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PHYTOCHEMICAL INVESTIGATION AND IN-VITRO ANTIOXIDANT ACTIVITY OF *BUTEA MONOSPERMA* LEAVES

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Abstract

Free radicals are chemical species which contains one or more unpaired electron. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The aim of the present study was to evaluate the *in vitro* antioxidant activities of *Butea monosperma* leaves extract. Petroleum ether, Ethyl acetate and methanol extract of *Butea monosperma* leaves was studied *in vitro* for DPPH free radical scavenging, nitric oxide, hydrogen peroxide, phenolic and flavonoid contents. Methanol extract of *Butea monosperma* leaves exhibited potent and concentration dependant free radical scavenging activity in all the tested parameters. Reducing ability was found to increase with increase in Methanol extract of *Butea monosperma* leaves concentration. Total phenol and flavonoidal content determination showed that the extract is rich in phenols and flavonoids. All the results of the *in-vitro* antioxidant assays reveal potent antioxidants and free radical scavenging activity of the *Butea monosperma* leaves equivalent to that of standard ascorbic acid and rutin. This potent antioxidant activity may be attributed to its high phenolic and flavonoidal contents.
***Keywords*:** Methanol extract; *Butea monosperma* leaves; DPPH; Hydrogen peroxide; Nitric oxide.

Introduction

Herbal medicine, also known as herbalism, is a medical practice based on the use of plants or plant extracts that may be taken orally or applied to the skin. Since ancient times, herbal medicine has been used by many different cultures throughout the world to treat illness and to assist bodily functions. Nearly all cultures from ancient times to the present day have used plants as a source of medicines. As a result, different remedies tended to develop in different parts of the world. Throughout humankind evolution, the importance of natural products for medicine and health has been enormous. Earliest ancestors chewed certain herbs to relieve pain, or wrapped leaves around wounds to improve healing. Natural products were the sole means to treat diseases and injuries (Agrawal & Prabakaran 2005).

The modern tools of chemistry and biology have currently allowed scientists to detail the exact nature of the biological effects of natural compounds on the human body, as well as to uncover possible synergy, which holds much promise for the development of new therapies against many devastating diseases, including dementia and cancer. Modern chemistry has ushered in a new era for the study and use of natural products (Benzie, 2003; Chaurasia *et al.,* 1995). Analytical and structural chemistry has provided the tools to purify various compounds and to determine their structures, which, in turn, has given insights into their exploit on the human body. In 1805, a German pharmacist Friedrich Wilhelm Serturner (1783–1841) isolated morphine from opium, and it became both the first pure naturally derived medicine and the first to be commercialized, by Merck in 1826. Medicinal plants, moreover as extracts, pure compounds or as derivatives, offer unlimited opportunities for the innovation of new drugs (Deniel *et al.,* 1998; Fang *et al.,* 2002). *Butea monosperma* is a species of *Butea* native to humid and sub-tropical parts of the Indian Subcontinent and Southeast Asia, ranging athwart India, Bangladesh, Nepal, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, and western Indonesia (Halliwell & Gutteridge 1999).

Common names include flame-of-the-forest, palash and bastard teak. It is a small-sized dry-season deciduous tree, growing to 15 m (49 ft) tall. It is a fast-growing tree: young trees have a growth rate of a few feet per year. The leaves are pinnate, with an 8–16 cm (3.1–6.3 in) petiole and three leaflets, each leaflet 10–20 cm (3.9–7.9 in) long. The flowers are 2.5 cm (0.98 in) long, bright orange-red, and produced in racemes up to 15 cm (5.9 in) long (Irshad & Chaudhuri 2002; Manavalan & Ramasamy 2001). The fruit is a pod 15–20 cm (5.9–7.9 in) long and 4–5 cm (1.6–2.0 in) broad. It is used for timber, resin, fodder, medicine, and dye. The wood is dirty white and soft. Being durable under water, it is used for well-curbs and water scoops (Lee *et al.,* 2004). Spoons and ladles made of this tree are used in various Hindu rituals to pour ghee into the fire. Good charcoal can be obtained from it. The leaves are usually very leathery and not eaten by cattle. The leaves were used by earlier generations of people to serve food where plastic plates would be used today (Nadkarni, 1954).

