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In Vitro Antioxidant activity of *Butea monosperma* Flowers Fractions

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Abstract: Antioxidant activity of *Butea monosperma* flowers was essayed through some *in vitro* models such as the radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay, reducing power assay, nitric oxide scavenging activity and antioxidant capacity by phosphomolybdenum method. The n-butanolic and ethyl acetate fractions were obtained by liquid-liquid partition from methanolic extract of *Butea monosperma* flowers. The n-butanolic fraction showed the highest scavenging activity and reducing power followed by ethyl acetate fractions in a dose dependent manner. Results were compared to standard antioxidants such as ascorbic acid. The present study reveals that fractions of *Butea monosperma* flowers posse strong antioxidant activity and it might be useful in the management of various pathophysiological conditions associated with oxidative stress.

Keywords: *Butea monosperma*, Antioxidant, DPPH, Reducing power, Oxidative stress

Introduction

Oxidative stress is involved in the pathogenesis of various chronic diseases.[1] Antioxidants have ability to neutralize excess oxidant production including enzymes that convert oxidants into less harmful or harmless species and small molecules that serve as oxidant sinks or scavengers.[2] The carcinogenic adverse effects of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) has led to a growing interest towards natural antioxidants of plant origin in recent years.[3,4,5,6]

Plants are rich in photochemical. Common dietary antioxidants primarily include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are principally due to the antioxidant vitamins and large number of phytochemicals, some with greater antioxidant properties.[7] These phytochemicals mainly includes secondary constituents or metabolites like carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols and are believed to have an crucial role in the maintenance of human health because endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species in certain conditions like cancer, cardiovascular disease, atherosclerosis, diabetes, cataracts, arthritis, immune deficiency diseases, aging and central nervous system disorders.[8,9]

Butea monosperma is commonly known as 'Flame of forest', belongs to the family Fabaceae.[10] It is locally called as Palash, Palas, Mutthuga, Bijasneha, , Khakara, Dhak Chichra, Bastard Teak, Bengal Kino, Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts.[10,11,12] Almost all the parts of plant including flowers, seeds, leaves and barks possess medicinal property.[13] The plant holds a significant place because of its medicinal and other miscellaneous uses of economic value.[10] Flowers are large, in a rigid racemes 15 cm long, 3 flowers together form the tumid nodes of the dark olive-green velvety rhachis.[10,11] Flowers used as vegetable by tribals and used for the preparation of dye for colouring garments and for making skin antiseptic ointments.[10] Flowers are rich in triterpene[14], butein, butin, isobutrin[13,15], coreopsin, isocoreopsin (butin 7-glucoside)[16], sulphurein, monospermoside (butein 3-β-D-glucoside) and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin)[17,16,18], steroids, sugar and amino acids[19,20], myricyl alcohol, stearic, palmitic, arachidic and lignoceric acids[21]. Traditionally flowers are used in various conditions like astringent to bowel, increase "Vata" cure "Kapha", leprosy, strangury, thirst, gout, skin diseases, burning sensation, enlargement of spleen, diarrhea, to reduce body heat and chronic fever, to prevent pus from urinogenital tracts of males, leucorrhoea, flower juice is useful in eye diseases.[10,11,12] Flower is bitter, aphrodisiac, expectorant, tonic, emmenagogue, diuretic, good in biliousness, inflammation and gonorrhea.[10] Plant parts has been evaluated for various activities such as antistress activity[22,23], anticonvulsive activity[24], nootropic activity, antiestrogenic activity[25], hypoglycemic and antidiabetic activity[26,27,28], osteogenic activity[16,29] and postcoital anticonceptive activity[30], antiovulatory and anti-implantation activities[31], leprosy, leucorrhoea and gout[32], chemopreventive and anti-cancer properties[32,33], anti-inflammatory[34,35,36], anthelmintic activity[37,38], antioxidant[39], aphrodisiac activity[40], antimicrobial[41], wound healing activity[42,43], thyroid inhibitory and antiperoxidative[44] and antidiarrhoeal activity.[45]

Hence, the present study aimed to assess antioxidant potential and free radical scavenging activities of various fractions of *Butea monosperma* flowers.

MATERIALS & METHODS

Chemicals

1- diphenyl-2-picryl hydrazly (DPPH) was purchased from Sigma Aldrich, India. Trichloroacetic acid (TCA), N-(1-Naphthyl)ethylenediamine dihydrochloride were obtained from S.D. Fine chemicals, India. Ascorbic acid, Butylated hydroxyl anisole (BHA), Potassium ferricyanide, Ferric chloride ($FeCl_3$), Sodium nitroprusside, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium chloride, Ammonium molybdate, Sodium phosphate, Sulphuric acid were purchased from Loba chieme, India. Methanol, Sulphanilamide , Phosphoric acid were obtained from Qualigens Fine Chemicals, India.

