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ANTIOXIDANT ACTIVITY OF *BUTEA MONOSPERMA* LEAF EXTRACTS

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ABSTRACT
Antioxidants are substances which help to defend the body against cell damage caused by various free radicals. Free radicals are unstable oxygen molecules containing unpaired electrons. Reactive oxygen species, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, have a causal relationship with oxidative stress.
Role of free radicals has been implicated in several diseases such as liver cirrhosis, atherosclerosis, Cancer, aging, arthritis, diabetes etc. the aim of the study was to investigate the antioxidant activity of *Butea monosperma*. The quantities of the *Butea monosperma* aqueous and benzene extracts needed for in vitro inhibition of hydroxyl radicals and lipid peroxidation were relatively similar to the known antioxidant ascorbic acid.
Key words: Reactive oxygen species, *Butea monosperma,* hydroxyl radicals, lipid peroxidation.

INTRODUCTION

Antioxidants are substances which help to defend the body against cell damage caused by various free radicals. Free radicals are unstable oxygen molecules containing unpaired electrons. Broadly possible mechanisms by which antioxidants may protect against reactive oxygen species toxicity are (i) prevention of reactive oxygen species formation (ii) interception of reactive oxygen species attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistivity of sensitive biological targets to reactive oxygen species attack (iii) facilitating the repair of damage caused by reactive oxygen species (iv) providing a favorable environment for effective functioning of other antioxidants. The capacity to detoxify reactive oxygen species is of critical importance in all aerobes. In the human body a complex combination of enzymatic and nonenzymatic systems function to minimize the stress induced by reactive oxygen species. These antioxidants may be classified as endogenous antioxidants, those which are physiological origin and exogenous antioxidants are those which cannot be produced by the human body but may protect against prooxidant forces when administered as supplements[1].
Reported pharmacological activities of *Butea monosperma* plant are antifungal[2] anti-inflammatory[3], antimicrobial[4], anticonvulsive[5], antifertility[6] and anti-diarrheal activity[7]. The present study reports the antioxidant activity of *Butea monosperma* leaf extracts.

MATERIALS AND METHODS

Plant Material

The dried leaves of *Butea monosperma* plant were collected from local market of Gulbarga. The plant leaves were authenticated at Pharmacognosy department of HKES's College of Pharmacy, Gulbarga. The leaves were dried in shade, powdered and stored in air tight containers for the studies.

Preparation of Plant Extract

The powdered material of *Butea monosperma* leaves were evenly packed in a Soxhlet extractor for extraction for about 36 hours with water and benzene. The extracts were then concentrated by distilling the solvent. The concentrated extracts were air dried at room temperature, weighed and percentage yield was calculated.

Experimental Animals

Albino rats (Wistar strain) of either sex weighing between 160-220g were procured from central animal house M. R. Medical College, Gulbarga for experimental purpose. After procuring, the animals were acclimatized for seven days under standard husbandry condition with 12 hours light/dark cycle[8, 9].
The animals were fed with standard diet manufactured by Amrut laboratories Pranava Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. After obtaining prior permission from Institutional Animal Ethical Committee (IAEC) registration number HKECOP/IAEC/04/2008-09, all animals' studies were performed in accordance to guidelines of CPCSEA.

Chemicals

2-Deoxy-D-Ribose, EDTA, Ferric chloride, sodium dodecyl sulphate, thiobarbituric acid, Ascorbic acid, NaH_2PO_4 and Na_2HPO_4 were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. Hydrogen peroxide, pyridine, Tris-HCl and ammonium ferrous sulphate were purchased from Qualigen Fine Chemicals, Mumbai. Acetic acid and n-butanol were purchased from Nice chemicals Pvt. Ltd., Kochin.

Methods

Determination of hydroxyl radical scavenging activity by deoxyribose degradation method

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances

(TBARS)[10]. Fenton reaction mixture consisting of 1 ml of ferrous sulphate ($FeSO_4.7H_2O$) (10 mM), 1 ml of EDTA (10 mM) and 1 ml of 2-Deoxy-D-Ribose (10 mM) and was mixed with 6 µl of phosphate buffer (pH 7.4) and 1 ml of various dilutions of extract. Thereafter, 1 ml of H_2O_2 (10 mM) was added before the incubation at 37 °C for 1 hour. Then 1 ml of this Fenton reaction mixture was treated with 0.2 ml of Sodium dodecyle sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of acetic acid (20%).

The total volume was then made to 5 ml by adding distilled water and kept in oil bath at 100 °C for 1 hour.

