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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIOXIDANT PROPERTY OF *BUTEAMONOSPERMA* (PALASH)

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Abstract

Buteamonosperma is a plant species of Lamnaceae family native to tropical and subtropical regions of India. *Buteamonosperma* is commonly known as Palash and found abundantly in Asian region. It possesses various therapeutic properties such as; anti-diarrheal, anti-dysenteric, astringent, purgative, anthelmintic and aphrodisiac activities. It is also used for making commercial timber, resin and dye, etc. Bark, flowers, leaves, and seeds are utilized for their medicinal properties. Considering the importance of this plant, the present study was planned to evaluate phytochemical and antioxidant properties of Palash. The study confirmed the presence of phyto-constituents responsible for the antioxidant property of the plant.

Key-Words: Plant, *Buteamonosperma*, Palash, Anti-oxidant

Introduction

Buteamonosperma is a plant of traditional value and possessing enormous medicinal properties. The bark of the plant is used for indigestion and also offers benefits in colitis. The plant acts as *Kaphapittashamak* thus utilized for treating many health ailments associated with vitiation of *Kapha* and *Pitta*. The constituents of the plant such as flavonoids and saponins

considered responsible for anti-inflammatory and antioxidant activities. Plant also used for pimples, haemorrhage, acts as an astringent, diuretic and helps to suppress pathogenesis of diabetes [1-5]. The Ayurveda property of plant is depicted in **Figure 1**.

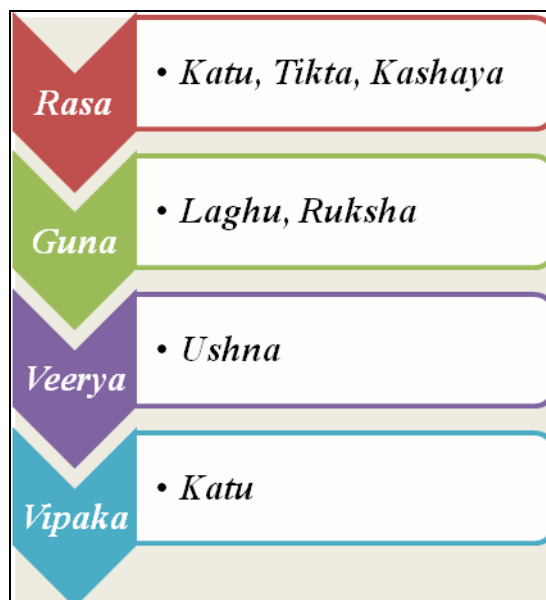


Figure 1: Ayurveda property of *Butea monosperma*

Botanical Descriptions:

The plant appears as long tree (**Figure 2**), can reach heights of 5 to 20 metres and trunk is crooked and tortuous with rough and fibrous bark that is greyish-brown in colour but exudes a reddish fluid.

The leaves are trifoliate; petiole is 7.5–20 cm long with little stipules. The leaflets are leathery, with terminal shaped having 7-8 pairs of lateral veins. Flowers are 5–40 cm long, towards the top on mostly leafless branchlets; calyx with campanulate tube and corolla 5-7 cm long.

Fruit a non-decomposing pod, stalked, with short brown hairs covering them, turning pale yellowish-brown or grey when ripe, with a solitary seed towards the tip, seeds are ellipsoid-shaped [4-7].



Figure 2: *Buteamonosperma* Tree

Scientific Classification of *Buteamonosperma* Lam

- Kingdom: Plantae
- Order: Fabales
- Family: *Fabaceae*
- Genus: *Butea*
- Species: *B monosperma*

Material and Methods:

Collection, Identification and Authentication of Plant Material:

The plant was collected from Indore districts and identified by Head department of Dravyaguna, Govt. Asthang Ayurveda College, Indore, (M.P.).

EXTRACTION:

The plant material cleaned and ground to powder or cut into small pieces, that after soaked in petroleum ether first for defatting with occasional shaking at room temperature for about 4-5 days.

Successive solvent extraction:

Soxhlet apparatus is used for the successive extraction of plant material, in this process powdered plant material was extracted sequentially with petroleum ether, chloroform and methanol.

Phytochemical Analysis of crude extracts:

The presence of specific phyto-constituents was confirmed by various tests described for phytochemical analysis. Different tests were conducted to perform qualitative analysis for the presence of Alkaloid, Flavonoid, Tannins, Steroids and Terpinoids.

