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Comparative anti-oxidant activity studies of *Buchanania lanzan* methanolic extract

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ABSTRACT

Traditionally used medicinal plants used for antioxidant activity has significance as it can target ROS (Reactive Oxygen Species) implicated in many disease conditions. Drugs of plant origin are gaining increasing popularity sense it is directly related to increasing resistance of an organism to non specific stress. Some plants like *Buchanania lanzan* are found to be potential herbs. *In vitro* screening of methanolic extracts of the leaf and bark of *Buchanania lanzan* was carried out using DPPH, ABTS and H_2O_2 radicals. The *in vitro* screening was carried out by using discoloration assay, which is evaluated by addition of anti oxidant to a solution of colored free radical. Antioxidant activity of both the extracts determined against DPPH, ABTS and H_2O_2 radicals. The bark and leaf extracts exhibited potent inhibition against ABTS radical generation. The extracts showed good activity against DPPH radical, but in H_2O_2 method leaf extract failed to show inhibition even at highest test concentration, whereas bark extract, inhibited H_2O_2 moderately.

Key words: *Buchanania lanzan,* DPPH, ABTS, ROS, free radical.

INTRODUCTION

Medicinal plants form an important part of Ayurveda practiced in India and other traditional systems of medicine used by two thirds of the world population. Realizing their importance, extracts of plant parts are extensively explored for different bioactivities including antioxidants[1]. In all forms of aerobic living systems, free radicals are produced endogenously during cellular metabolism, e.g. oxidative phoshorylation in mitochondria, liver mixed function oxidases, bacterial phagocytosis, xanthine oxidase activity, transition metal catalysis, drug and xenobiotics metabolism[2,3]. They are implicated to play a significant role in the pathogenesis of many disorders. Production of free radicals may be greatly induced by exogenous factors like environmental pollutants, drugs, radiation, and pathogens[4,5]. These reactive oxygen species (ROS) create homeostatic imbalance which generate oxidative stress and cause cell death and tissue injury. Involvement of ROS is implicated in neurodegenerative and other disorders, e.g. Alzheimer's disease, Parkinson's disease, multiple sclerosis, Down's syndrome, inflammation, viral infection, autoimmune pathology, and digestive ulcers. To circumvent the damage caused by ROS, multiple defence systems collectively called antioxidants are present, with protective efficiency depending on the balance between ROS and availability of antioxidants in the microenvironment of a cell. Traditionally, medicinal plants are used for more than one disease and they may possess very high bioactivity against common targets[6-8]. In this context, antioxidant property has significance as it can target ROS implicated in many disease conditions. Herbal health promoters and teas with defined bioactivity are very popular among the masses and their antioxidant potential has been

worked out in our laboratory. Bark from the tree is easily available throughout the year and does not destroy the parent plant unlike roots, *etc*. Therefore, the present study was to check the antioxidant activity of *Buchanania lanzan* herb by multiple *in vitro* assays and to compare the antioxidant activity of the leaves and the bark of *Buchanania lanzan.*

MATERIAL AND METHODS

Anti oxidant studies

DPPH Assay

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. The free radical DPPH is purple in color in methanol and is reduced to the corresponding hydrazine, which is yellow in color, when it reacts with hydrogen donor.

It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in the absorbance is measured at 490 nm.

Reagents

DPPH solution (100 µM): 22mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml with methanol to obtain 100 µM DPPH solution.

Preparation of Test and Standard solutions

The extracts and the standards, ascorbic acid and rutin (21 mg each) were separately dissolved in 5 ml of freshly distilled DMSO. These solutions were serially diluted with freshly distilled DMSO to obtain the lower dilutions.

Procedure

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of various concentrations of the extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ºC for 30 min. Absorbance was measured at 517nm using ELISA reader. Free radical scavenging activity of curcuminoids.

Scavenging of 2, 2'-azino-bis (3-ethylbezothiazoline-6-sulfonic acid) diammonium salt) (ABTS) radical cation Assay

ABTS assay is relatively recent one, which involves a more drastic radical, chemically produced and, is often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS radical for the estimation of the antioxidant activity.

Preparation of test and standard solutions

13.5 mg of each of the extracts and the standards, ascorbic acid and rutin were accurately weighed and separately dissolved in 2ml of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions.

