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Research Article

ANTIOXIDANT ACTIVITY AND HEPATOPROTECTIVE POTENTIAL OF *BALANITES ROXBURGHII* FRUITS

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ABSTRACT

Objectives: The objective of the present study was to evaluate antioxidant activity and hepatoprotective potential of a hydroalcoholic extract of *Balanites roxburghii* (BR) fruits.

Methods: Extraction was performed by triple maceration using ethanol:water as a solvent. The extracts were vaporated using rotavapor. Then antioxidant capacities were tested using superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Determination of hepatoprotective model was performed by thiocetamide induced liver models.

Results: The hydroalcoholic extract of BR fruits produced a dose-dependent percentage inhibition on tested free radicals, i.e., superoxide anion (50% inhibition concentrations [IC_{50}] value 190.50 µg/ml), hydroxyl radical (IC_{50} value 266.30 µg/ml), and DPPH radical (IC_{50} value 175 µg/ml). After treatment with silymarin and hydroalcoholic extract of BR in respective groups, they had showed good protection against thioacetamide induced liver toxicity.

Conclusion: The results of the present investigation clearly indicate the free radical scavenging activity and hepatoprotective potential of a hydroalcoholic extract of BR fruits and this activity is comparable with that of the standard drugs ascorbic acid and silymarin.

Keywords: *Balanites roxburghii*, Antioxidant activity, Hepatoprotective activity, Ascorbic acid.

INTRODUCTION

Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, and chlorophylls. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins, and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system.

Active oxygen and related species

Superoxide anion (O^{2-}.), hydroxyl radical (OH.), nitric oxide (NO.), hydrogen peroxide (H_2O_2), lipid radical (L.), lipid peroxyl radical (LO_2.), and lipid alkoxyl radical (LO.) play a vital role in biological processes of energy production, phagocytosis, and signal transduction. There is an increasing evidence to show that active oxygen species may also play a causative role in various diseases such as atherosclerosis, ischemia-reperfusion injury, inflammation carcinogenesis, cataracts, brain dysfunction, immune-system decline, cardiovascular disease, and rheumatoid arthritis, endogenous antioxidant enzymes, catalase, superoxide dismutase, and glutathione peroxidase defend against oxidative damage caused by active oxygen and related radicals. In addition to the enzymatic antioxidant defenses, nutritional antioxidants in the diets may have protective effects to prevent oxidative stress-related diseases [1].

Balanites roxburghii (BR) belongs to the family Zygophyllaceae, commonly known as Heeng, is a spiny, woody evergreen tree, and perennial species are mostly found in tropical and subtropical countries (China, India, Sri Lanka, and West Tibet). The BR has been used traditionally in the treatment of malaria, syphilis, jaundice, liver, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, spleen problems, epilepsy, and yellow fever [2-4]. However, literature survey indicated no published reports on the antioxidant and hepatoprotective activities of the BR fruits. In the view of the lack of study on selected plant, the author planned to study *in-vitro* antioxidant and hepatoprotective activities of the hydroalcoholic extract of BR fruits. An *in-vitro* antioxidant activity using different free radicals (superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl [DPPH]) and hepatoprotective activity using thioacetamide (TAA) induced liver toxicity model.

METHODS

Chemicals

All the chemicals and reagents used were of analytical Grade-1, DPPH was purchased from Sigma Chemical Company, St. Louis, USA, Riboflavin from Loba Chemicals Pvt. Ltd., Bombay), deoxyribose and nitrobluetetrozolium were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, silymarin, and sodium carboxymethyl cellulose (CMC) from Biochem Pharma Labs Ltd., serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total protein, total bilirubin, and alkaline phosphate (ALP) kits from Autospam Liquid Gold Pvt., Ltd.

Plant material

The plant material was collected in November 2010 at Kadapa district of Andhra Pradesh, India, and authenticated by Dr. P. Prayaga Murthy, the taxonomist. The specimens were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University.

Preparation of extract

The freshly collected fruits of the BR were shade dried and powdered. The powdered material was then subjected to triple maceration with ethanol:water (70:30). The extract thus obtained was concentrated under vacuum at a temperature of 45°C by using Rota vapor (Buchi), dried extract was weighed and stored in a desiccator.