Material and Method

Selection and Collection of Plant

The plant material was selected on the basis of Ethano botanical survey. Leaves of *Butea monosperma* collected from the Pinnacle Biomedical Research Institute, Bhopal Campus.

Authentication of Plant

The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science, Bhopal. A voucher specimen number was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Solvent Extraction

Hot Soxhlet Extraction Method

In this method, the leaves of *Butea monosperma* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. The whole or coarsely powdered plant material of *Butea monosperma* were successively extracted by solvent like petroleum ether, ethyl acetate and methanol in increasing polarity order for different period of time (6 h, 8 h, and 10 h). The powder was placed "thimble" in chamber of the Soxhlet apparatus. The extracting solvent in flasks was heated, and its vapours condense in condenser. The condensed extract and drips into the thimble containing the powder, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon drop into flask. This process was continuous and was carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The extract thus obtained were filtered and concentrated to dryness, weighed and stored for further use. The yield of the extract is calculated by using the following formula.

$$\text{Yield}\ (\%) = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Phytochemical Investigation – Qualitative Test

Detailed qualitative phytochemical analysis was performed to identify presence or absence of different phytoconstituents. The color intensity or the precipitate formation was used as analytical responses to these tests. Following standard procedures were used.

Test for Carbohydrates

Molisch's Test: To 1 ml of aqueous solution of the extract mixed with few drops of Molish reagent (α naphthol) and conc. H_2SO_4 (sulphuric acid) was added along the wall of the tube. Formation of purple colored ring at junction indicated the presence of carbohydrates.

Fehling's Test: Equal volume of Fehling A and Fehling B solution were mixed (1ml each) and 2ml of aqueous solution of extract was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown colored precipitate due to formation of cuprous oxide indicated presence of reducing sugar.

Benedict's Test: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presences of reducing sugar.

Barfoed's Test: To the aqueous solution of extract, 1 ml of Benedict solution was added and heated almost to boiling. Red colour due to formation of cupric oxide indicates the presence of monosaccharides.

Tests for Alkaloids

Dragendorff's Test: To 1 ml of extract dissolved in alcohol was shaken well with a few drops of acetic acid and Dragendroff's reagent. An orange red precipitate formed indicated the presence of alkaloids.

Wagner's Test: To 1 ml of extract dissolved in acetic acid, a few drops of Wagner's reagent were added. A reddish-brown precipitate formed indicated the presence of alkaloids.

Mayer's Test: To 1 ml of extract dissolved in acetic acid, a few drops of Mayer's reagent were added. A dull white precipitate formed indicated the presence of alkaloids.

Hager's Test: To 1-2 ml of extract dissolved in acetic acid, 3 mL of Hager's reagent was added; the formation of yellow precipitate indicated the presence of alkaloids.

Test for Saponins

Froth Test: To 1ml of extract, distilled water was added and shaken. Stable froth formation indicated the presence of saponin.

Test for Triterpenoids and Steroids

Libermann-Burchard Test: The extract was dissolved in chloroform, 1 mL of acetic acid and 1 mL of acetic anhydride were added, then heated on a water bath and cooled. Few drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicated the presence of steroids.

Salkowski Test: The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicated the presence of steroids.

Test for Tannin and Phenolic Compounds

Ferric Chloride Test: Some amount of extract was dissolved in distilled water, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour showed the presence of tannins.

Gelatin Test: Some quantity of extract was dissolved in distilled water. Add 2ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicates presences of phenolic compounds.

Lead Acetate Test: Some amount of extract dissolved in distilled water, few drops of lead acetate solution were added. Formation of white precipitate indicates presences of phenolic compounds.

Test for Flavonoids

Shinoda's Test: To the 1 ml of extract in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicated the presence of flavonoids.

Test for Glycosides

Borntragers Test: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benezene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color

in ammonical layer indicates presence of anthraquinone glycosides.