Plant material

Flowers of *Butea monosperma* were collected from Toranmal, Maharashtra, India. The plant was botanically identified and authenticated by Dr. D. A. Patil botanist SSVPS college, Dhule, Maharashtra, India and a voucher

specimen was deposited at the departmental herbarium.

Extraction and fractionation

The Flowers were shade dried at room temperature and the dry material was ground to a fine powder using pulverizer. The powdered plant material was extracted using methanol by soxhlet extraction. Solvent was removed with a rotary evaporator (Equitron Rotaeva-8703). The extract was filtered and concentrated. The dried extract was suspended in water and fractionated with pet ether, n-butanol and ethyl acetate. The n-butanolic and ethyl acetate fractions and the remaining aqueous phase were concentrated. All the solvent were removed with a rotary evaporator to obtain the fractions in the yield of 5.5 % gm for n-butanolic and 1.1% for ethyl acetate.

Photochemical prospective

The phytochemical tests of fractions were performed to detect the presence of saponins, tannins, flavonoids, steroids, triterpenes, glycosides, proteins, carbohydrates and alkaloids. The tests were based on the visual observation of a change in color or formation of precipitate after the addition of specific reagents.

DPPH radical scavenging activity[46,47,48]

The capacity to scavenge the ''stable'' free radical DPPH by n-butanolic and ethyl acetate fraction was measured according to Hanato *et al* (1998) which is based on the reduction of methanolic solution of the coloured free radical of 1, 1- diphenyl-2-picryl hydrazyl (DPPH). A methanol DPPH solution (0.1 mM, 1 ml) was mixed with serial dilutions (10, 20, 40, 60, 80µg/ml) of the n-butanolic fraction and ethyl acetate fraction and incubated for 30 min at room temperature (25^0C). For each concentration the assay was run in triplicate and the absorbance was read at 517 nm using microplate reader (Powerwave XS, Biotek, USA). Ascorbic acid (Loba chiemie, India) was used as standard to compare with fractions. IC_{50} (the antiradical dose required to cause a 50% inhibition) for ascorbic acid, n-butanolic and ethyl acetate fraction was determined. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_{DPPH} - A_{test}) / A_{DPPH} \times 100$$

Where A_{DPPH} is absorbance of 0.1mM DPPH solution and A_{test} is absorbance of fractions.

Reducing power assay[3,49,50,51]

The reducing power was determined according to the method of Oyaizu (1986). Various concentration of the n-butanolic and ethyl acetate fraction (10, 20, 40, 60, 80µg/ml) were mixed with 2.5ml of 200 mmol/L sodium phosphate buffer (p^H 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50^0C for 2 min. Then 2.5 mL of 10% tricloroacetic acid (w/v) were added, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5mL) was mixed with 2.5mL of demonized water and 0.5 mL of 0.1% of ferric chloride and the absorbance was measured spectrophotometrically at 700 nm using microplate reader (Powerwave XS, Biotek, USA). Increase in the absorbance of the reaction mixture indicates reducing ability of fractions and which is compared with ascorbic acid as a standard.

Nitric oxide scavenging activity[49,51,52,53]

The nitric oxide scavenging activity was determined according to method reported by Sreejayan (1997). The method is based on inhibition of nitric oxide (NO) radicals generated from sodium nitroprusside solution at physiological pH. Sodium nitroprosside (1ml of 10mM) was mixed with 1ml fractions of different concentrations (150-300µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 25^{0}C for 150 min. To 1 ml of incubated solution, 1ml of Griess reagent (α-naphthyl-ethylenediamine dihydrochloride 0.1% in water and sulfanilamide 5% in H_3PO_4) was added. The same reaction mixture without fractions but equivalent amount of distilled water was served as control. Absorbance was measured at 546 nm using microplate reader (Powerwave XS, Biotek, USA) and percentage inhibition was calculated using following formula:

$$\% \text{ Inhibition} = (A_{Control} - A_{fraction}) / A_{Control} \times 100$$

Where $A_{Control}$ is absorbance of control and $A_{fraction}$ is absorbance of fractions.

The antioxidant activity of the factions was expressed as IC_{50} value was defined as concentration (in µg/ml) of fractions that inhibits the formation of nitric oxide radicals by 50%.