Acute toxicity study
The acute toxicity study was conducted for hydroalcoholic extracts of the BR fruits as per Organization for Economic Co-operation and Development (OECD) guidelines 420 (OECD 2001).

***In-vitro* antioxidant activity**
The hydroalcoholic extract of BR fruits was screened for antioxidant activity against superoxide radical, hydroxyl, and DPPH radicals. The percentage inhibition and 50% inhibition concentrations (IC_{50}) were calculated.

Calculation of percentage inhibition
The percentage inhibition of superoxide production by the extract was calculated using the formula:

Formula 1: $$\text{Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of control; A_1 is the absorbance with the addition of plant extract/ascorbic acid.

Calculation of IC_{50}
The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the IC_{50} of extract/ascorbic acid.

Superoxide radical scavenging activity [5]
Superoxide radical scavenging activity of the BR extract was measured according to McCord and Fridovich method. It depends on light-induced superoxide generation by riboflavin and the corresponding reduction of nitro blue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560 nm. The percentage inhibition was calculated from the above formula.

Hydroxyl radical scavenging activity [6]
Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao 1990, by studying the competition between deoxyribose and test extract for hydroxyl radicals generated by Fenton's reaction. The damage imposed on deoxyribose due to the free radicals was determined calorimetrically by measuring the thiobarbituric acid reactive substances at 532 nm. Percentage of inhibition was calculated using formula.

DPPH radical scavenging activity [7]
DPPH radical scavenging activity was measured according to the method of Braca *et al*., 2003, an aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed and incubated at 37°C for 30 minutes and absorbance of the test mixture was read at 517 nm. The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using formula.

Hepatoprotective activity

Animals

Adult wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12 hrs light and dark cycle. Animals were fed pellet diet and water *ad-libitum*. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/ Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA]) following the guidelines of the CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

Grouping and treatment
Rats were divided into five groups, each group consisting of six animals.
Group I: Control received the vehicle *viz*. sodium CMC.
Group II: Negative control received the vehicle sodium CMC and administered TAA 200 mg/kg, s.c on 8th day.
Group III: Received silymarin 50 mg/kg p.o for 7 days and simultaneously administered TAA 200 mg/kg, s.c on 8th day.
Group IV: Received BR. 125 mg/kg p.o for 7 days and simultaneously administered TAA 200 mg/kg, s.c on 8th day.
Group V: Received BR. 250 mg/kg p.o for 7 days and simultaneously administered TAA 200 mg/kg, s.c. on 8th day.

At the end of experimental period, all the animals were sacrificed by using chloroform anesthesia. Blood samples were collected by retro-orbital puncture, allowed to clot for 45 minutes at room temperature. Serum was separated by centrifuging at 2500 rpm for 15 minutes and analyzed for various biochemical parameters.

Assessment of liver function [8-11]
Biochemical parameters, i.e., aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), ALP, total bilirubin, and total protein were analyzed according to the reported methods. The liver was removed, weighed, and morphological changes were observed.

Preliminary phytochemical screening [12-14]
The extract of BR was tested for preliminary phytochemical screening for the presence of different phytochemicals such as phytosterols, triterpenes, saponins, and flavonoids.

RESULTS AND DISCUSSIONS

Antioxidant activity

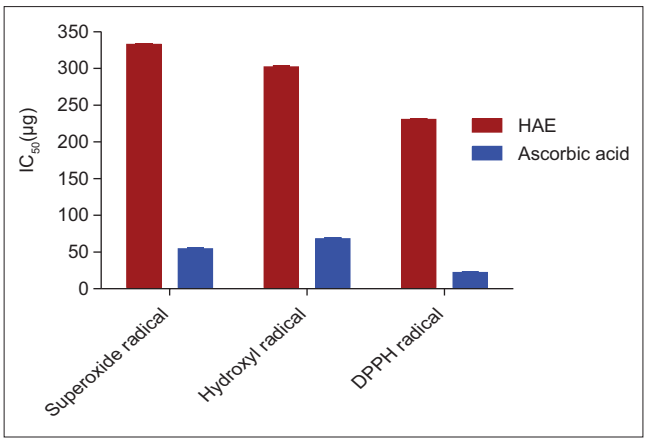
Superoxide radical

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. Therefore, studying the scavenging activity of plant extracts/ compounds on superoxide radical is most important.