Keller Killiani Test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5%ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presences of Cardiac glycosides.

Quantitative Phytochemical Assay

Total Phenolic Content Estimation (TPC)

Procedure - The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5 ml of a 10 fold dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. Line of regression from Gallic acid was used for estimation of unknown phenol content. From standard curve of gallic acid line of regression was found to be

$$y = 0.005x + 2.569 \text{ and } R^2 = 0.991$$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned GA (Nagendrappa, 2005).

Total Flavonoid Content Estimation (TFC)

Procedure

The amount of total flavonoids was measured by a colorimetric assay according to Dewanto *et al*. An aliquot of diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutine/g DW), through the calibration curve of Rutin. All samples were analysed in three replications (Nordberg & Amer 2001). Line of regression from rutin was used for estimation of unknown flavonoid content. From standard curve of rutin, line of regression was found to be

$$y = 0.001x - 0.020 \text{ and } R2 = 0.994$$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin.

***In Vitro* Antioxidant Activity**

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

Antioxidants reacts with DPPH, which is stable free radical and is reduced to DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in term of hydrogen donating ability. To assess the scavenging ability on DPPH, each extract (5-20mg/ml) in water and ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals (0.2 mM).The mixture was shaken vigorously and left to stand for 30 mins in the dark before measuring the absorbance at 517nm against a blank. Then the scavenging ability was calculated using the following equation:

$$I\% = 100\times (A \text{ blank} - A \text{ sample}/ A \text{ blank})$$

Where, I (%) is the inhibition percent, A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Nitric oxide assay

Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standard (Ray & Hussain 2002).

Hydrogen peroxide scavenging activity

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 2 ml (20-100 µg/ml) extract in phosphate buffer were added to 1 ml H_2O_2 (40 mM). Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of H_2O_2 scavenging of extract and ascorbic acid (standard compound) was calculated as: % Inhibition= [(A control – A sample)/A control] x 100

Result & Discussion

Phytochemical Investigation

Table 1: Qualitative Phytochemical Analyses

S. No.	Experiment	Result		
		Pet Ether Extract	**Ethyl acetate Extract**	**Methanolic Extract**
Test for Carbohydrates				
1.	Molisch's Test	-ve	+ve	+ve
2.	Fehling's Test	-ve	+ve	+ve
3.	Benedict's Test	-ve	+ve	+ve
4.	Barfoed's Test	-ve	+ve	+ve
Test for Alkaloids				
1.	Dragendorff's Test	-ve	-ve	-ve
2.	Wagner's Test	-ve	-ve	-ve
3.	Mayer's Test	-ve	-ve	-ve
4.	Hager's Test	-ve	-ve	-ve
Test for Triterpenoids and Steroids				
1.	Libermann-Burchard Test	+ve	+ve	+ve
2.	Salkowski Test:	+ve	+ve	+ve
Test for Saponins				
1.	Froth Test	+ve	+ve	+ve
Test for Tannin and Phenolic Compounds				
1.	Ferric Chloride Test	-ve	+ve	+ve
2.	Gelatin Test	-ve	+ve	+ve
3.	Lead Acetate Test	-ve	+ve	+ve
Test for Flavonoids				
1.	Shinoda's Test	-ve	+ve	+ve
Test for Glycosides				
1.	Borntragers Test	-ve	-ve	-ve
2.	Keller Killiani Test	-ve	-ve	-ve
Test for Protein				
1.	Biuret's Test	-ve	-ve	-ve
2.	Ninhydrin Test	-ve	-ve	-ve
3.	Millon's Test	-ve	-ve	-ve
Test for Oil		+ve	-ve	-ve

Total Phenolic Content of extract *Butea monosperma*

Table 2: Total Phenolic Content of extract BM

Extracts	**Total Phenolic content (mg/gm equivalent of Gallic acid)**
	BM
Ethyl acetate	99.833
Methanol	170.67

