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Biochemistry

ESTIMATION OF ANTIDIABTIC, ANTIOXIDANT AND PHYTOCHEMICAL CONSTITUENTS OF TWO SPECIES OF BUTEA MONOSPERMA (PALASH)

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ABSTRACT Butea monosperma had been observing as potent, efficient, ethno medicine for various ailments in Indian traditional system of medicine. The present study was focus to investigate the antidiabtic, antioxidant potential and Phytochemical screening of Palash. We have selected two types of palash plant the Butea monosperma yellow flower show highest antioxidant activity and Butea Monosperma red flower show lowest antioxidant activity.

KEYWORDS :

INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder of multiple etiologists characterized by high blood glucose level with disturbance of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action or insulin insensitivity. It's a complex metabolic disorder, caused by high levels of glucose in blood cells, defective insulin production and action[1].Multifactorial syndrome is associated with dysfunctions and failure of various organs especially eyes, kidney, nerves, heart and blood vessels, which is not normally controlled and managed in precise form. Diabetic subjects can control diabetes and lower the risk of complications by modifying their life-style and by strictly maintaining their blood glucose levels[2].

Antioxidants are substance which helps to define the body against cell damage caused by various free radicals. Free radicals are unstable oxygen molecule containing unpaired electron[3]. Experimental and clinical research studies enforces that oxidative stress shows us much important steps to finalize the pathogenesis of both types of diabetes mellitus[4]. Oxidative accentuation an imbalance between the generation of reaction of oxygen species & antioxidant immunity capacity of the body is closely related with ageing & number of disease including cancer, cardio vascular disease, and diabetic problems[5]. In traditional medicine there are various of natural crude drugs that have potential to treat many disaster diseases and disorders, one of them is Butea Monosperma belong to family Fabacae and commonly known as older name is Leguminosae[6]. The main contribution of this plant includes flavonoids, triterpene, alkaloids, sterols, lipid, inorganic and proteins, which are responsible for biological and pharmacological activities such as anti-hyperglycemic, anti-tumorous, anti-cancerous, and anti-oxidant, wound healing activity, anti-malarial impunity. *Butea Mono- Sperma* is widely used in Ayurveda and has become a milestone of modern medicine race[7].

In the present study we have chosen 2 verity of Palash red and yellow Palash for estimate their Phytochemical constituents, antioxidant and antidabtic activity.

MATERIALAND METHOD:

CHEMICALS:

Methanol (HPLC grade), Water (HPLC grade), Tris-HCL, Folin & Ciocaltus,2,2-Diphenyl-1-picryl(DPPH), Sodium acetate, Querecrtin, Molish reagent, Wagner reagent, Lead acetate solution, aluminium chloride etc.

SAMPLE COLLECTION OF PLANT MATERIALS:

The plants parts of *Butea Monosperma* were collected in Chithara near Majhgawan Satna (M.P.) and identified. All the plant parts (leave, stem, Flower) were collected, first washed with fresh water and then washed with methanol and dried under shade room temperature separately. The parts were grinded coarsely and then powdered. Filtered through 120 no sieve and take an air tight container for further use.

PREPARATION OF PLANT EXTRACT:

20gm powdered sample extracted with 100 ml HPLC grade methanol through open air reflux at 40 0C for 6 hour. The extract thought filter paper (what man no-1) to remove free extractable substance. The filter of plant extract were evaporated at room temperature up to dryness and preserved at 4-5 0C for further process.

DETERMINATION OF TOTAL POLYPHENOLIC CONTENT:

Total Polyphenolic content of different part of plant extract was measured by using Folin - Ciocaiteu reagent. The 25µl of plant extract diluted with 125µl water followed by addition of 150µlof Folin-ciocalteu (1N) & 25µl of Na_2CO_3 (20% W/V) and incubated at 45℃ for 60 min then absorbance was measured by spectrophotometrically at 765nm (Bio Tek Synergy H4 multimode micro plate reader Bio Tek instrument, Inc Winoosci, VT, USA). Absorbance was recorded triplicates. Quantification was performed with respect to the standard curve of querecrtin (equation). Result was expressed as milligram of querecrtin equivalent per ml of extract[8].

DETERMINATION OF DPPH:

The assay for free radical DPPH was done by using 1-picrylhydrazyl (DPPH) method. In brief a 96-well micro plate, 30µl of various dilutions (10-100µg/ml) of Methenolic Extract 100µl of tris-HCL buffer (0.1M, Ph 7.4) and 150µl of DPPH solution (0.004%W/V in methanol) were added. The reaction mixture was shaken well. The DPPH decolourization was recorded at 517nm on Biotek synergy H4 hybrid multimode micro plate reader (Biotek instruments, Inc Winoosci VT, USA), after 30 min incubation in dark. The percentage of DPPH scavenging by plant extracts obtained in terms of ascorbic acid equation[9].

ALPHA GLUCOSIDASE:

α glucusidase inhibitory activity was performed following the method of Tripathi el al In brief Rat-intestinal acetone powder was dissolved in 4 ml of 50 MM ice cold phosphate buffer and sonicated for 6 min at 4°c. After vortexing for 20 minutes, the suspension was centrifuged (10,000 rpm, 4°c,30 minutes) and the resulting supernatant was used for the assay. A reaction mixture containing 50µl of phosphate buffer (50mM: Ph 6.8), 20µl of sample of varying concentration was pre-incubated for 5 min at 37°c, and then 25µl of 3Mm PNPG was added to the mixture as a substrate. After incubation at 37 °c for 30 min enzymatic activity was quantified by measuring the absorbance at 405 nm in a micro titer plate reader (Bio-TEK, USA), Acarbose was used as a positive control.