The hydroalcoholic extract of BR produced dose-dependent inhibition of superoxide radicals ranging from 8.5±0.3 to 69.3±1.1. The concentration dependent percent inhibition of superoxide radical activity by a hydroalcoholic extract of BR was given in Table 1. The mean IC_{50} values for superoxide radical of BR and ascorbic acid were found to be 332.50 µg and 54.4 µg, respectively (Table 2 and Graph 1).

Hydroxyl radical

Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism. Due to the high reactivity, the radicals have a



Graph 1: *In-vitro* 50% inhibition concentration of hydroalcoholic extract of *Balanites roxburghii* and ascorbic acid on free radicals scavenging activity

Table 1: Concentration dependent percent inhibition of superoxide radical by hydroalcoholic extract of BR and ascorbic acid

Extracts/ compound	Percentage inhibition of superoxide radical					
	Quantity of extracts/ascorbic acid in µg					
	20	40	80	160	320	640
BR	8.5±0.3	18.5±1.0	30.4±1.1	42.51±1.2	56.15±1.2	69.3±0.5
Ascorbic acid	28.15±0.5	43.19±1.5	56.87±1.4	74.46±0.7	80.72±2.1	84.41±1.2

BR: *Balanites roxburghii*

Table 2: *In-vitro* IC_{50} of hydroalcoholic extract of BR on superoxide, hydroxyl, and DPPH free radical scavenging activity

Extract/ standard	Quantity of various extracts in µg		
	Superoxide radical	Hydroxyl radical	DPPH radical
BR	332.50±1.30	302.00±1.50	230.40±1.10
Ascorbic acid	54.4±1.1	68.00±1.3	22.0±0.5

BR: *Balanites roxburghii*, DPPH: 1,1-diphenyl-2-picrylhydrazyl, IC_{50}: 50% inhibition concentration

very short biological half-life. The generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain process via decomposition of lipid hydroperoxides. A single hydroxyl radical can result in the formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely, disrupts its function, and lead to cell death.

The hydroalcoholic extract of BR fruits produced dose-dependent inhibition of hydroxyl radicals ranging from 8.4±0.5 to 62.8±1.1.The standard drug Ascorbic acid showed the better percentage of inhibition of hydroperoxide radicals than the hydroalcoholic extract of BR. The results were given Table 3. The mean IC_{50} values for hydroxyl radical hydroalcoholic extract of BR and ascorbic acid were found to be 302 and 68, respectively (Table 2 and Graph 1).

DPPH radical

In the present study, hydroalcoholic extract of BR was found to possess concentration dependent scavenging activity on DPPH radicals and the standard drug ascorbic acid showed the better percentage of inhibition on DPPH radical. The hydroalcoholic extract of BR produced dose-dependent inhibition ranging from 9.7±0.4 to 78.25±1.8 (Table 4). The mean IC_{50} values for DPPH radical hydroalcoholic extract of BR and ascorbic acid were found to be 230.40 µg and 22 µg (Table 2 and Graph 1).

The hydroalcoholic extract of BR produced a dose-dependent inhibition of free radical generation of superoxide anion, hydroxyl radical, and DPPH radical in *in-vitro*. The results clearly indicate the free radical scavenging activity of a hydro alcoholic extract of BR and this activity comparable with that of the standard drug ascorbic acid.

Hepatoprotective activity

The liver is an important organ actively involved in metabolic functions and is a frequent target of a number of toxicants. One of the major functions of the liver is detoxification of xenobiotics and toxin. Because the liver performs many vital functions in the human body, damage of liver causes unbearable problems. SGPT and SGOT are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis [15]. Assay of serum ALP activity has been recognized as a suitable marker of skeletal and hepatobiliary disorder. Moreover, an elevated serum level of ALP activity is frequently associated with various pathological conditions. ALP is a non-specific tissue enzyme widely spread, mainly in the osteoblasts, live, and biliary canaliculi [16,17].