Evaluation of total antioxidant capacity by phosphomolybdenum method[54,55,56,57,58]

The total antioxidant capacity of fractions was evaluated by the method of Prieto *et al* (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (9). 0.1 ml of fraction (100-800 µg/ml) was mixed with 1 ml of the reagent solution (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) and sample was incubated at 95^{0}C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm using an UV/Vis spectrophotometer (Beckman DU-530). The values are presented as the means of triplicate analysis. The antioxidant capacity was expressed as ascorbic acid equivalent by using the standard ascorbic acid graph.

Statistical analysis

All determinations were run in triplicate and the results were reported as the mean and standard deviation. Statistical analysis was carried out with one way ANOVA followed by Dunnet using graph pad prism software.

Result

Photochemical Prospective

Table 1 shows presence of various metabolites such as flavonoids, glycosides, tannins, steroids, alkaloids, proteins, carbohydrate and phenol in extract and further fractions contains only flavonoids, steroids and phenols.

Table 1: Phytochemical prospective of extract and fractions of *Butea monosperma* flowers.

Metabolites	1	2	3	4	5	6	7	8
Methanolic Extract	+	+	+	+	+	+	+	+
n-butanolic Fraction	+	-	+	-	+	-	-	+
Ethyl acetate fraction	-	-	+	-	-	-	-	+

1.Steroids, 2.Alkaloids, 3.Flavonoids, 4.Flavonoids, 5.Glycosides, 6.Proteins, 7.Carbohydrate, 8.Phenols

+: Present, - : Absence.

Inhibition of DPPH radical

The scavenging activity of n-butanolic fraction and ethyl acetate fraction of *Butea monosperma* flowers is shown in figure 1 and compared with

that of ascorbic acid. The scavenging effect of extract, fractions and standard on the DPPH radical was expressed as percentage inhibition. Fractions show concentration dependant reduction in absorbance and exhibits effective antioxidant activity.

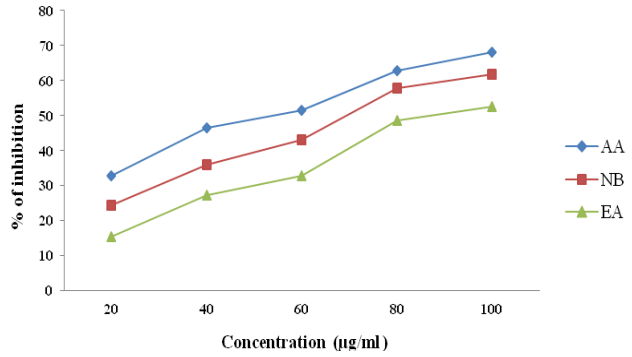

Figure 1: DPPH scavenging assay of n-butanolic (NB), ethyl acetate (EA) fractions of *Butea monosperma* flowers compared with standard ascorbic acid (AA).

Reducing ability

Figure 2 shows the reducing capabilities of n-butanolic and ethyl acetate fractions of *Butea monosperma* flowers compared with ascorbic acid. The reducing power of fractions increased with increase in concentration.

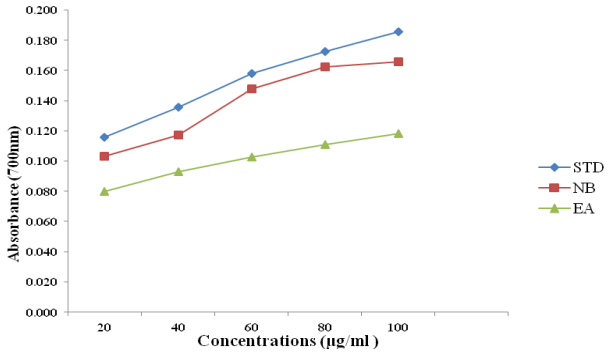

Figure 2: Reducing ability of n-butanolic (NB), ethyl acetate (EA) fractions of *Butea monosperma* flowers compared with standard ascorbic acid (STD).

Inhibition of nitric oxide radical

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by fractions of *Butea monosperma* flowers when compared with ascorbic acid. Figure 3 shows significant reducing capabilities of n-butanolic fraction.