Total Flavonoid Content of extract *Butea monosperma*

Table 3: Total Flavonoid Content of extract BM

Extracts	**Total Flavonoid content (mg/gm equivalent of rutin**
	BM
Ethyl acetate	28.33
Methanol	93.667

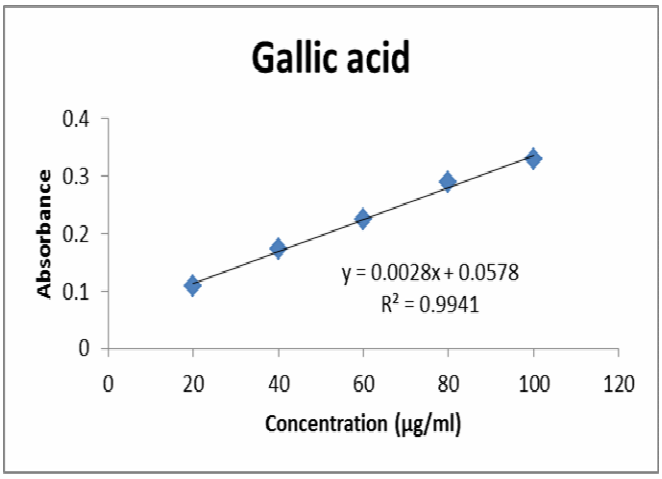


Fig. 1: Standard curve of gallic acid

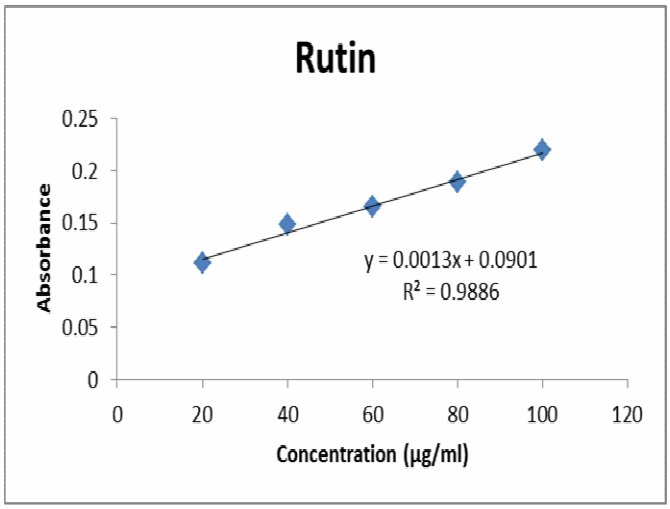


Fig. 2: Standard curve of rutin

DPPH Anioxidant Assay

Table 4: DPPH radical scavenging activity of Std. Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.251	54.36
2.	40 µg/ml	0.228	58.55
3.	60 µg/ml	0.197	64.18
4.	80 µg/ml	0.137	75.09
5.	100 µg/ml	0.089	83.82
IC_{50}		**14.42 µg/ml**	

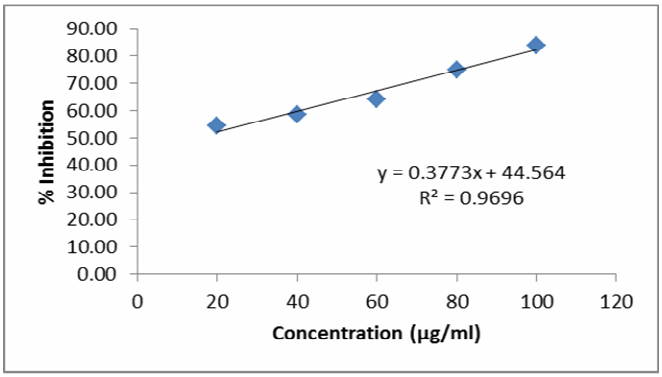


Fig. 3: Standard curve of ascorbic acid

DPPH radical scavenging activity of Petroleum Ether extract of *Butea monosperma*

