ANTI-OXIDANT ACTIVITY OF *Buchanania lanzan* Spreg. F: ANACARDIACEAE

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Summary

This study is designed to examine the in vitro antioxidant activity of phenolic compounds in the methanol and acetone extract of *Buchanania lanzan* Spreng. (Root) by three methods, based on a kinetic and thermodynamic approach: namely Iron (III) to Iron (II) reduction assay, cyclic voltametry and DPPH. In the ferric to ferrous reduction assay the electron donation capacity (reflecting the reductive power) of the sample were assessed and compared to that of BHT. Both extracts shows good degree of electron donation capacity in terms of relative reductive efficiency (RRE) but methanolic extract shows more RRE (0.79) value as compared to acetone extract (0.60) due to more content of pheolics. In cyclic voltametry measurement lower oxidation potential of methanol extract shows higher antioxidant efficacy. In DPPH system, the strongest radical scavenging activity was exhibited by the methanolic extract (EC_{50}=0.24±0.02). These results reveal that *Buchanania lanzan* Spreng. (Root) exerts a promising antioxidant potential against free radical induced oxidative damage.

Key words: *Buchanania lanzan* Spreng., DPPH, reduction assay, Cyclic voltametry

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Introduction

The traditional medicine all over the world is now a days revalued by an extensive activity of research on different plant species and their therapeutic principles [1]. Experimental evidence shows that reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen have been implicated in degenerative disease such as cancer, inflammation, atherosclerosis and ageing [2, 3]. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease [4]. Many species of fruits, vegetables, herbs, cereals, sprouts and seeds have been investigated for antioxidant activity during the past decade [5, 6,7]. Herbs and spices are, in general, harmless sources for obtaining natural antioxidants. Plant tissue antioxidant capacity is clearly associated with the activity of ‘‘free radical scavenging enzymes’’ (superoxide dismutase, catalase, peroxidase, etc.) and with the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid [8]. It is evident that there is an increasing demand to evaluate the antioxidant properties of direct plant extract [9].

Buchanania lanzan Spreng., family Anacardiaceae, commonly known as Char, Chirauli. It is widely distributed in hot and dry parts of India. It is a drug of the ayurveda and the Unani system of medicine [10]. It is known to have tonic, cardiotonic and astringent properties. Literature survey reveals that there is presence of glycosides, carbohydrates, sterols and flavonoids present in *Buchanania lanzan* Spreg (Root). On the other hand, there is no information on the antioxidant properties of *Buchanania lanzan* Spreng. root,in the literature, except its phytochemical constituents.

The aim of this work is to evaluate the antioxidant properties of the methanol and acetone extracts of *Buchanania lanzan* Spreng. (Root) by DPPH, Iron (III) to Iron (II) reduction assay and Cyclic voltametry. Additionally total phenolic contents of the methanol and acetone extract have been identified.

Materials and Methods

Chemicals and reagents

Potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), methanol, sulphuric acid, Potassium chloride, Sodiumphosphate, ammonium molybdate and trichloroacetic acid (TCA) were purchased from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated Hydroxyl Toluene (BHT), Ascorbic acid (AA) and gallic acid were purchasedfrom Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents are of analytical grade.

Plant Materials and Preparation of extract

Buchanania lanzan Spreg. plant was collected from the campus of BIT Mesra, Ranchi, Jharkhand, India, during september and identified by Dr S. Jha (Department of Pharmacognosy, Birla institute of Technology, Mesra, Ranchi, India), according to morphological characteristics. A voucher specimen was kept at the Herbarium of the Department of Pharmacognosy BIT Mesra, Ranchi. The dried and powdered (root) materials (20 g) each were extracted with 100 ml of methanol and 100ml of toluene for 30 minutes with the help of a sonicator at room temperature and concentrated under reduced pressure to yield 7.25% w/w of methanolic and 6.76% w/w acetone extract.

Total Polyphenol estimation

Total phenolic compound amount in extracts were determined by Folin-Ciocalteu method [11]. Different concentrations of Gallic acid (50µg/ml-250 µg/ml) were prepared for standard curve. 0.1 ml of these different concentrations of Gallic acid was taken in different test-tubes and to it 0.5 ml Folin-Ciocalteu reagent was added. After 1 min, 1.5 ml 20 % (w/v) anhydrous sodium carbonate (Na_2Co_3) was added and volume was made up to 10 ml with water. After 1 hour incubation at 25^0C, the absorbance was measured at 760 nm. Plot of absorbance versus concentration was plotted to prepare the standard curve. For total polyphenol estimation in the extract, 0.1 ml (Conc.1mg/ml) of extract was taken and followed as above. The results are means of three repetitions expressed in the form of gallic acid equivalents/gm of extract.

