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Research Article

IN VITRO RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF BUTEA *MONOSPERMA* BARK

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ABSTRACT

Free radical scavenging activity of bark of *Butea monosperma* was evaluated by *in vitro* methods like radical scavenging activity by DPPH reduction, superoxide radical scavenging activity and nitric oxide radical scavenging activity. In the present study, different extracts of bark of *Butea monosperma*, viz, methanol, ethanol and aqueous extracts were analyzed for antioxidant efficiency. It was observed that ethanolic extract has the potent antiradical activity. The results were significant when compared with that of standards. These findings suggest that the ethanolic extract of *Butea monosperma* possess *in vitro* antioxidant activity as an effective free radical scavenger and might be a valuable source of antioxidant in medicine.

Keywords: *Butea monosperma*, Antioxidants, Free radicals, Phenolic content

INTRODUCTION

Reactive oxygen species are highly reactive compounds with a short half-life. ROS are generated continuously in the body by both endogenous and exogenous factors. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates[1,2,3] and this lead to a number of physiological disorders. Oxidative stress has been linked to various disorders such as cancer, cataracts, diabetes mellitus, inflammation, renal failure, cardiovascular diseases, hepatitis, inflammation, neurodegenerative diseases and aging[4]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation. Recent studies showed that a number of plant products including polyphenolic substances and various plant extracts exert potent antioxidant actions[5,6].

Butea monosperma (Lam.) Taub., commonly called the flame of the forest, is considered as one of the most beautiful trees of India due to its gorgeous canopy of scarlet flowers which looks like a flame[7]. *Butea monosperma* is also known as Palash. It belongs to the family Fabaceae[8]. It is a well known traditionally used medicinal plant reported to possess antidiabetic, anti-inflammatory, antihelmintic, antimicrobial and antidiarrhoeal properties[9,10,11,12].

MATERIALS AND METHODS

Plant material

Bark of *Butea monosperma* Lam. were collected from Kappukadu (Kottoor), Trivandrum, India. The plant material was identified and authenticated by Dr.Valsala Devi, Department of Botany, University of Kerala, India. A voucher specimen (Voucher No. KUBH 5803) has been deposited at the herbarium of Department of Botany, University of Kerala.

Preparation of plant extract

The bark of *Butea monosperma* was shade dried and then powdered in a mechanical grinder. The powdered material was extracted with different solvents such as methanol, ethanol and H_2O. The extracts were filtered and concentrated under reduced pressure. Yield of methanol, ethanol and aqueous extracts were 5.1%, 3.8% and 4.6% w/w respectively.

Preliminary phytochemical analysis

Butea monosperma bark was screened for phytochemical constituents using standard procedures of analysis[13, 14].

DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl)[15]. Different concentrations of each extracts and standard were taken in different vials. 2.8 ml of DPPH solution (45mg/ml) were rapidly mixed with plant extracts. The absorbance was read at 517 nm. Gallic acid was used as reference standard. The radical scavenging activity was expressed as percent inhibition and was calculated using the following formula.

$$\% \text{ Inhibition} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Superoxide radical scavenging activity

Superoxide anion scavenging activity was measured according to the modified method of Robak and Gryglewski[16]. All the reagents were prepared in 100 mM phosphate buffer (pH 7.4). 1 ml of NBT (156 µM), 1 ml of NADH (468µM) and different concentrations of extracts were mixed. The reaction was started by adding 100 µl of phenazine methosulphate (60 µM) and the mixture was then incubated at 25ºC for 5 min followed by measurement of absorbance at 560 nm. Quercetin was used as a reference standard. The percentage inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside in aqueous solution under physiological pH and the extent of nitric oxide scavenged by the test samples were measured by the Griess reaction[17]. Ascorbic acid was used as the reference standard.

$$\% \text{ Inhibition} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Estimation of total phenolic content

Total soluble phenolics in the extracts were determined using Folin-Ciocalteau reagent[18]. Percentage of total phenolic content was calculated from calibration curve of gallic acid and was expressed as percent equivalent to gallic acid.

Estimation of total flavonoid content

Total soluble flavonoid content of the extracts was determined with aluminium chloride using quercetin as the standard[19]. The total flavonoid content in the extracts was expressed as percent equivalent to quercetin by using the standard quercetin graph.

Statistical analysis

All experiments were performed in three times and results were expressed as mean ± SD. All statistical analyses were performed with SPSS 11.5.

RESULTS AND DISCUSSION

Free radicals play an important role in the genesis of various diseases such as cardiovascular diseases, diabetes, cataract, arthritis, immune deficiency diseases, cancer and aging[20]. Antioxidants are capable of neutralizing the deleterious effects of free radicals.

Synthetic antioxidants, such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are suspected to be carcinogenic. So, the importance of search for natural antioxidants has greatly increased in the recent years. Medicinal plants are rich sources of various antioxidant phytochemicals. Epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases. The studies in recent years have shown that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage.

Preliminary phytochemical analysis

Preliminary phytochemical analysis revealed the presence of tannins, saponins, terpenoids, flavonoids, phenols and steroids.

