FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC ROOT EXTRACT OF THE PLANT *CROTOLARIA BURHIA*.LINN

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Summary

The present study was undertaken to evaluate the free radical scavenging potential of the roots of *Crotolaria burhia* by using different antioxidant models of screening. The methanolic extract at 1280µg/ml showed significant scavenging of the radical cation; DPPH (1, 1-diphenyl-2-picryl hydrazyl), superoxide anion ($O_2^{\cdot-}$) and lipid peroxidation potential. The percentage scavenging effect was maximum in case of superoxide anion followed by DPPH and lipid peroxidation. In conclusion, *C.burhia* posses antioxidant activity which was found to be concentration dependent (increases with increase in the concentration of the extract) and thereby justifies the therapeutic value of the plant in the present era.

Key Words: *Crotolaria burhia,* antioxidant activity, reactive oxygen species.

Introduction

Free radicals are involved in number of pathophysiological conditions as they have the ability to damage lipids, proteins and nucleic acid, the essential components of life. They are referred to as Reactive Oxygen Species (ROS). ROS such as superoxide anions ($O_2^{\cdot-}$), hydroxyl radical(OH^-) and nitric oxide (NO)inactivate essential enzymes and thereby causing tissue injury through covalent binding and lipid peroxidation [1].

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An imbalance between free radical generating and free radical scavenging systems results in oxidative stress, a condition that has been associated with the cell injury seen in many pathologic conditions [2,3]. Free radical mediated damage contributes to such varied processes as chemical and radiation injury, ischemia reperfusion injury, cellular ageing and microbial killing by phagocytes [4-10]. Natural products are becoming the cynosure to inhibit and scavenge these reactive oxygen species. The plant *Crotolaria burhia* (Fabaceae) commonly known as Dronnu and Chag, is undershrub, fibrous plant. It has been used by native tribes in Rajasthan for the treatment of gout, hydrophobia and inflammation. Selection of the plant was based on the fact that other species of the genus *Crotolaria juncea* showed potent antiovulatory[11], antispermogenetic[12], anti-inflammatory and antiulcerogenic activities[13]. The study of literature showed no report on antioxidant potential of the plant. Taking this into consideration, the present study has been undertaken on this plant for preliminary investigation of antioxidant potential.

Materials and Methods

Plant material and preparation of extract

The plant *Crotolaria burhia* was collected from the roadside near Sangali fields, Udaipur, Rajasthan and was authenticated by a botanist of our college. The roots were separated, dried under shade, powdered in a grinder and was extracted with methanol for 72 hours. The different concentrations of the extract (1-1280µg/ml) were prepared and were used throughout the experimental studies.

Chemical and solvents

All the chemicals employed for the experimental studies were of analytical grade and were purchased from CDH, New Delhi. DPPH was procured from Sigma chemicals, Mumbai. The chemicals used were Nitroblue tetrazolium dye, EDTA, Riboflavin, ascorbic acid, sodium dodecadecyl sulfate, thiobarbituric acid, acetic acid, ferric chloride, ferrous ammonium sulfate and phosphate buffer.

Experimental

DPPH radical scavenging activity:- DPPH scavenging activity was measured spectrophotometrically[14]. To 1 ml each of various concentrations (1-1280µg/ml) of methanolic extract in methanol 1 ml of DPPH solution (100mµ) was added and incubated for 30 minutes at 37°C. After 30 minutes, the reduction in absorbance was measured at 545 nm and the percentage inhibition was calculated and compared with the standard, Ascorbic acid. The experiment was performed in triplicate.

Inhibition of superoxide radical production: - Nitroblue tetrazolium reduction method [15] was employed to measure the effect on the superoxide radical production. The methanolic extract (1-1280µg/ml), EDTA (5µM), Riboflavin 45(µM) and phosphate buffer (75µM) was taken and the final volume was made upto 1ml. the tubes were illuminated for 20 minutes with the help of an incandescent lamp and then measured at 530 nm. The percentage inhibition in superoxide anion production was calculated by the following formula

% inhibition = $[(A_o-A_1)/ A_o]X100$

Where A_o was the absorbance of the control and A_1 was the absorbance of the extract and the standards [16].

