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Antioxidant activity of *Balsamodendron Mukul* hook extract

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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 (ROS), 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 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, *Balsamodendron mukul* hook, 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 defined as chemical species possessing unpaired electrons in their outer orbital which are generally reactive [1,2]. If a radical reacts with a non radical, another free radical must be produced. This implication is reflected continuously in cells either during phagocytosis or pathological condition. The most important free radicals include: superoxide anion (O_2^-), alkoxyl, hydroxyl radical (HO), and alkoxyl singlet oxygen ($^1O_2$) [3, 4] These oxygen derived free radicals are capable of damaging reversibly or irreversibly the compounds of all biochemical classes, including nucleic acids, proteins, free amino acids, lipids, lipoproteins, carbohydrates and connective tissue macromolecules[5].

In the present era natural products have a crucial role in the therapeutics and human clinical trials. A number of plant constituents possessing antioxidant potential have been reported.

Reported pharmacological activities of *Balsamodendron mukul* hook are Antihypercholesterolaemic activity, Antiamoebic activity, Antiinflammatory activity, Antibacterial and Antiallergic activity, Antidiabetics activity, Anticoagulant activity, Antiobesity activity etc.

MATRIALS AND METHODS

Collection of Plant Material

The plant extract of *Balsamodendron mukul* was procured from standard ayurvedic drug supplier from local market as this extract is used in its natural form for treatment by ayurvedic practitioners.

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.

PROCEURE

1. Determination of hydroxyl radical scavenging activity by deoxyribose degradation method[6]

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).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. 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.

Calculation of percentage inhibition:

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

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.

2. Determination of lipid peroxidation inhibiting activity by Fe^{2+}/ascorbate system[7]

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.

RESULTS

Hydroxyl radical scavenging activity

The extract of *Balsamodendron mukul* and ascorbic acid at different concentrations (25 - 500µg) scavenged the hydroxyl radicals in a dose dependent manner **(Table 1).** The quantity of *Balsamodendron mukul* extract and ascorbic acid needed for 50% inhibition of hydroxyl radicals was found to be (µg**)** 245 and 270 respectively.

Inhibition of lipid peroxidation

The extract of *Balsamodendron mukul* and ascorbic acid at different concentrations (25 - 500µg) scavenged the lipid peroxidation in a dose dependent manner **(Table 2).** The quantity of *Balsamodendron mukul* extract and ascorbic acid needed for 50% inhibition of hydroxyl radicals was found to be (µg**)** 332 and 342.5 respectively.

DISCUSSION

Role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc [8] and the compounds that can scavenge free radicals have great potential in ameliorating these disease processes [9].

The antioxidant activity of ascorbic acid is well established [10]. The quantities of the *Balsamodendron mukul* extract needed for *in vitro* inhibition of hydroxyl radicals and lipid peroxidation were relatively significant to the known antioxidant ascorbic acid.

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

Extract/AA	Quantity (µg)					
	25	50	100	200	300	400
***Balsamodendron mukul* extract**	6.88±0.36	12.83±1.49	23.78±2.44	41.61±2.56	59.23±2.12	75.29±2.86
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 extract / ascorbic acid *in vitro* studies

Extract/AA	Quantity (µg)					
	25	50	100	200	300	400
***Balsamodendron mukul* extract**	3.14±0.56	7.39±1.62	17.40±2.90	30.46±3.32	47.14±2.48	60.81±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 3: IC_{50} Values of extract / ascorbic acid

Extract/AA	Hydroxyl radical scavenging activity	Lipid peroxidation inhibition activity
***Balsamodendron mukul* extract**	245 µg	332 µg
Ascorbic acid	270 µg	342.5 µg

CONCLUSION

The extract of *Balsamodendron mukul* has antioxidant activity comparable to standard ascorbic acid. The extract of *Balsamodendron mukul has* shown antioxidant activity in both the models of studies i.e. Hydroxyl radical scavenging activity and inhibition of lipid peroxidation. The results of our study indicate that further extension of studies with this extract in other species and by other models would throw more light on this extract for explaining its potential use in human beings.

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