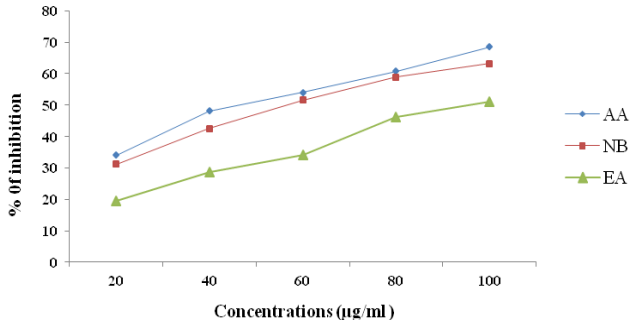

Figure 3: Nitric oxide scavenging activity of n-butanolic (NB), ethyl acetate (EA) fractions of *Butea monosperma* flowers compared with standard ascorbic acid (AA).

Total antioxidant capacity

Figure 4 illustrates the total antioxidative capacities of various concentrations of n-butanolic and ethyl acetate fractions of *Butea monosperma* flowers.

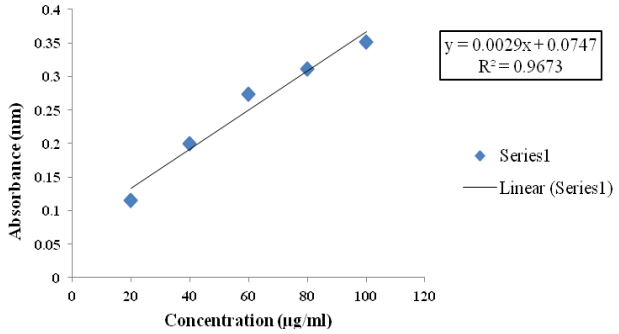

Figure 4: Total antioxidant capacity of n-butanolic and ethyl acetate fractions of *Butea monosperma* flowers.

Discussion

Oxidative stress is classically defined as a redox unbalance with an excess of oxidants or a fault in antioxidants.[59] The sources of oxidants are abundant and are generally extremely reactive and unstable.[60] Most oxidants are derived from enzymatic or chemical reactions that produce

superoxide anion, hydroxyl, peroxyl (RO2*), alkoxyl (RO*), and hydroperoxyl (HO2*) or nitric oxide (NO). Further these are converted to secondary very reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydrogen peroxide, hypochlorous acid (HOCl), hypobromous acid (HOBr) and per-oxynitrite ($ONOO^-$).[2,60,61] In normal physiology ROS and RNS serve as important regulators of signal transduction and protein function and both produced in a well regulated manner to maintain homeostasis at the cellular level in the normal healthy tissues.[62] However elevated levels of ROS or RNS can damage vital cellular components such as structural and regulatory proteins, membrane lipids, DNA.[2,63] Cellular antioxidants include non-enzymatic endogenous antioxidants like vitamins C and E and coenzyme Q, b-carotene, and glutathione and enzymatic antioxidant defenses like superoxide dismutase, glutathione peroxidase and catalase that convert free radicals to more benign molecules.[64,65] These molecules are capable to donate an electron and neutralize free radicals but are destroyed upon oxidation.[2] Different mechanisms such as neutralizing reactive species (scavenging activity), sequestering transition metal ions (chelation activity), inhibiting enzymes involved in the over production of reactive species and modulating gene expression (e.g. ARE/Nrf-2 pathway) are responsible for its protective effect as antioxidant.[65] In recent years herbal drugs were found to be effective in combating free radical induced physiologic and pathologic conditions.

Previous animal study has shown that dietary phytochemical antioxidants like phenolic and polyphenolic compounds such as flavonoids and catechin from edible plant exhibit potent antioxidant activities and capable of removing free radicals. Phytochemical are effective in the management of conditions associated with ROS formation like β-carotene reacts with a peroxyl radical to form a resonance-stabilized carbon-centered radical within its conjugated alkyl structure thereby arresting the chain propagation effect of ROS. Brussels sprouts (300 g/d) markedly minimizes the urinary excretion of 8-hydroxydeoxyguanosine in humans indicating a reduction of DNA oxidation.[66,67] Phytochemical investigation of extract and fractions shows that both are rich in the phenolic and polyphenolic compounds.

Antioxidant behavior can be assessed either by activity in foods or bioactivity in humans. Antioxidant activity cannot be measured directly but rather by the effects of the antioxidant in controlling the extent of oxidation. DPPH radical bleaching is one of the methods used to evaluate the antioxidant properties of phytoconstituents and is based on the capacity of herbal extract to bleach the DPPH radical, a nitrogen-centred free radical. Figure 1 shows the results of scavenging DPPH radical ability of n-butanolic and ethyl acetate fractions of *Butea monosperma* flowers in comparison with same doses of ascorbic acid as a standard. The IC_{50} value of n-butanolic and ethyl acetate fractions was 69.09 and 95.16 showed dose-dependent DPPH radicals scavenging activity. This decrease in absorbance is due to scavenging of the formed radicals by donated hydrogen from antioxidants. Similar effects have been reported by many authors.[68]