In the present study, rats treated with TAA developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes such as SGPT, SGOT, ALP, total protein, and total bilirubin when compare to normal control (Table 5). After treatment with silymarin and hydroalcoholic extract of BR in respective groups, had showed good protection against TAA induced toxicity to the liver. Decrease in levels of biomarker enzymes after treatment with the extract of BR indicated the effectiveness of the extract in normalizing the functional state of the liver. The total protein concentration of serum was lesser in Group-II animals, and it was significantly increased in groups treated with the extract of BR.

The % protection against a rise in SGOT levels by a hydroalcoholic extract of BR at doses of 125 mg/kg and 250 mg/kg was found to be 63.16% and 73.10%, respectively.

The % protection against a rise in SGPT levels by a hydroalcoholic extract of BR at doses of 125 mg/kg and 250 mg/kg was found to be 57.98% and 77.35%, respectively.

The % protection against a rise in ALP levels by a hydro alcoholic extract of BR at doses of 125 mg/kg and 250 mg/kg was found to be 74.12% and 81.82%, respectively.

The % protection against a rise in total bilirubin levels by a hydroalcoholic extract of BR at doses of 125 mg/kg and 250 mg/kg was found to be 30.26% and 48.21%, respectively.

The % protection against a decline in total protein levels by a hydroalcoholic extract of BR at doses of 125 mg/kg and 250 mg/kg was found to be 13.18% and 27.6%, respectively.

Preliminary phytochemical screening

Preliminary phytochemical screening of the hydroalcoholic extract of BR showed the presence of phytosterols, triterpenes, saponins, flavonoids, etc. (Table 6). Natural antioxidants such as plant phenols and flavonoids possess potent antioxidant activity [18]. Sterols like β-sitosterol have been reported for antioxidant activity. Terpenoids are also reported to possess antioxidant activity. These active constituents alone or in combination may be responsible for the observed antioxidant activity [19].

CONCLUSIONS

Findings of the present investigation adequately prove the hepatoprotective potential of BR. Decrease in levels of biomarker enzymes after treatment with the extract of BR fruits indicated the effectiveness of the extract in normalizing the functional state of the liver. The therapeutic potential shown by BR in the management of hepatic dysfunction may be due to its phytochemical constituents acting synergistically. Extraction, isolation, and characterization of the constituents responsible for the therapeutic efficacy of BR followed by evaluation of their pharmacological action against liver damage can be carried out to identify an even efficient hepatoprotective drug.

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Table 3: Concentration dependent percent inhibition of hydroxyl radical by hydroalcoholic extract of BR and ascorbic acid

Extracts/ compound	Percentage inhibition of hydroxyl radical					
	Quantity of extracts/ascorbic acid in µg					
	20	40	80	160	320	640
BR	8.4±0.5	16.5±1.1	25.4±0.5	36.1±1.2	51.3±1.0	62.8±1.1
Ascorbic acid	24.32±1.0	35.12±0.4	55.61±1.1	65.31±1.2	76.25±1.2	82.11±0.7

BR: *Balanites roxburghii*

Table 4: Concentration dependent percent inhibition of DPPH radical by hydroalcoholic extract of BR and ascorbic acid

Extracts/ compound	Percentage inhibition of DPPH radical					
	Quantity of extracts/ascorbic acid in µg					
	20	40	80	160	320	640
BR	9.7±0.4	15.8±1.1	20.6±0.5	32.7±1.1	56.8±1.2	78.25±1.8
Ascorbic acid	48±0.5	88.08±1.0	90.68±0.3	93.63±0.5	94.21±0.3	94.74±1.1

BR: *Balanites roxburghii*, DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 5: Percentage protection and effect of hydroalcoholic extract of BR on SGPT, SGOT, ALP, total protein, and total bilirubin levels in TAA induced hepatotoxic rats

