# *In-Vitro* antioxidant and free radical scavenging activity of *Butea Frondosa* Roxb. Flower.

**Giridhari lal, Manasi Mantri***

*Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, SVKM's NMIMS University, Mumbai-400 056 India.*

## ABSTRACT

In this present study we aim to evaluate *in vitro* antioxidant and free radical scavenging potential of different extract of *Butea Frondosa Roxb.* Flowers in different system viz. Radical scavenging activity by DPPH reduction and lipid peroxidation assay (TBARS). All extracts have different level of antioxidant activity. The activity was expressed as the inhibitory concentration ($IC_{50}$). In conclusion the present study indicates that alcoholic extract and *n*-butanol fraction of alcoholic extract which is obtained by solvent partition of alcoholic extract of *Butea Frondosa Roxb.* flower showed significant activity by both methods and can be a potential source of natural antioxidants.

**Key words:** *Butea Frondosa Roxb.*, Antioxidant activity, DPPH radical, Lipid peroxidation assay and TBARS.

## INTRODUCTION

In recent years, phyto-medicine is in great demand as food supplement for age related chronic diseases, because of their multi-targeted action and lesser side effects.[1] *Butea Frondosa Roxb.* (Family: Fabaceae) is commonly known as "palash". The Sanskrit word palasa literally means that which looks like a flesh or blood. It has various synonyms in Ayurvedic texts. Like kimsuka – the flowers resemble to the parrot's beak, triparna – 3 – foliate leaves, bija sneha – the seeds are oily and many other, describing its usefulness for yajna i.e. rituals and worships performed infront of fire viz. yajniya, samidvara, brahmapadapa, samiduttama etc. Maharsi Susruta has categorized it as visaghna (anti-toxin), sukrasodhana (purifies seminal fluids) and stambhana – astringent and anti-diarrhoeal. Two varieties of palasa – white flowered and red flowered, are mentioned in Samhita.[1]

It holds an important place because of its medicinal and other miscellaneous uses of economic value. Flowers are astringent to bowel, increase "Vata" cure "Kapha", leprosy, strangury, gout, skin diseases, thirst, burning sensation; flower juice is useful in eye diseases. Flower is bitter, aphrodisiac, expectorant, tonic, emmenagogue, and diuretic good in biliousness, inflammation and gonorrhoea. Flowers also show good antistress, anticonvulsive, antihepatotoxic, antiestrogenic and anthelmintic activity. The dye is useful in enlargement of spleen. Flowers are depurative, as a poultice they are used to disperse swelling and to promote menstrual flow.[1,2,3]

Phytochemical investigations of the dried flowers of *Butea frondosa Roxb* revealed the presence of at least seven flavones and flavanoid constituents and also four free amino acids. These includes Triterpene, butein, butrin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, monospermoside (butein 3-e-D-glucoside) and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin) and steroids.[4,5]

Oxygen involved in the respiratory process can be transformed under some condition into superoxide anion, hydroxyl radical, singlet oxygen and hydrogen peroxide.[6] These reactive oxygen species are implicated in some diseases such as inflammation, cancer, ageing, anaemia, degenerative diseases and atherosclerosis.[7] A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant property. Several studies have been to asses the antioxidant properties of natural products.[8] Scientific information on chemical constituent and antioxidant properties of various plants less widely used in the medicine is still rather scarce. Hence assessment of chemical constituent and such properties remain an interesting and useful task particularly for finding new source

***Address for correspondence:**
E-mail: mantrimanasi@yahoo.co.in

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of natural antioxidant, functional food and neutraceuticals including polyphenolic such as flavonoid, tannin, proanthocyanidin.[9] The objective of the present study was to investigate the *in vitro* antioxidant activity of alcoholic extract of *Butea Frondosa Roxb.* including its *n*-butanol and water fraction.