NO is one of the most common signaling molecules and involved in various cellular metabolic pathways in the body. Physiologically it is essential for regulating the relaxation and proliferation of vascular smooth muscle cells,

platelet aggregation, leukocyte adhesion, angiogenesis, thrombosis, vascular tone, hemodynamics, neurotransmitter synthesis and immune response. However increase level of No causes oxidation of various biomolecules (e.g., protein, amino acids, lipid, and DNA) which leads to cell injury and death. Removal of such free radicals is achieved through enzymatic (SOD, GSH, GSH peroxidases, glutathione reductase, catalase) and non-enzymatic reactions in the body.[61,66] The n-butanolic and ethyl acetate fractions of flowers of *Butea monosperma* significantly scavenges the NO formed from sodium nitroprusside. Figure 3 illustrates the percentage inhibition of nitric oxide generation by fractions.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant.[3,5] Fe^{3+}/Fe^{2+} transformation was observed in the presence of fractions for the measurements of the reductive ability. Okuda *et al* reported that tannins inhibits the formation of lipid peroxides and thereby prevents liver damage.[50] Reducing power of the n-butanolic fraction was found to be significant as that of standard ascorbic acid.

Phosphomolybdenum assay used to determine the total antioxidant capacity of fractions which is based on formation of phosphate/Mo (V) complex because of reduction of Mo (VI) by the fraction.[54,55] Figure 4 illustrates the antioxidative capacities of various concentrations of factions. The n-butanolic fraction of *Butea monosperma* flowers presented a strong total antioxidant activity. The antioxidant activity of fraction might be attributed to the presence of antioxidant phytochemicals such as flavonoids and phenolic compounds.

Till date over eight thousand naturally occurring phenolic compounds are known. Major classes of plant phenolics with 'the type of carbon skeleton, class name (example)' format include: C6, simple phenols (resorcinol); C6-C1, phenolic acids (p-hydroxybenzoic acid); C6-C2, acetophenones and phenylacetic acids; C6-C3, hydroxycinnamic acids (caffeic acid); C6-C4, hydroxyanthraquinones (physcion); C6-C2-Molecules 2007, 12 1498C6, stilbenes (resveratrol); C6-C3-C6, flavonoids (quercetin); (C6-C3)2, lignans (matairesinol); (C6-C3-C6)2, biflavonoids (agathisflavone); (C6-C3)n, lignins; (C6-C3-C6)n, condensed tannins (procyanidin).[67,69] The admirable antioxidant capacity of polyphenols is due to presence and distribution of numerous hydroxyl groups in the chemical structure. They either chelate transition metal ions or inhibit the activity of many enzymes participating in the formation of free radicals.[70,71] Among polyphenols, flavonoids constitute the most important single group, including more than 5000 compounds that have been thus far identified and exhibits a broad spectrum of chemical and biological activities including radical scavenging properties.[57,67,72] The potent antioxidative activity of fractions of *Butea monosperma* flowers ascribed to their free-radical scavenging capacity as well as their ability to form chelation with metal ions or both.

Conclusion

The n-butanolic fraction and ethyl acetate fractions were found to have potent free radical scavenging activity and significant in vitro antioxidant activity. The observed activity may be due to the steroids, flavonoids and phenolic content presents in the fraction. The component

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and exact mechanism responsible for antioxidant potential is still needs to evaluate. Determination of these natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES

1) Simone R, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biology & Medicine* 2010; 49: 1603-1616.
2) Jennifer SM, Michael BR. Oxidative stress, chronic disease, and muscle wasting. *Muscle & Nerve* 2007; 35: 411-429.
3) Muruhan S, Selvaraj S, Viswanathan PK. In vitro antioxidant activities of *Solanum Surattense* leaf extract. *Asian Pac J Trop Biomed* 2013; 3(1): 28-34.
4) Yerra R, Senthil Kumar GP, Gupta M, Mazumder UK. Studies on in vitro antioxidant activities of methanol extract of *Mucuna Pruriens*(Fabaceae) seeds. *European Bulletin of Drug Research* 2005; 13(1): 31-39.
5) Sonia M, Mohamed D. in vitro antioxidant activities of aloe vera leaf skin extracts. *J. Soc. Chim. Tunisie* 2008; 10: 101-109.
6) Irina IK, Teris A van Beek, Jozef PH Linssen, Aede de Groot, Lyuba NE. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal* 2002; 13:8-17.
7) Boskou D. Sources of natural phenolic antioxidants. *Trends in Food Science & Technology* 2006; 17:505–512.
8) Duduku K, Rosalam S, Awang B. phytochemical antioxidants for health and medicine – a move towards nature. *Biotechnology and Molecular Biology Review* 2007; 1(4): 097-104.
9) Kwang-Geun Lee, Alyson EM, Takayuki Shibamoto. determination of antioxidant properties of aroma extracts from various beans. *J. Agric. Food Chem* 2000; 48: 4817−4820.
10) Burli DA, Khade AB. A comprehensive review on *Butea monosperma* (Lam.) Kuntze. *Pharmacognosy Reviews* 2007; 1(2): 333-337.
11) Sindhia VR, Bairwa R. Plant review: *Butea monosperma*. *International Journal of Pharmaceuticaland Clinical Research* 2010; 2 (2): 90-94.
12) Parashar B, Dhamija HK. Botaical, Phytochemical and biological investigation of *Butea monosperma* (Lam.) Kuntze. *Pharmacologyonline* 2011, 3: 192-208.
13) Thomas M, Emilie D, Virginie P, Isabelle R, Laure P, Patrice A, Claire E. Two-step centrifugal partition chromatography (CPC) fractionation of *Butea monosperma* (Lam.) biomarkers. *Separation and Purification Technology* 2011 , 80 : 32–37.
14) Kasture VS, Kasture SB, Chopde CT. Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. *Pharmacology, Biochemistry and Behavior* 2002; 72: 965–972.
15) Wagner H, Geyer B, Fiebig M, Kiso Y, Hikino H. Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers. *Planta Med* 1986; 52(2): 77-79.
16) Gupta SR, Ravindranath B, Seshadri T. The glucosides of *Butea monosperma*. *Phytochemistry* 1970; 9(10): 2231-35.
17) Mishra M, Shukla YN, Kumar S. Chemical constituents of *Butea monosperma* flowers. *J. Med. Aro. Plant Sci* 2000; 22(1): 16.
18) Yadava RN, Lata Tiwari. New antifungal flavone glycoside from *Butea monosperma* O. *Journal of Enzyme Inhibition and Medicinal Chemistry* 2007; 22(4): 497–500.
19) Shah KC, Baxi AJ, Dave KK.Isolation and identification of free sugars and free amino

acids from *Butea frondosa* Roxb. flowers. *Indian Drugs*1992; 29 (9): 422-23.

20) Mazumder PM, Das MK, Das S. *Butea Monosperma* (Lam.) Kuntze - a comprehesive review. *International Journal of Pharmaceutical Sciences and Nanotechnology* 2011; 4(2): 1390-1393.

21) Murti P, Bhaskara R, Krishnaswamy H. Proceedings - Indian academy of sciences 1940. section A(12A): 472-476.

22) Bhatwadekar AD, Chintawar SD, Logade NA, Somani RS, Kasture VS, Kasture SB. Antistress activity of *Butea monosperma* flowers. *Ind. J. Pharmacol* 1999; 31: 153-55.

23) Gawale NS, Pal SC, Kasture VS, Kasture SB. Effect of *Butea monosperma* on memory and behaviour mediated via monoamine neurotransmitters in laboratory animals. *J. Nat. Remedies* 2001; 1(1): 33-41.

24) Kasture VS, Kasture SB, Chopde CT. Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. *Pharmacol. Biochem. Behav* 2002; 72: 965-72.

25) Shah KG, Bakxi AJ, Sukla VJ, Dave KK, De S, Ravishankar B. Phytochemical studies and antiestrogenic activity of *Butea frondosa* (*Butea monosperma*) flowers. *Ind. J. Pharm* Sci 1990; 52: 272-75.

26) Jamkhande PG, Patil PH, Surana SJ. Evaluation of n-butanolic fractions *of Butea monosperma* flowers on dexamethasone induced hyperglycemia and hyperlipidemia in mice. *International Journal of Phytopharmacy Research* 2010; 1(1): 5-10.

27) Somani R, Kasture S, Singhai AK. Antidiabetic potential of *Butea monosperma* in rats. *Fitoterapia* 2006; 77: 86–90.

28) Deore SL, Khadabadi SS, Daulatkar VD, Deokate UA, Farooqui IU, Evaluation of hypoglycemic and antidiabetic activity of bark of *Butea monosperma*. *Phcog Mag* 2008; 4(13): 434-38.