CHROMATOGRAPHIC PURIFICATION:

Thin layer chromatography (TLC) was performed for eluting the constituent presence in extract. The methanol extract was concentrated by removing the solvent and dried. The origin of spot is commonly noted by drawing a thin line with a pencil across the bottom of the plate. The fraction of the sample was dissolved in a volatile solvent. A little amount of sample solution was applied to the plate using a glass capillary tube, confining the sample in small area. The spots were visualized in ultraviolet (UV) lamp on the TLC plate.

High-performance thin layer chromatography (HPTLC):

The HPTLC analysis was performed using different mobile phases and final mobile phase was selected on the trial basis analysis. The sample application was done using micro-injector and gradient elution technique was used for the separation of mixture present in plant extract.

Infrared spectroscopy:

IR spectroscopy was performed to check the functional group present in plant extract, in this regard KBr pellet technique was used for sample application and structure elucidation was performed on the basis of spectra obtained after the absorption of IR radiation by specific functional groups present in plant sample.

IN-VITRO ANTI-OXIDANT ACTIVITY:

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) technique was used to test the anti-oxidant activity of the fractions eluted during column chromatography.

Preparation of standard solution:

The required amount of ascorbic acid was dissolved in methanol to produce concentrations of 50, 100, 250, 500 and 750 µg/ml.

Preparation of test sample:

Sample stock solutions were made by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to obtain a concentration of 1 mg/ml.

Preparation of DPPH solution:

4.3 milligram of DPPH was dissolved in 3.3 ml methanol, and the test tubes were covered with aluminium foil to shield it from light.

Protocol for estimation of DPPH scavenging activity:

For the control reading, 50 µl of DPPH stock solution were added to 3 ml of methanol and the absorbance was measured at 516 nm immediately. Different concentrations of test sample (50, 100, 250, 500, 750 µg/ml) were screened, and 200 µl of each dose was prepared using methanol as solvent. Each test tube received a 50 µl of DPPH solution. After 20 minutes of incubation, absorbance was measured at 516 nm in a UV-Visible spectrophotometer (Systronics) using methanol as a blank. The following formulas were used to calculate the percentage reduction and the IC₅₀ value [7-9].

$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$
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Results and Discussion

The present study involves phytochemistry and pharmacology analysis of selected plants. The dried plant bark material was grinded using electronic grinder and extracted

consecutively with solvents of increasing polarity namely chloroform, petroleum ether and methanol in Soxhlet apparatus. The petroleum ether extract appears as dark brown and oily, methanolic extract possesses deep green and sticky appearance while green and sticky appearance observed with chloroform extract of plant. The various tests were performed for the phyto-chemical analysis of constituents present in plant extract and result of same is depicted in **Table 1**.

Table 2: Phyto-chemical analysis of plant extracts

Phyto-chemical	Petroleum ether extract	Methanol Extract	Chloroform Extract
Alkaloid	+	+	-
Flavonoid	-	+	+
Tannins	-	+	-
Steroids	-	+	
Terpinoids	-	-	+

Thin layer chromatography (TLC)

The result of separation of bioactive constituents from methanol fraction of *Butea monosperma* shows that in CHCl₃:MeOH (95:5) the residue with R_f value 0.67 and in CHCl₃:MeOH (80:20) the residue with R_f value of 0.37, while in other eluting solvents no residue was observed as depicted in **Figure 3**.

High-performance thin layer chromatograph (HPTLC)

The HPTLC analysis showed different retention time for various constituents present in *Butea monosperma* (Lam) extract; Alkaloids appeared at 4.771 min. in HPTLC graph, Flavonoids at 36.970 min., Terpenoids at 14.622 min., Steroids at 16.412 min. and the

retention time for Saponins was found to be 21.785 min. (**Figure4**). The peaks at various intervals confirmed presence of many phyto-constituents in plant extract.