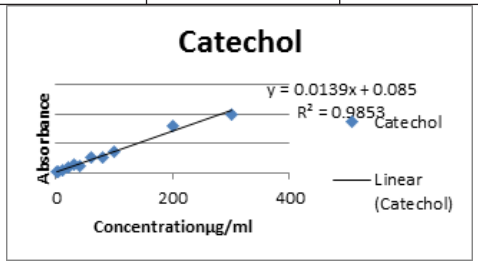
RESULT AND DISCUSSION:

It was reported that phenolic compound were associated with

antioxidant activity and play an important role in stabilizing lipid per oxidation. Total phenolic content of selected medicinal parts were shown in table 1.1 and graphical representation of standard curve of catechol for estimation of total Polyphenolic were shown in graph 2.1 . In this study DPPH scavenging activity of Palash plants was compared with standard ascorbic acid. Graph 2.2 representing graph of % inhibition of DPPH assay ascorbic acid. graph 2.3 and 2.4 show the % inhibition of DPPH aasay. Table show 1.2 show the IC_{50}value of selected plant and ascorbic acid. In this study alpha glucusidase activity of Palash plant was compared with Acarbose. Graph 2.5 show standard curve of Acarbose. table 1.3 show the IC_{50} value of plant and graph 2.6 and 2.7 show % inhibition of alpha glucusidase assay.

Table 1.1: Total Polyphenolic content of Palash Plant:

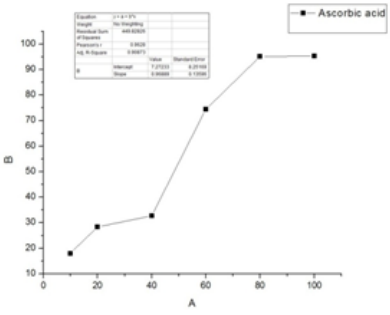
S.NO	Plant parts	T.P.C ug/ml
1.	P. yellow stem bark	1.46
2.	Leave	22.69
3.	flower	12.23
4.	p. red stem bark	1.15
5.	p. red leave	14.30
6.	flower	6.92



Graph 2.1: Standard curve of catechol

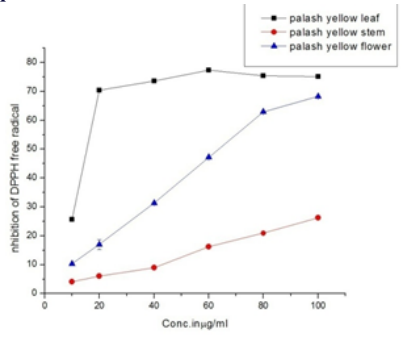
Table 1.2: DPPH Scavenging Activity of Palash plants:

S.No	Plant name	IC_{50}µg/ml
1.	Ascorbic acid	49.35
2	Palash red leave	15.61
3	stem bark	11.68
4	flower	63.39
5	Palash yellow leave	48.49
6	stem bark	34.26
7.	flower	12.12



Graph 2.2 : Standard curve of Ascorbic acid for DPPH.

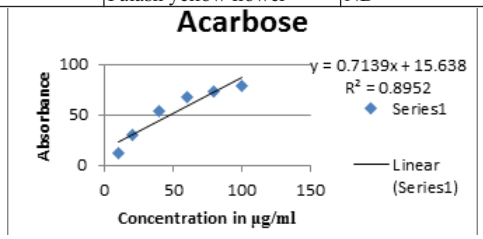
Graph 2.3: Percentage inhibitory curve of DPPH activity for *Butea monosperma*



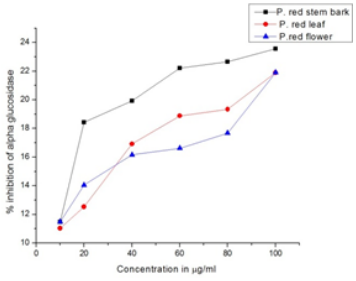
Graph 2.4: Percentage inhibitory curve of DPPH activity for *Var. Butea monosperma lutea*

Table 1.3: Alpha glucosidase activity of Palash plants:

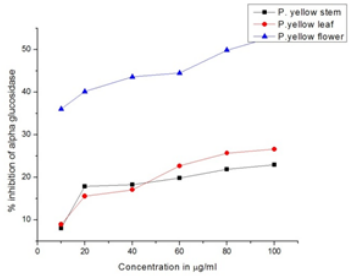
S.No.	Plant name	IC_{50} µg/ml
1.	Palash red leave	ND
2.	stem bark	ND
3.	flower	81.54
4.	Palash yellow leave	ND
5.	Palash yellow stem bark	ND
6.	Palash yellow flower	ND



Graph 2.1: Standard curve of catechol for Alpha glucosidase



Graph 2.6: Percentge inhibitory curve of Alpha glucosidase activity of Butea monosperma



Graph 2.7: Percentage inhibitory curve of Alpha glucosidase activity of *Var. Butea monosperma lutea*

CONCLUSION:

The present investigation demonstrates yellow Palash plant show better antioxidant activity as compared to red Palash. Highest Polyphenolic content was found in Palash red stem bark. Present study conclude that active principal present in palash (yellow and red part) have the capacity to inhibit the formation of free redicals in living oraganism.

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