worth full study.

Table 3. Antibacterial activity of *B. roxburghii* extracts.

Name of the extract	Dose (µg/ cup)	Zone of inhibition# (Diameter in mm)					
		gram (+)ve			gram (–)ve		
		S.e	*B.m*	*L.a*	*E.c*	*S.t*	*K.p*
Hexane	100	7	8	-	7	8	7
	200	9	9	7	8	10	9
	400	12	10	8	9	12	11
	800	14	13	10	9	13	12
Ethyl acetate	100	8	-	7	7	8	7
	200	10	8	8	9	10	9
	400	12	11	9	12	12	11
	800	14	14	12	14	15	13
Hydro-alcoholic	100	7	-	7	7	7	-
	200	9	9	10	10	9	8
	400	11	12	12	13	11	12
	800	15	15	16	17	14	15
Rifampcin	50	24	26	22	20	22	24
DMSO		-	-	-	-	-	-

- = No activity; # Values Includes the cup diameter (4mm)

4. Conclusion

The inhibition of tested bacterial growth allows us to conclude that the hydroalcoholic extract of *B. roxburghii* have the more antibacterial potentiality.

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Conflicts of interest

Author has none to declare.

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