Figure3: TLC Plate of fraction of *Buteamonosperma*

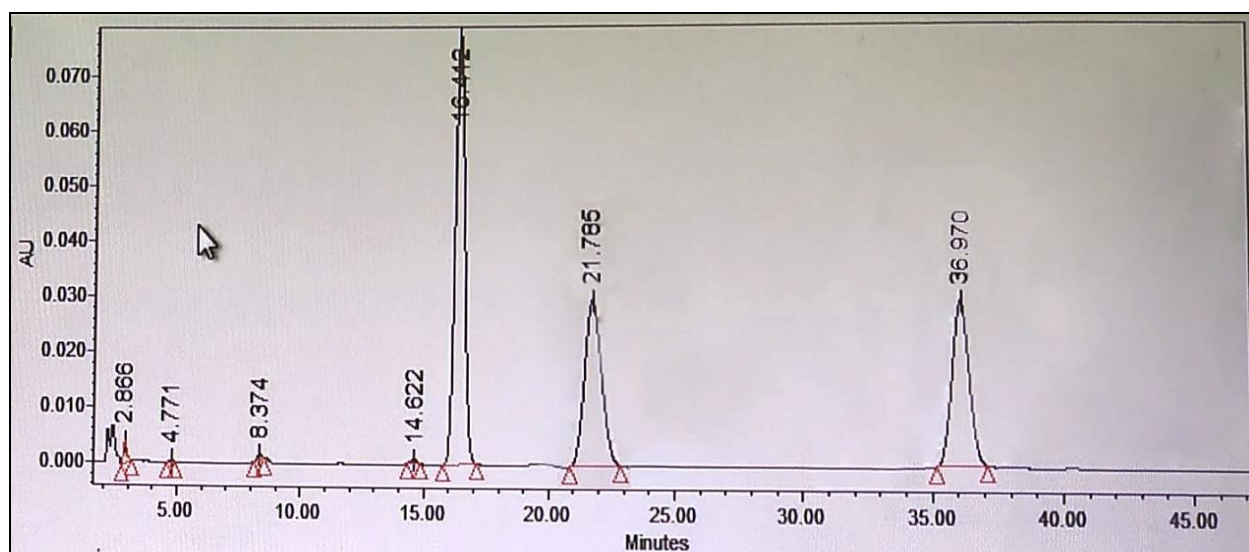


Figure4: HPTLC of *Buteamonosperma*(Lam.)

IR-Spectroscopy:

The IR spectra of plant extract confirmed presence of several functional groups including aromatic ring, carbonyl group and hydroxyl group, etc. The in plane C-H bending vibration was observed as 932 cm^{-1} , peak at 3474 cm^{-1} observed for O-H stretch, 2926 cm^{-1} for -C-H stretch of aromatic ring, 1622 cm^{-1} (-C=C stretch) 1478 cm^{-1} (-O-H deformation vibration) and peak at 1274 cm^{-1} observed for -C-O carbonyl stretch (**Figure 5**).

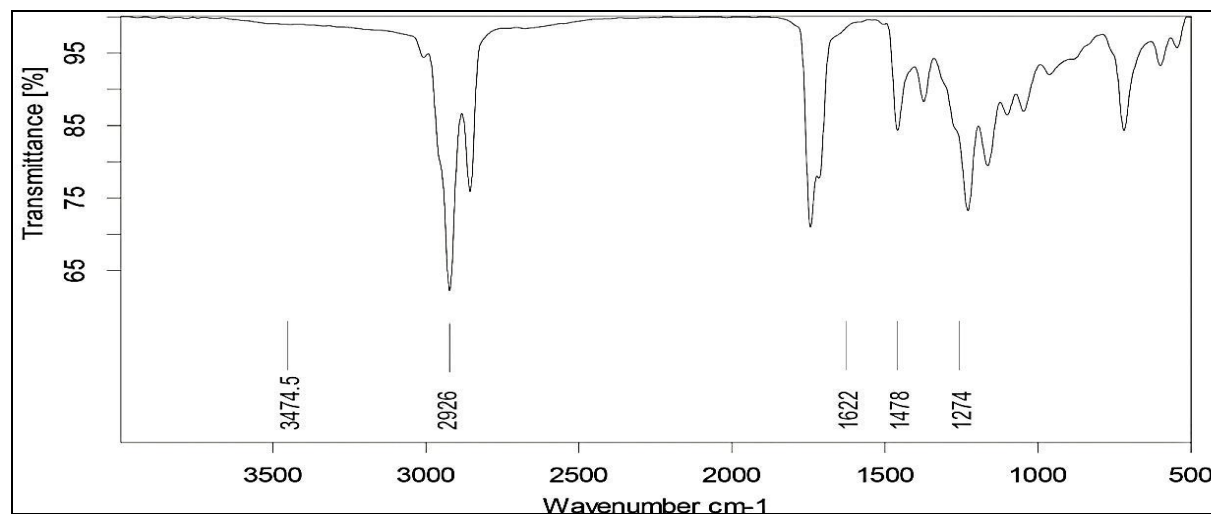


Figure5: IR spectrum of isolated compound of *Buteamonosperma* Lam extract
INVITRO ANTIOXIDANT ACTIVITY