Table 5: DPPH radical scavenging activity of PEBM

PEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.411	25.27
2.	40 µg/ml	0.395	28.18
3.	60µg/ml	0.353	35.82
4.	80µg/ml	0.332	39.64
5.	100µg/ml	0.306	44.36
IC_{50}		**121.93µg/ml**	

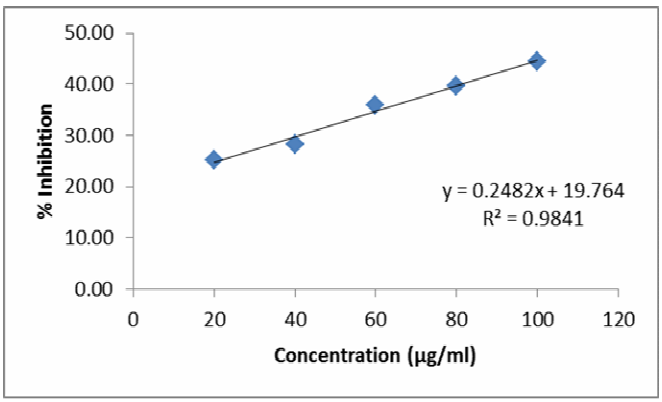
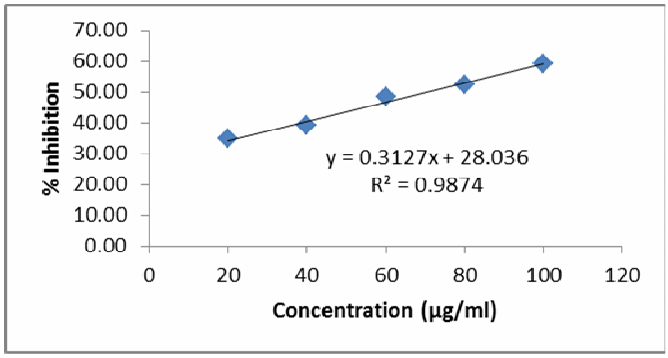


Fig. 4: DPPH radical scavenging activity of PEBM

DPPH radical scavenging activity of Ethyl Acetate extract of *Butea monosperma*

Table 6: DPPH radical scavenging activity of EABM

EABM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.359	34.73
2.	40 µg/ml	0.335	39.09
3.	60µg/ml	0.284	48.36
4.	80µg/ml	0.261	52.55
5.	100µg/ml	0.224	59.27
IC_{50}		**70.41 µg/ml**	



Fig, 5: DPPH radical scavenging activity of EABM

DPPH radical scavenging activity of Methanol extract of *Butea monosperma*

Table 7: DPPH radical scavenging activity of MEBM

MEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.28	49.09
2.	40 µg/ml	0.25	54.55
3.	60µg/ml	0.232	57.82
4.	80µg/ml	0.197	64.18
5.	100µg/ml	0.159	71.09
IC_{50}		**25.18µg/ml**	

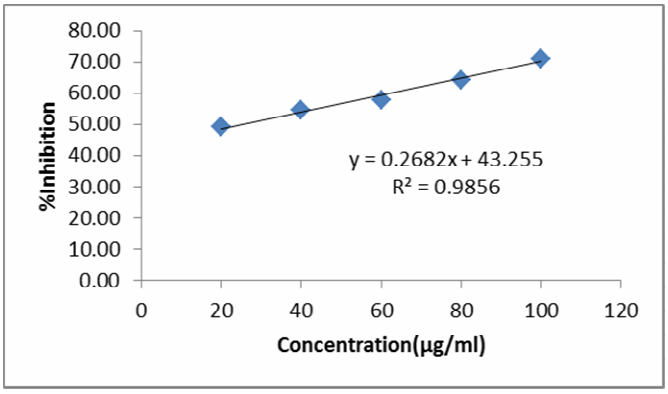


Fig. 6: DPPH radical scavenging activity of MEBM

H_2O_2 Antioxidant Assay

Table 8: H_2O_2 radical scavenging activity of Std. Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.911	43.486
2.	40 µg/ml	0.77	52.233
3.	60µg/ml	0.65	59.677
4.	80µg/ml	0.479	70.285
5.	100µg/ml	0.38	76.426
IC_{50}		**17.59µg/ml**	