Groups	Levels of various biochemical parameters (mean±SEM)				
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total protein (g/dl)
Group I vehicle control	96.17±2.85	56.00±1.46	217.50±1.06	0.17±0.01	7.40±0.19
Group II negative control (TAA)	330.50±2.67	173.00±2.13	715.33±17.45	2.12±0.11	4.87±0.32
Group III standard silymarin+TAA	124.00±1.59, (88.12%)	69.83±1.56, (88.18%)	279.33±3.94, (87.58%)	0.92±0.05, (61.71%)	6.88±0.10, (79.64%)
Group IV BR HAE 125 mg/kg+TAA	182.50±3.69, (63.16%)	105.17±1.14, (57.98%)	346.33±6.00, (74.12%)	1.53±0.06, (30.26%)	5.20±0.07, (13.18%)
Group V BR HAE 250 mg/kg+TAA	152.17±1.01, (76.10%)	82.50±0.76, (77.35%)	308.00±2.35, (81.82%)	1.18±0.03, (48.21%)	5.57±0.04, (27.60%)

TAA: Thioacetamide, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphate, BR: *Balanites roxburghii*, SEM: Standard error of mean

Table 6: Nature of phytochemical constituents present in BR hydroalcoholic extract

Name of the test	BR fruit extract
Phytosterols	+
Triterpenes	+
Glycosides	+
Saponins	+
Flavonoids	–
Tannins	–
Carbohydrates	–
Alkaloids	+

+: Present, –: Absent, BR: *Balanites roxburghii*

REFERENCES

1. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet 1994;344:721-4.
2. Nadkarni AK, Nadkarni KM. Indian Materia Medica. Bombay: Popular Prakashan; 1976. p. 1166.
3. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: CSIR; 1956. p. 92.
4. Kirtikar KR, Basu BD. Indian Medicinal Plants. Deheradun: International Book Distributors; 1933. p. 31823-4.
5. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.
6. Elizabeth K, Rao MN. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990;58:237-40.
7. Braca A, Fico G, Morelli I, De Simone F, Tomè F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. J Ethnopharmacol 2003;86(1):63-7.
8. Viswanath KM, Queseshi AA, Ramachandra Settee S. Hepatoprotective activity of flowers of *Calotropis aprocera* in paracetamol induced hepatic injury in rats. Scientific Abstracts 57 I.P.C. EP.44:261; 2005.
9. Reitman S, Frankel S. Determination of serum glutamate oxaloacetate and glutamate pyruvic acid transaminase. Am J Clin Pathol 1957;28:56-63.
10. King EJ, Armstrong AR. Determination of serum and bile phosphatase activity. Can Med Assoc J 1957;31:56-63.
11. Jendrassik L, Gróf P. Simplified photometric methods for the determination of Blutbilirubins . Biochem J 1938;297:81-9.
12. Rao BG, Rao YV, Rao TM. Hepatoprotective and antioxidant capacity of *Melochia corchorifolia* extracts. Asian Pac J Trop Med 2013;6(7):537-43.
13. Rao TM, Rao GB, Rao YV. Antioxidant activity of *Spilanthes acmella* extracts. Int J Phytopharmacol 2012;3(2):216-20.
14. Ethadi S, Pragada R, Battu G. Evaluation of anti-inflammatory and hepatoprotective activities of different extracts of *Cleome chelidonii* root in albino rats. Int J Pharm Bio Sci 2013;4(4):111-9.
15. El-Gazzar UB, El-Far AH, Abdel Maksoud HA. The ameliorative effect of *Phoenix dactylifera* extract on carbon tetrachloride hepatotoxicity in New Zealand rabbits. J Appl Sci Res 2009;5(9):1082-7.
16. Poole A, Leslie GB. A Practical Approach to Toxicological Investigation. Cambridge: Cambridge University Press; 1989. p. 44-86.
17. Ringler DH, Dabich L. Haematology and clinical biochemistry. In: Barker HJ, Lindsey JR, Weisbroth SH, editors. The Laboratory Rat. Vol. I. London: Academic Press; 1979. p. 105-18.
18. Khatua S, Roy T, Acharya K. Antioxidant and free radical scavenging capacity of phenolic extract from *Russula laurocerasi*. Asian J Pharm Clin Res 2013;6(4):156-60.
19. Paloi S, Acharya K. Evaluation of antioxidative activity and chemical composition of ethanolic extract from *Amanita vaginata* (Bull.) Lam.: An *in vitro* study. Asian J Pharm Clin Res 2014;7(2):88-92.