29) Maurya R, Dinesh KY, Geetu Singh, Biju Bhargavan, Narayana Murthy PS, Sahai M, Singh MM. Osteogenic activity of constituents from *Butea monosperma*. *Bioorganic & Medicinal Chemistry Letters* 2009; 19: 610-613.

30) Bhargava SK. Strogenic and postcoital anticonceptive activity in rats of butin isolated from *Butea Monosperma* Seed. Journal of Ethnophorrnacoiogy 1986; 18: 95-101.

31) Shah NC. My experiences with herbal plants and drugs as i knew XXXI : observations on *Butea monosperma* (Palash, Dhak) and *B. Superb* (Bari okhat) from Madhya Pradesh. *Herbal Tech Industry* 2011; 23-28.

32) *The Wealth of India-Raw Materials*. PID, CSIR, New Delhi; 1988. 341-346.

33) Sehrawat A, Khan TH, Prasad L, Sultana S. *Butea monosperma* and chemomodulation: protective role against Thioacetamide-mediated hepatic alterations in wistar rats. *Phytomedicine* 2006; 13:157–163.

34) Shahavi VM, Desai SK. Anti-inflammatory activity of *Butea monosperma* flowers. *Fitoterapia* 2008; 79: 82–85.

35) Carey MW, Krishna MG. Antiinflammatory and analgesic activity of *Butea Monosperma* (Lam) stem bark in experimental animals. *Pharmacologyonline* 2007, 2: 88-94.

36) Mengi SA, Deshpande SG. Anti-inflammatory activity of *Butea frondosa* leaves. *Fitoterapia*1999; 70: 521-522.

37) Prashanth D, Asha MK, Amit A, Padmaja R. Anthelmintic activity of *Butea monosperma*. *Fitoterapia* 2001; 72: 421-422.

38) Zafar Iqbal, Muhammad L, Abdul J, Muhammad NG, Gilani AH. In vivo anthelmintic activity of *Butea Monosperma* Against Trichostrongylid Nematodesin sheep. *Fitoterapia* 2006; 77: 137-140.

39) Nidhi Sharma, Veena Garg. Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan induced diabetic mice. *Indian journal of Biochemistry and Biophysics* 2009; 46: 99-105.

40) Ramachandran S, Sridhar Y, Kishore Gnana Sam S, Saravanan M, Thomas Leonard J, Anbalagan N, Sridhar SK. Aphrodisiac activity of *Butea frondosa* Koen. ex Roxb. extract in male rats. *Phytomedicine* 2004; 11: 165–168.

41) Gurav SS, Gulkari VD, Duragkar NJ, Patil AT. Antimicrobial activity of *Butea monosperma* Lam. Gum. *Iranian Journal of Pharmacology & Therapeutic* 2008; 7: 21-24.

42) Habbu PV, Joshi H, Patil BS. Potential wound healers from plant origin. *Pharmacognosy Reviews* 2007, 1(2): 271-82.

43) Miriyala S, Panchatcharam M, Lochin S. Efficacy of *Butea monosperma* on dermal wound healing in rats. *The International Journal of Biochemistry & Cell Biology* 2005; 37: 566–573.

44) Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 2009; 80: 123–126.

45) Gunakkunru A, Padmanaban K, Thirumal P, Pritila J, Parimala G, Vengatesan N, Gnanasekar N, James BP, Sharma SK, Pillai KK. Anti-diarrhoeal activity of *Butea monosperma* in experimental animals. *Journal of Ethnopharmacology* 2005; 98: 241–244.

46) Bhuiyan MAR, Hoque MZ, Hossain SJ. Free radical scavenging activities of *Zizyphus mauritiana*. *World Journal of Agricultural Sciences* 2009; 5(3): 318-322.

47) Marian Naczk, Ryszard Amarowicz, Ryszard Zadernowski, Ronald BP, Fereidoon Shahidi. Antioxidant activity of crude phenolic extracts from Wild Blueberry leaves. *Pol. J. Food Nutr. Sci* 2003; 12/53(1): 166–169.

48) Sharma OP, Bhat TK. DPPH antioxidant assay revisited. *Food Chemistry* 2009; 113: 1202–1205.

49) Hassan Mahmood Kzar Jindal, Jamaludin Mohamad. Antioxidant activity of *Ardisia crispa* (Mata pelanduk). *Sains Malaysiana* 2012; 41(5): 539–545.

50) Kumar TS, Shanmugam S, Palvannan T, Bharathi Kumar VM. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. leaves. *Iranian Journal of Pharmaceutical Research* 2008; 7(3): 211-215.