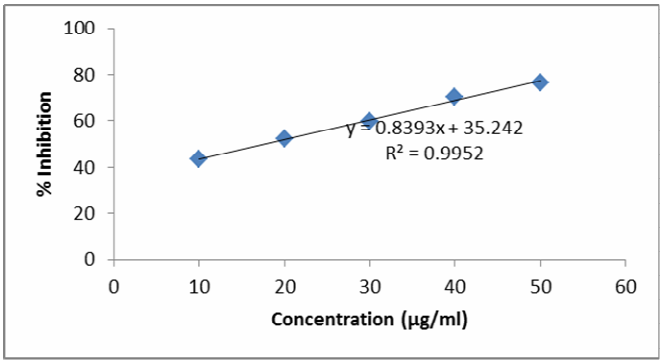


Fig. 7: H_2O_2 radical scavenging activity of Std. Ascorbic acid

H_2O_2 radical scavenging activity of Petroleum Ether extract of *Butea monosperma*

Table 9: H_2O_2 radical scavenging activity of PEBM

PEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	1.211	24.875
2.	40 µg/ml	1.141	29.218
3.	60µg/ml	1.099	31.823
4.	80µg/ml	0.996	38.213
5.	100µg/ml	0.963	40.260
IC_{50}		**73.19µg/ml**	

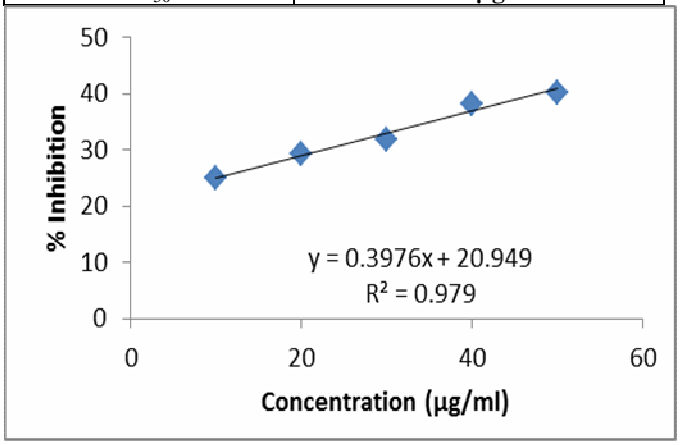


Fig. 8: H_2O_2 radical scavenging activity of PEBM

H_2O_2 radical scavenging activity of Ethyl Acetate extract of *Butea monosperma*

Table 10: H_2O_2 radical scavenging activity of EABM

EABM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	1.145	28.970
2.	40 µg/ml	1.101	31.699
3.	60µg/ml	0.982	39.081
4.	80µg/ml	0.971	39.764
5.	100µg/ml	0.788	51.116
IC_{50}		**52.75µg/ml**	

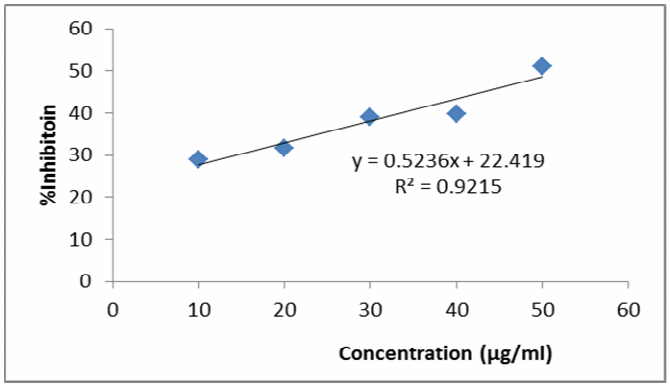


Fig. 9: H_2O_2 radical scavenging activity of EABM

H_2O_2 radical scavenging activity of Methanol extract of *Butea monosperma*

Table 11: H_2O_2 radical scavenging activity of MEBM

MEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	1.139	29.342
2.	40 µg/ml	1	37.965
3.	60µg/ml	0.978	39.330
4.	80µg/ml	0.792	50.868
5.	100µg/ml	0.644	60.049
IC_{50}		**38.74µg/ml**	