## MATERIALS AND METHODS

### Chemicals

1, 1-diphenyl-2-picryl hydroxyl (DPPH) purchased from Sigma Aldrich Ltd. Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were purchased from Loba chemie laboratory reagents. Potassium chloride purchased from Merck Ind. Ltd. All other chemicals or solvents either used for extraction or assay purpose were purchased from Qualigens fine chemicals. All the chemicals required are of analytical grade.

### Plant material and extraction

*Butea Frondosa Roxb.* Flowers were purchased from D.G. Ayurvedic Sangrah Andheri, Mumbai, India and the voucher specimen (No. 268) was deposited in the department for future reference.

The flowers were further dried in shadow and powered. The powder obtained was passed through sieve no. 85, weighed and stored in tightly closed container. 250 gm of coarsely powdered flowers of *Butea frondosa* were subjected to simple percolation method of extraction with 3 different solvent viz. 95% ethanol, methanol and water separately for 72 hours. The resulting extracts were concentrated under reduced pressure using rotary vacuum evaporator at 40-50°C to get the semisolid mass. These masses were transferred in petridish & allowed to dry in an oven for about 2 to 3 hours. Maximum yield as well as no. of compounds was obtained in ethanolic extract. Hence ethanolic extract selected for evaluation of antioxidant activity.

For initial screening investigations 10 gm of the total ethanolic extract was partitioned between water and ethyl acetate (3 times). After removal of the ethyl acetate fraction, the remaining water phase was treated with *n*-butanol (3 times). Both the ethyl acetate and the *n*-butanol fractions were dried with sodium sulphate. The solvent was removed by drying in oven at 40-50°c to get the semisolid masses of ethyl acetate, water and *n*-butanol fraction. Each fractions obtained were tested for presence of various phytoconstituents with the help of TLC. Finally whole ethanolic extract, and *n*-butanol fraction & water fraction of ethanolic extract were selected for evaluation of antioxidant activity. Percentage yield of the alcoholic extracts was found to be 16.37%.

### Free radical scavenging activity

#### *DPPH scavenging activity*

The free radical scavenging activity was measured *in vitro* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Blois.[10] About 0.36 mg/ml solution of DPPH in 100% methanol was prepared and 200 µl of this solution was added to 2.7 ml of the extract dissolved in methanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 20 min and the absorbance was measured at 517 nm using a spectrophotometer. The $IC_{50}$ value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radicals was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A Test}}{\text{A control}} \times 100$$

*Where,*

*A control is the absorbance of the control reaction mixture.*
*A test is the absorbance of sample of the extracts at different concentrations.*

Graph of % Inhibition vs. Concentration was plotted to obtain $IC_{50}$ value.

### Lipid peroxidation inhibitory activity by TBARS method

Antilipid peroxidation abilities of *Butea frondosa* was evaluated by Thiobarbituric acid reacting substances (TBARS) using the method of Ohkawa.[11] It is one of the most frequently used tests for measuring the peroxidation of lipids. This method is carried out on liver homogenates of wistar strain albino mice. Curcumin was used as reference standard.

Mouse was sacrificed using anaesthetic ether. The liver was quickly removed and chilled in ice cold saline. After washing with ice cold saline the liver was homogenized in 0.15 M KCl to get 10% liver homogenate.

Fresh liver homogenate (0.2 ml) was mixed with 0.15 M KCl (0.1 ml) and Tris buffer (0.4 ml). The extracts were then added in various concentrations. *In vitro* lipid peroxidation was initiated by addition of ferrous sulphate (10 mM) and ascorbic acid (100 mM), 0.1 ml each. After incubation for 1 hour at 37°C, reaction was terminated by addition of Thiobarbituric acid reagent (2 ml) and boiled for 15 minutes for development of coloured complex. Tubes were centrifuged at 4000 rpm for 10 minutes and cooled. The colour was estimated spectrophotometrically at 532 nm. % reduction of thiobarbituric acid reacting substances was calculated with respect to control to which no sample has been added. The inhibition of lipid peroxidation was