Lipid peroxidation assay **[17]*:-***
Egg phosphotidylcholine (20mg) in chloroform (2ml) was dried under vacuum in a rotary evaporator to give a thin homogenous film and further dispersed in normal saline (5ml) with a vortex mixture. The mixture was sonicated to get a homogeneous suspension of liposome. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid to a mixture containing liposome (0.1ml). 150 mM potassium chloride, 0.2 mM ferric chloride, drug solution (1-1280 µg/ml) were added separately in a total volume of 1ml. The reaction mixture was incubated for 40 min at 37ºC. After incubation, the reaction was terminated by adding 1ml of ice cold 0.25M sodium hydroxide containing 20% w/v TCA, 0.4% w/v TBA and 0.05% w/v BHT. After keeping in boiling water bath for 20 min, the samples were cooled. The pink chromogen was extracted with constant volume of n-butanol and absorbance of the upper organic layer was measured at 532nm. The experiment was performed in triplicate.

Results and Discussions

Different concentrations (1-1280µg/ml) of the methanolic extract of the roots of *C .Burhia* were tested for their free radical scavenging activity *in vitro* models. It has been found that the test compounds scavenge free radicals in all the models (Table1, Table2 and Table3). The maximum percentage inhibition was observed with superoxide anion (96.66%) followed by DPPH (94.85%) and lipid peroxidation (89.68%) when compared to the standard, (Fig 1, Fig 2 and Fig 3)Ascorbic acid (99.75%). ROS has been found to be one of the prevalent causes in pathophysiological conditions including heart diseases, diabetes, cancer, inflammatory conditions and ageing (2). Free radical scavengers help to scavenge these species by inhibition of lipid peroxidation and by scavenging free radicals and thereby preventing several conditions [18]

Table 1: DPPH scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid

Sr. no.	Concentration (µg/ml)	% Scavenging	
		DPPH	Ascorbic acid (std)
1.	1	3.65	62.52
2.	10	7.48	68.39
3.	20	12.23	73.28
4.	40	26.53	79.63
5.	80	37.68	82.87
6.	160	49.93	88.53
7.	320	77.25	92.15
8.	640	94.57	98.32
9.	1280	94.85	99.75

Fig 1: DPPH radical scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid

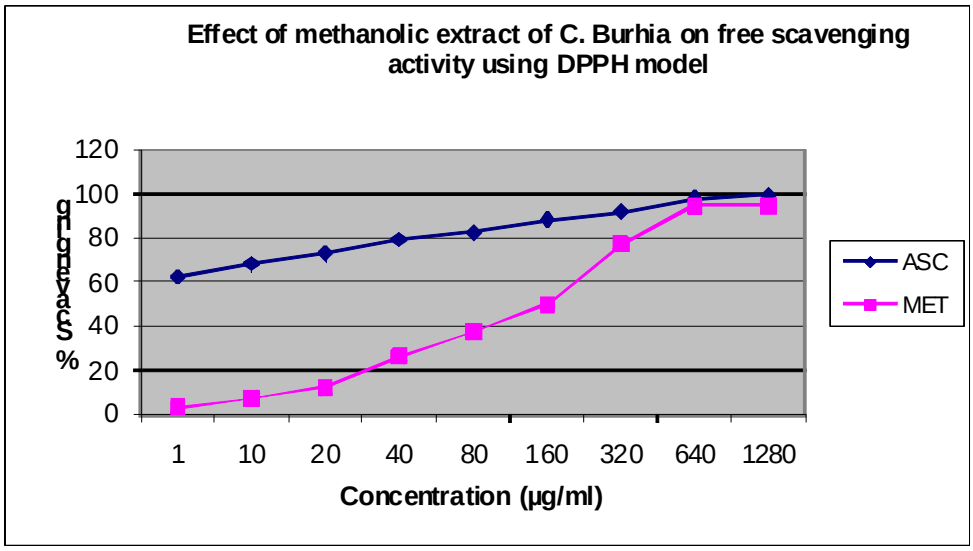


Table 2: Superoxide radical scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid

Sr. no.	Concentration (µg/ml)	% Scavenging	
		Superoxide radical	Ascorbic acid (std)
1.	1	2.45	62.52
2.	10	9.28	68.39
3.	20	14.73	73.28
4.	40	29.26	79.63
5.	80	45.73	82.87
6.	160	6725	88.53
7.	320	92.15	92.15
8.	640	96.32	98.32
9.	1280	96.66	99.75

Fig 2: Superoxide radical scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid

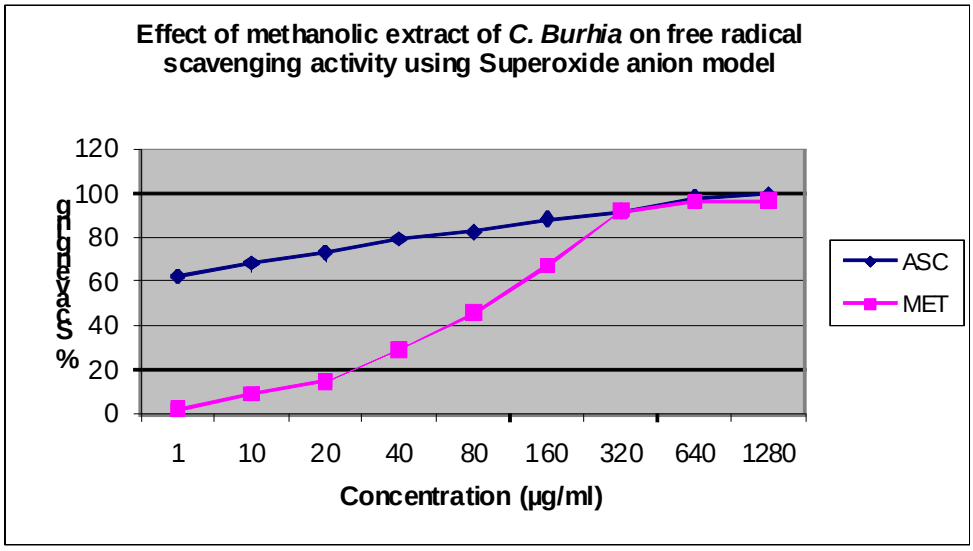
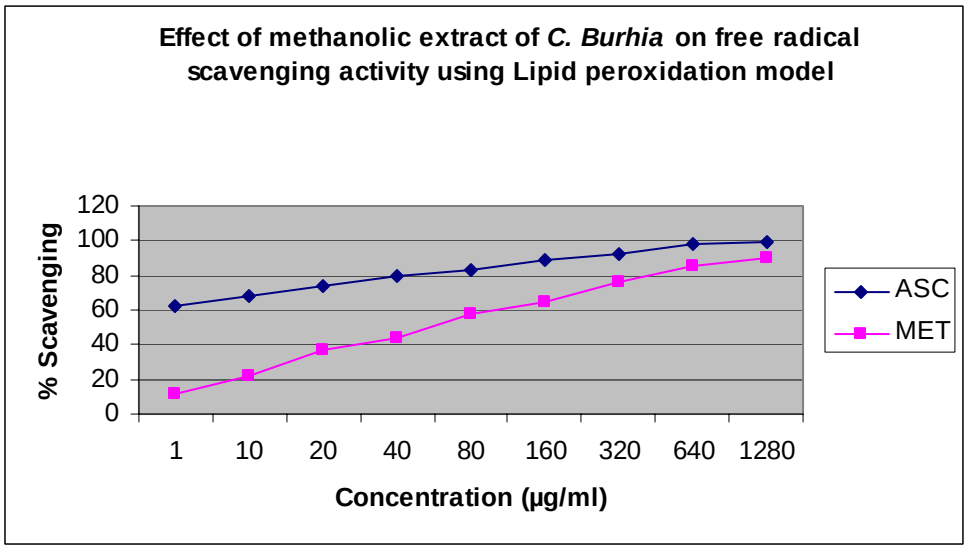


Table 3: Lipid peroxide scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid

Sr. no.	Concentration (µg/ml)	% Scavenging	
		Lipid peroxidation	Ascorbic acid (std)
1.	1	11.76	62.52
2.	10	22.29	68.39
3.	20	37.43	73.28
4.	40	43.28	79.63
5.	80	57.82	82.87
6.	160	64.28	88.53
7.	320	76.65	92.15
8.	640	85.35	98.32
9.	1280	89.68	99.75

Fig 3: Lipid peroxide scavenging activity of various concentrations of *C. Burhia* and Ascorbic acid



Conclusion

DPPH and superoxide radical assays were based on percentage scavenging activity of Free radical scavengers towards DPPH and superoxide radical respectively. From the present study, it may be concluded that *C .Burhia* converts DPPH to hydrazine in the presence of Free radical scavengers [19] and by inhibiting superoxide radical, respectively. While the inhibition of lipid peroxidation may be due to the inhibition of ferryl- perferryl[20] complex formatiom, scavenging of $^{\cdot}OH$, superoxide radical which was initiated by ferrous ammonium sulfate either through ferryl- perferryl complex or through $^{\cdot}OH$ by fenton reaction [21].

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