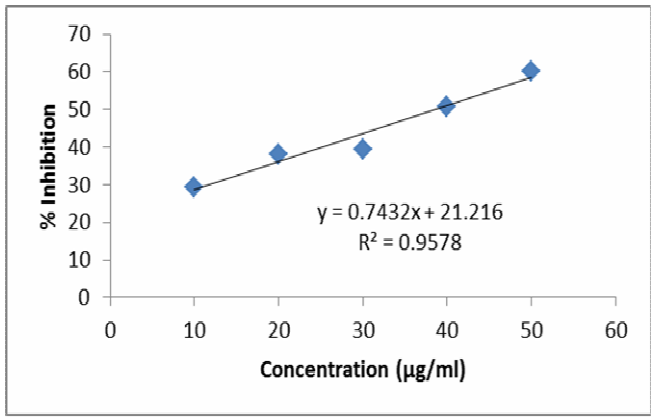


Fig. 10: H_2O_2 radical scavenging activity of MEBM

Nitric Oxide Assay

Table 12: NO radical scavenging activity of Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.28	49.367
2.	40 µg/ml	0.244	55.877
3.	60µg/ml	0.225	59.312
4.	80µg/ml	0.195	64.737
5.	100µg/ml	0.174	68.535
IC_{50}		**9.74µg/ml**	

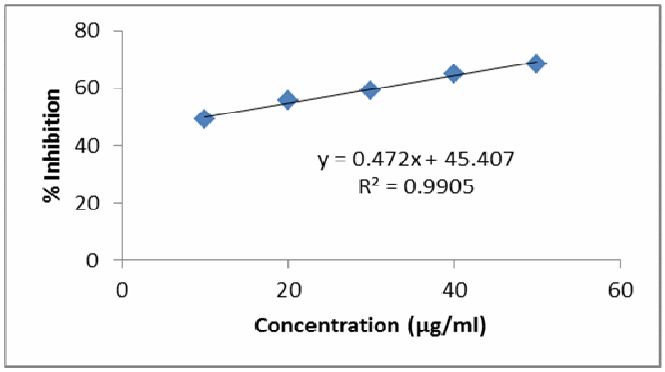


Fig. 11: NO radical scavenging activity of Ascorbic acid

NO radical scavenging activity of Petroleum Ether extract of *Butea monosperma*

Table 13: NO radical scavenging activity of PEBM

PEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.455	17.721
2.	40 µg/ml	0.429	22.423
3.	60µg/ml	0.392	29.113
4.	80µg/ml	0.381	31.103
5.	100µg/ml	0.372	32.730
IC_{50}		**90.43µg/ml**	

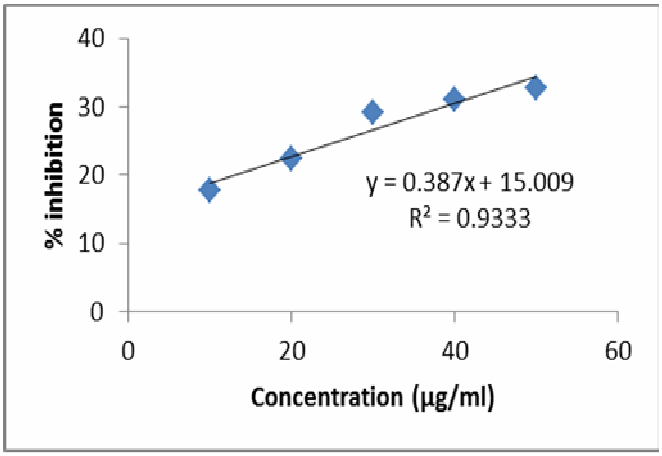


Fig. 12: NO radical scavenging activity of PEBM

NO radical scavenging activity of Ethyl Acetate extract of *Butea monosperma*

Table 14: NO radical scavenging activity of EABM

EABM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.464	16.09403
2.	40 µg/ml	0.411	25.67812
3.	60µg/ml	0.385	30.37975
4.	80µg/ml	0.366	33.81555
5.	100µg/ml	0.333	39.783
IC_{50}		**67.58µg/ml**	