The DPPH radical scavenger analysis of plant extract was performed using ascorbic acid as standard and an IC_{50} value was recorded with non linear regression analysis. The higher percentage inhibition of DPPH and lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH radical scavengers. Highest percentage inhibition and lower IC_{50} value was found in methanolic extract. The IC_{50} value was found to be $478.41\mu\text{g/ml}$ for methanol extract. The DPPH radical scavenging activity was checked at different concentration levels (50, 100, 250, 500, $750\mu\text{g/ml}$) and more than 50% inhibition was observed at concentration level of $500\mu\text{g/ml}$ (**Figure 6**). The IC_{50} value was calculated at $478.41\mu\text{g/ml}$.

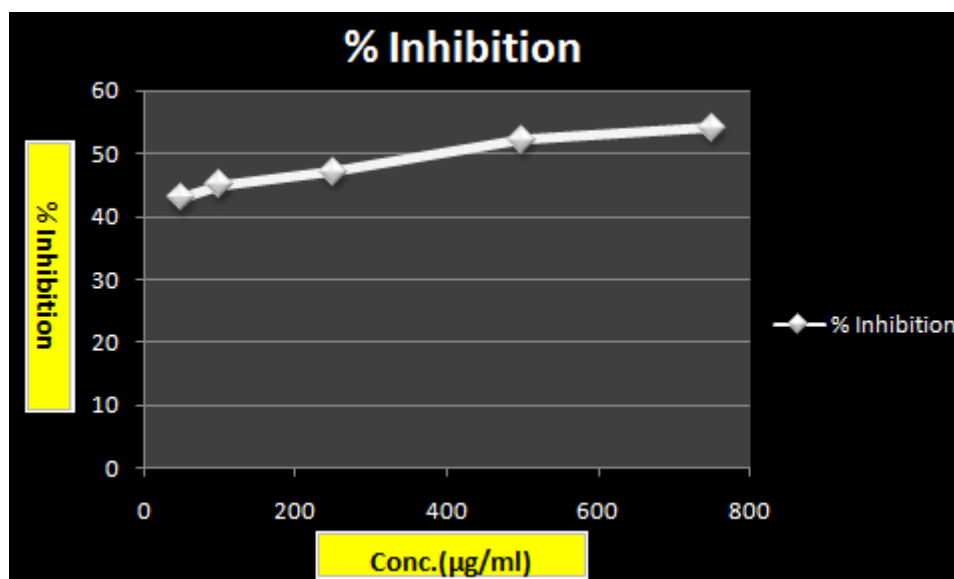


Figure6: DPPH inhibition curve of methanol extract of *Buteamonosperma*

Discussion

The methanol extract of *Buteamonosperma* confirms the presence of saponins, terpenoids and flavonoids. HPTLC results confirm the presence of natural products saponins and terpenoids. The constituents present in the bark of *Buteamonosperma* are flavonoids and saponins, which contributed remarkably towards the antioxidant activity of plant extract. The antioxidant activity was observed in dose-dependent manner and study confirmed presence of phyto-constituents responsible for antioxidant property of plant [10-12].

Conclusion

Buteamonosperma is commonly known as Palash and found abundantly in Asian region. It possesses various therapeutic properties such as; anti-diarrheal, anti-dysenteric, astringent, purgative, anthelmintic and aphrodisiac activities. Plant contains alkaloids and flavonoids. The HPTLC analysis showed presence of several phyto-constituents including terpenoids, steroids and saponins, etc. The peaks at various intervals confirmed presence of many phyto-constituents in plant extract. The finding of anti-oxidant activity revealed more than 50% inhibition of free radicals at concentration level of 500 µg/ml of plant extract. The IC_{50} value was calculated at 478.41 µg/ml. Low IC_{50} value

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ensure potent anti-oxidant capacity of plant extract. The Flavonoid contents in *Buteamonosperma* can be considered as responsible for antioxidant activity.

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