Iron (III) to Iron (II) reduction assay:

The reductive capacities of the methanolic and acetone extracts were assessed using ferric to ferrous reductive activity as determined spectrophotometrically from the formation of perl,s Prussian blue colored complex [12]. Different concentrations (0.0-1mg/ml) of extract (1 ml) extract were mixed with 2.5ml phosphate buffer (0.2M, PH 7.0) and 2.5ml of a 1% (w/v) potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. After 30 min incubation at 50^0C, 2.5ml (10%, w/v) tri-chloroacetic acid was added and the mixture was centrifuged for 10 min (1800 rpm). Finally, 2.5ml of the upper layer were mixed with 2.5ml water and 0.5ml (0.1%, w/v) ferric chloride and the absorbance was recorded at 700nm. BHT was taken as standard. Finally relative reductive efficiency (RRE) was calculated by taking the ratio of the slope of sample to that of BHT.

Cyclic voltametry:

A Cyclic voltammeter (Ecochemie, Twente, model PGSTAT 30, Holland) with three electrode system viz. Calomel as reference electrode, a Platinum electrode as working electrode and a platinum wire as a counter electrode was used for measurement of oxidation potential. Cyclic voltametry tracing were recorded from 0.2 to 1 V at a scan rate of 100 mV/s [13]. Data were analyzed using GPES 3.2 software from Ecochemie, running on a P-III personal computer.

DPPH radical scavenging effect

The DPPH assay was carried out by Brand-Williams and his co-workers (1995) [14]. 25, 50, 75, 100, 250, 500 µg of the sample and standard (Ascorbic acid) solution was prepared in methanol. 4 ml of various concentrations of the extracts in methanol were added to a 1 ml solution of DPPH radical in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min in room temperature the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated in following way $I(\%) = 100\times (A_0-A_1) / A_0$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test compound), and A_1 is the absorbance of the test compound.

Result and Discussion

Total phenolic content in methanolic and acetone extract of *Buchanania lanzan* Spreg. (root) as estimated by Folin-ciocalteu Reagent method shows 510.79±8.308 and 475.03±7.290 mg gallic acid equivalent per 100 gm of root powder respectively. The result indicates that both methanolic and acetone extract of *Buchanania lanzan* Spreg. (Root) contain satisfactory amount of phenolic compounds but phenolic compounds present in methanolic extract is more.

In the ferric to ferrous reduction assay the electron donation capacity (reflecting the reductive power) of the sample were assessed and compared to that of BHT, which is known to be a strong reducing agent. Both extracts shows good degree of electron donation capacity in terms of RRE shown in figure-1 but methanolic extract shows more RRE value as compared to acetone extract due to more content of pheolics.

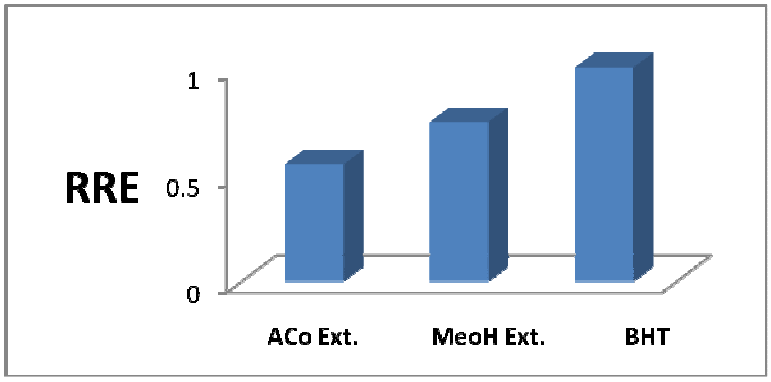

Fig. 1: RRE of various extracts with respect to BHT

The redox properties are crucial for better understanding of the electron transfer process. Cyclic voltammetry is an established instrumental tool for the measurement of electron transfer efficiency and in turn antioxidant efficacy of a test compound. Thus, we have measured the oxidation potential of the different extract. By and large lower the oxidation potential of a test compound higher the antioxidant efficacy. The low oxidation potential value (Figure 2-A, B, C) of the methanolic extract confirmed have efficient oxidative radical scavenger.