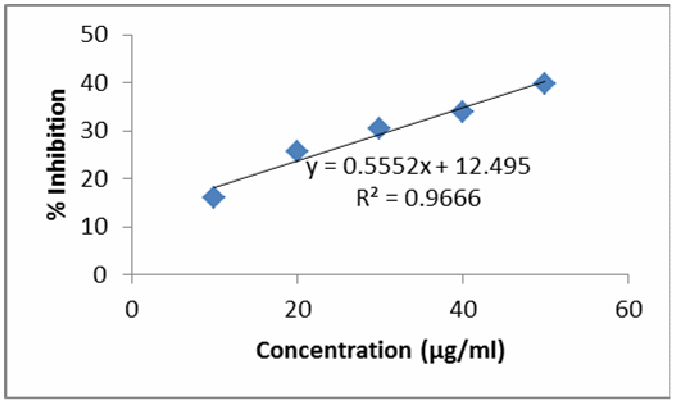


Fig. 13: NO radical scavenging activity of EABM

NO radical scavenging activity of Methanol extract of *Butea monosperma*

Table 15: NO radical scavenging activity of MEBM

MEBM			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 µg/ml	0.418	24.4123
2.	40 µg/ml	0.39	29.47559
3.	60µg/ml	0.362	34.53888
4.	80µg/ml	0.32	42.13382
5.	100µg/ml	0.3	45.75045
IC_{50}		**56.67µg/ml**	

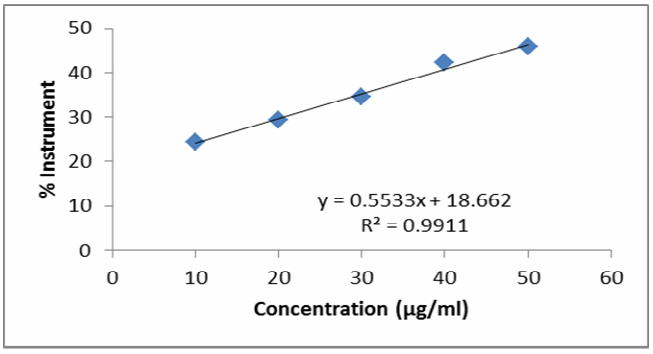


Fig. 14: NO radical scavenging activity of MEBM

Phytochemical screening of the plant extract revealed the presence of alkaloids, saponins, tannins, carbohydrates and protein. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25^{0}C for 150 min resulted in the generation of NO. The Methanol extract of *Butea monosperma* effectively reduced the generation of NO. In the

DPPH redical Scavenging activity of the extract the IC_{50} was found to be 14.42 and 25.18 µg/ ml for MEBM and for standard, ascorbic acid, respectively (Table 4-7). MEBM was capable of scavenging H_2O_2 in an amount and concentration dependent manner. The scavenging ability of the extract and standard, ascorbic acid are shown in Table 8. H_2O_2 scavenging activity of MEBM was closer to that of ascorbic acid at doses of 60, 80 and 100 µg/ml. The content of total phenolics in EETFG was determined using folin ciocalteau assay, calculated from regression equation of calibration curve of gallic acid. Phenolic content of MEBM was found to be 40 g gallic acid equivalent / mg. the total flavonoid content of MEBM was found to be 88 g rutin equivalent / mg.

Conclusion

Based on the various *in vitro* assays, it can be concluded that the MEBM posses string antioxidant activity, evidenced by the free radical scavenging property, hydrogen peroxide activity, and nitric oxide activity which may be due to the presence of phenolic components in the extract. Overall, the plant extract is a source of natural antioxidant that can be important in disease prevention, health preservation and promotion of longevity promoter.

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