Calculation of percentage inhibition

$$\text{Percentage Inhibition} = \frac{\text{Average of the control O.D} - \text{Test sample O.D}}{\text{Average of the control O.D}} \times 100$$

Calculation of 50% inhibition concentration

The optical density obtained with each concentration of the extracts and ascorbic acid was plotted on a graph taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the concentration needed for 50% inhibition.

Determination of lipid peroxidation inhibiting activity by Fe^{2+}/ascorbate system

Inhibition of lipid peroxidation was determined by the method developed by Ohkawa H *et al*., 1979. Rat liver tissue weighing 10 g was homogenized with a poly homogenate and centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of plant extract of different concentrations, followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06mM) and 0.1 ml ammonium ferrous sulphate (0.16 mM) and incubated for one hour at 37 °C. The reaction mixture was treated with 0.2 ml of Sodium dodecyle sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of acetic acid (20%). The total volume was then made to 4 ml by adding distilled water and kept in oil bath at 100 °C for 1 hour.

After cooling, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured a 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control and sample and calculated for Hydroxyl radical scavenging activity.

After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured a 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extract was determined by comparing the absorbance values of the control and experimental tubes.

RESULTS

Inhibition of Hydroxyl radicals

The herbal aqueous and benzene extracts of *Butea monosperma* leaves and ascorbic acid at different concentrations (25 - 400µg) scavenged the hydroxyl radicals in a dose dependent manner (Table 1). The quantity of herbal aqueous and benzene extracts of *Butea monosperma* leaves and ascorbic acid needed for 50% inhibition of hydroxyl radicals was found to be 235µg, 310µg and 270µg respectively (Table 3).

Inhibition of lipid peroxidation

The herbal aqueous and benzene extracts of *Butea monosperma* leaves and ascorbic acid at different concentrations (25 - 500µg) scavenged the lipid peroxidation in a dose dependent manner (Table 2). The quantity of herbal aqueous and benzene extracts of *Butea monosperma* leaves and ascorbic acid needed for 50% inhibition of hydroxyl radicals was found to be 320µg, 375µg and 342.5µg respectively (Table 3).

Table 1: Percentage inhibition of hydroxyl radical by extracts / ascorbic acid *in vitro* studies

Extracts/Ascorbic Acid	Quantity (µg)					
	25	50	100	200	300	400
Butea monosperma aqueous extract	7.09±1.86	14.39±1.88	23.88±1.46	43.48±1.33	61.21±2.98	77.79±1.96
Butea monosperma benzene extract	4.38±1.59	9.07±2.53	15.54±2.90	33.06±1.93	48.8±2.39	62.78±2.41
Ascorbic acid	6.05±1.34	12.09±2.07	21.27±2.66	39.11±1.37	56.10±2.71	72.26±2.08

Table 2: Percentage inhibition of lipid peroxidation by extracts / ascorbic acid *in vitro* studies

Extracts/ Ascorbic Acid	Quantity (µg)					
	25	50	100	200	300	400
Butea monosperma aqueous extract	5.71±0.52	9.41±1.46	16.79±1.24	32.82±1.20	48.26±2.82	62.15±2.84
Butea monosperma benzene extract	2.91±0.17	5.82±1.48	8.06±1.62	24.75±1.78	41.20±1.80	55.32±1.29
Ascorbic acid	6.38±0.28	10.30±1.90	16.91±2.97	31.02±1.69	46.25±2.06	58.79±2.94

Table 2: IC_{50} Values of extracts / ascorbic acid

Extracts/ Ascorbic Acid	Hydroxyl radical scavenging activity	Lipid peroxidation inhibition activity
Butea monosperma aqueous extract	235 µg	320 µg
Butea monosperma benzene extract	310 µg	375 µg
Ascorbic acid	270 µg	342.5 µg

DISCUSSION

Role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc[11,12] and the compounds that can scavenge free radicals have great potential in ameliorating these diseases processes[13].

The antioxidant activity of ascorbic acid was well established[14]. The quantities of the *Butea monosperma* aqueous and benzene extracts needed for in vitro inhibition of hydroxyl radicals and lipid peroxidation were relatively similar to the known antioxidant ascorbic acid.

CONCLUSION

The *Butea monosperma* extracts has antioxidant activity comparable to standard ascorbic acid.

The quantities of the *Butea monosperma* extracts needed for the in vitro inhibition (IC50) of hydroxyl radicals and lipid peroxidation were closer to the known antioxidant ascorbic acid which evidenced that *Butea monosperma* extracts possesses significant antioxidant activity.

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