DPPH radical scavenging activity

DPPH is one of the stable free radicals generally used for testing preliminary radical scavenging activity of a compound or a plant extract[21]. Methanol, ethanol and aqueous extracts were found to exhibit better inhibition of DPPH radical with an IC_{50} value of 14.69 ± 1.35, 8.61 ± 0.79 and 13.53 ± 1.23 µg/ml respectively. The results were compared with reference standard gallic acid (IC_{50} : 5.13 ± 0.47 µg/ml). In the present study, ethanolic extract of *Butea monosperma* showed a good antiradical activity than methanolic and aqueous extracts by scavenging DPPH radical.

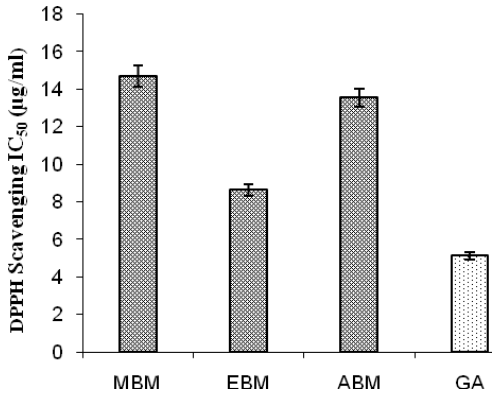

Fig. 1: DPPH radical scavenging activity of bark of *Butea monosperma*

MBM - Methanol extract, EBM - Ethanol extract

ABM - Aqueous extract, GA - Gallic acid

Superoxide radical scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species[22]. The superoxide radical scavenging activity of *Butea monosperma* bark extracts were studied and compared with quercetin. Methanol, ethanol and aqueous extracts showed superoxide radical scavenging activity**,** as indicated by their IC_{50} values 202 ± 18.43, 69.96 ± 6.38, and 76.88 ± 7.02 µg/ml respectively compared to quercetin which showed an IC_{50} 10.25 ± 0.94 µg/ml. The ethanolic extract was found to be an efficient scavenger of superoxide radical generated in riboflavin-NBT-light system *in vitro* than methanolic and aqueous extracts.

Nitric oxide radical scavenging activity

Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, neural signal transmission, immune response, control vasodialation and control of blood pressure etc[23,24,25,26]. *In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. The nitric oxide radical scavenging activity of *Butea monosperma* bark extracts were studied and compared with ascorbic acid (IC_{50} : 33.52 ± 3.07 µg/ml). Concentration of methanol, ethanol and aqueous extracts required for 50% inhibition was found to be 52.89 ± 4.83, 49.42 ± 4.5 and 51.66 ± 4.71 µg/ml respectively. Ethanolic extract of *Butea monosperma* has better nitric oxide radical scavenging activity than methanolic and aqueous extracts in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions.

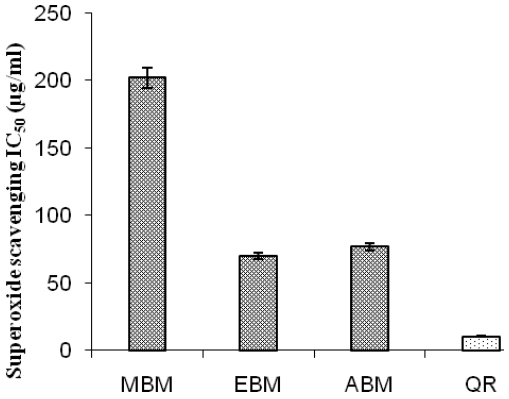

Fig. 2: superoxide radical scavenging activity of bark of *Butea monosperma*

MBM - Methanol extract, EBM - Ethanol extract

ABM - Aqueous extract, QR - Quercetin

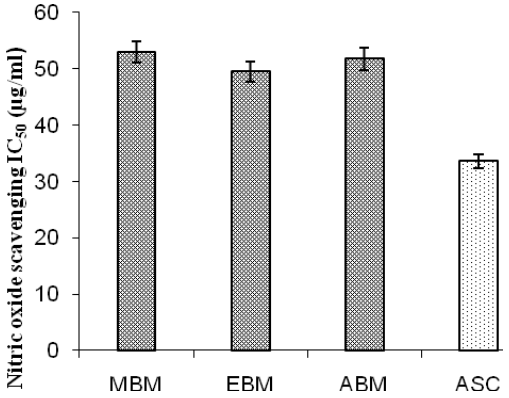

Fig. 3: Nitric oxide radical scavenging activity of bark of *Butea monosperma*

MBM - Methanol extract, EBM - Ethanol extract

ABM - Aqueous extract, ASC - Ascorbic acid

Total phenolic content

The total phenolic content in methanol, ethanol and aqueous extracts were found to be 34.88, 52.38 and 40.2 % w/w respectively.

Total flavonoid content

The total flavonoid content present in methanol, ethanol and aqueous extracts were found to be 10.17, 20.23 and 13.88 % w/w respectively.

CONCLUSION

In the present investigation, we evaluated comparative antioxidant activity of various extracts of *Butea monosperma* bark**.** Ethanolic extract of *Butea monosperma* showed superior radical scavenging activity than methanol and aqueous extracts. The overall antioxidant

activity of *Butea monosperma* might be attributed to its phytochemical constituents and polyphenolics . The results clearly indicate that *Butea monosperma* bark is effective against free radical mediated diseases. This promissory plant needs further investigations for the isolation of active constituents and *in vivo* studies are necessary to utilize the potent antioxidant activity of this plant.

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