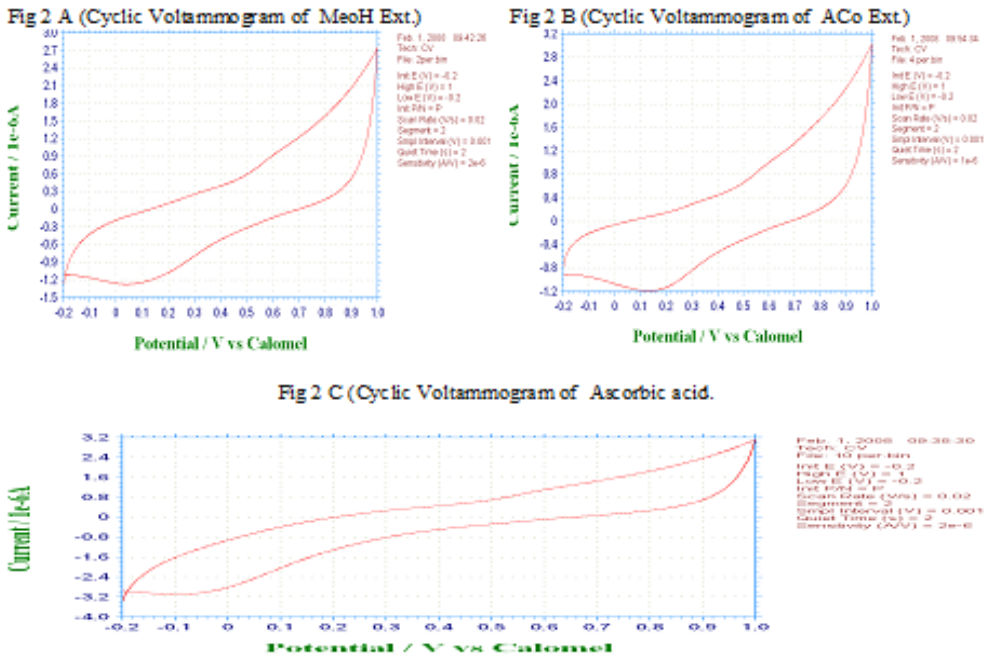

Figure 3 shows the DPPH radical scavenging activity of different extracts of *Buchanania lanzan* Spreg. (Root). This method evaluates the radical scavenging activity by its reaction with the stable radical DPPH. The assay was carried out in methanol and the results expressed as EC_{50}, which represents the antioxidant concentration necessary to decrease the initial DPPH concentration by 50%. The EC_{50} values were found to be 0.24±0.02 and 0.29±0.01 for methanol and toluene extracts respectively.

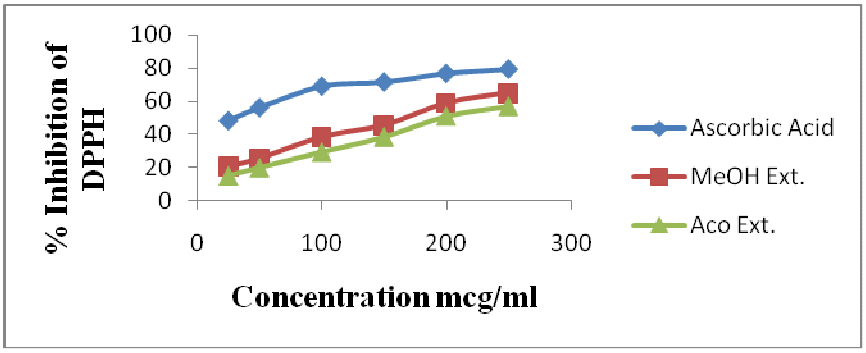

Figure 3: Free radical scavenging activity of methanol and acetone extract of *Buchanania lanzan* Spreg. (root) with respect to Ascorbic acid.

Conclusion

All the three methods used, although base on different approaches, shows the antioxidant potentioal of *Buchanania lanzan* Spreg. (root). Literature report suggests that there was no pharmacological work on the root of *Buchanania lanzan* Spreg. Both methanol

and acetone extract shows very good antioxidant activity so this plant may be helpful for various degenerative diseases.

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