Procedure

ABTS (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm.

Scavenging of hydroxyl radical by Deoxyribose method

The sugar deoxyribose (2-deoxy-D-ribose) is degraded on exposure to hydroxyl radical generated by irradiation or by Fenton systems. If the resulting complex mixture of products is heated under acid conditions, malondialdehyde (MDA) is formed and may be detected by its ability to react with thiobarbituric acid (TBA) to form a pink chromogen.

Preparation of test and standard solutions

16 mg of each of the extracts and BHA were accurately weighed and separately dissolved in 2 ml of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions.

Procedure

To the reaction mixture containing

Table 1: *In vitro* antioxidant activity of test extracts

Extracts	IC_{50} values ± SE mg/ml* by methods																							
/ Standards	Conc.				DPPH	ABTS H_2O_2											Conc.							
	in	% Inhibition			IC_{50} in ug/ml				Conc.In ug/ml	% Inhibition			IC_{50} in ug/ml				in ug	% Inhibition			IC_{50} in ug/ml			
		1	2	3	1	2	3	AVG		1	2	3	1	2	3	AVG		1	2	3	1	2	3	AVG
BARK	62.50	94.25	89.26	92.00	25	25	25	25	4.12	94	87	90	2.1	2.0	1.8	1.	500	51	55	52	482	465	471	47
extract	31.25	85.00	83.15	85.00					2.06	56	72	69				96	250	23	28	31				2.6
	15.62	13.05	12.50	11.85					1.03	48	44	45					125	6	9	8				
	7.80	6.25	6.00	9.86																				
LEAF extract	62.50	78.76	71.69	76.56	26	23	25.	24.	5.51	81	80	81	3.5	3.9	3.9	3.	1000	38	42	36	>10	>10	>10	>1
	31.25	66.05	65.50	71.25			5	8	4.12	49	44	45				76	500	21	19	15	00	00	00	00
	15.62	24.00	28.50	25.75					2.06	21	24	25					250	02	00	00				0
	7.80	12.56	16.25	11.50																				
Standards																								
Ascorbic acid		2.69 ± 0.05						11.25 ± 0.49								187.33 ± 1.93								
Rutin		-						0.51± 0.01								36.66 ± 0.22								

*Average of three determinations, values was mean ± S.E.M.

deoxyribose (3 mM, 0.2 ml), ferric chloride (0.1 mM, 0.2 ml), EDTA (0.1 mM, 0.2 ml), ascorbic acid (0.1 mM, 0.2 ml) and hydrogen peroxide (2 mM, 0.2 ml) in phosphate buffer (pH, 7.4, 20 mM), 0.2 ml of various concentrations of extracts or standards in DMSO were added to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37^{0}C. After incubation, ice-cold trichloro acetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v), in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm.

RESULTS AND DISCUSSION

The antioxidant capacity of methanolic extracts from *Buchanania lanzan* were studied by multiple *in vitro* assays. Superoxide anion is a free radical generated by one electron transfer and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems. ABTS radical cation decolorisation assay could measure the relative antioxidant ability to scavenge the radical ABTS+ as compared with Trolox, and is an excellent tool for determining the antioxidant capacity of hydrogen donating antioxidants. The results obtained from this study show that multiple *in vitro* methods targeting different radical species are important for testing antioxidant potential of a standardized herbal extract. Employment of more than one test method specific to a radical species gives a better estimate of comparative antioxidant potential of a test compound. In our study, The extracts showed (Table. 1) good activity against DPPH radical with almost similar IC50 values, i.e IC50 values 25 and 24.8 µg/ml by bark and leaf extracts, respectively. The bark and leaf extracts exhibited potent inhibition against ABTS radical generation with IC50 values 1.96 and 3.76 µg/ml, respectively. But, in H_2O_2 method leaf extract failed to show inhibition even at highest test concentration i.e 1000 ug/ml. whereas bark extract, inhibited H_2O_2 moderately with IC50 value 472.6 µg/ml.

CONCLUSIONS

From these studies, bark extract proved to have better antioxidant properties than leaf

extract. The findings of this study support this view that *Buchanania lanzan* bark was promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. The presence and absence of correlation between chemical constituents and antioxidant potential give new insight into defences against free radical mechanisms in plants.

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