51) Sushant Kumar Mondal, Goutam Chakraborty, Gupta M, Mazumder UK. In vitro antioxidant activity of *Diospyros malabarica* Kostel bark. *Indian Journal of Experimental Biology* 2006; 44: 39-44.

52) Francis MA, Andrew WV, Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. Journal *of Medicinal Plants Research* 2010; 4(24): 2479-2487.

53) Bibhabasu Hazra, Santanu Biswas, Nripendranath Mandal. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complementary and Alternative Medicine* 2008; 8:63.

54) Farhana Islam, Tasdique Mohammad Quadery, Sharmin Reza Chowdhury, Mohammad Abul Kaisar, Md. Gias Uddin, Mohammad A. Rashid. Antioxidant and cytotoxic activities of *Mussaenda macrophylla*. *Bangladesh Pharmaceutical Journal* 2012; 15(1): 69-71.

55) Hanane El Hajaji, Nadya Lachkar, Katim Alaoui, Yahya Cherrah, Abdellah Farah, Abdesslam Ennabili, Brahim El Bali, Mohammed Lachkar.. Antioxidant properties and total phenolic content of three varieties of Carob tree leaves from Morocco. *Rec. Nat. Prod* 2010; 4(4): 193-204.

56) Laetitia Meot-Duros, Christian Magne, Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiology and Biochemistry* 2009; 47: 37–41

57) Guddadarangavvanahally K. Jayaprakasha, Bhabani SJ, Pradeep SN, Kunnumpurath KS,. Evaluation of antioxidant activities and antimutagenicity of Turmeric oil: a byproduct from curcumin production. *Verlag der Zeitschrift für Naturforschung, Tübingen* 2002; 0939-5075:0900-0828.

58) Ruchi GM, Majekodunmi O Fatope, Ramla Al Mahrooqi, Varma GB, Hussain Al Abadi, Suad Khamis S. Al-Burtamani. Antioxidant capacity of some edible and wound healing plants in Oman. *Food Chemistry* 2007; 101: 465–470.

59) Virginia BC Junqueira, Silvia BM Barros, Sandra S Chan, Luciano Rodrigues, Leandro Giavarotti, Ronaldo L Abud, Guilherme P Deucher. Aging and oxidative stress. *Molecular Aspects of Medicine* 2004; 25: 5–16.

60) Yun-Zhong Fang, Sheng Yang, Guoyao Wu. Free radicals, antioxidants, and nutrition. *Nutrition* 2002; 18:872-879.

61) Jens Lykkesfeldt, Ove Svendsen, Oxidants and antioxidants in disease: oxidative stress in farm animals. *The Veterinary Journal* 2007; 173: 502–511.

62) Devasagayam TPA, Tilak JC, Boloor KK, Ketaki S Sane, Saroj S Ghaskadbi, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. *JAPI* 2004; 52: 794-804.

63) Hopps E, Noto D, Caimi G, Averna MR. A novel component of the metabolic syndrome: the oxidative stress. *Nutrition, Metabolism & Cardiovascular Diseases* 2010; 20: 72-77.

64) Vanessa Fuchs-Tarlovsky. Role of antioxidants in cancer therapy. *Nutrition* 2013; 29: 15-21.

65) Sofia Benfeito, Catarina Oliveira, Pedro Soares, Carlos Fernandes, Tiago Silva, José Teixeira, Fernanda Borges. Antioxidant therapy: still in search of the 'magic bullet'. *Mitochondrion* 2013; xxx–xxx.

66) Johanna W Lampe. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr* 1999; 70: 475S–90S.

67) Reşat Apak, Kubilay Güçlü, Birsen Demirata, Mustafa Özyürek , Saliha Esin Çelik, Burcu Bektaşoğlu, K. Işıl Berker, Dilek Özyurt. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; 12: 1496-1547.

68) Sudhakar Singh, Singh RP. In vitro methods of assay of antioxidants: an overview. Food *Reviews International* 2008; 24: 392–415.

69) Jeffrey B. Harborne, Herbert Baxter, Gerard Peter Moss. Phenolics. *Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants*, Taylor & Francis, London; 1998, p. 359.

70) Amit Kunwar, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health. *J Med Allied Sci* 2011; 1(2): 53-60.

71) Gzella AG, Dudek-Makuch M, Matławska I. DPPH radical scavenging activity and phenolic compound content in different leaf extracts from selected Blackberry species. *Acta Biologica Cracoviensia* 2012; 54(2): 32–38.

72) Gupta VK, Sharma SK. Plants as natural antioxidants. *Natural Product Radiance* 2006; 5(4): 326-334.

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