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PHARMACOGNOSTICAL, PHYTOCHEMICAL, HPTLC AND ETHANO-BOTANICAL STUDY OF *BAUHINIA PURPUREA* L. POD

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Keywords:

Bauhinia purpurea L. Pod, Microscopy, Physicochemical analysis, HPTLC, Phenolic, Flavonoids *etc*

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ABSTRACT: Background: *Bauhinia purpurea* L. is a flowering plant belongs to family Leguminosae, also known as purple orchid plant. All parts of this plant including leaves, stem bark, flower and pods are edible and used for various medicinal purposes. Detailed pharmacognostic profiling of various parts has been performed except plant Pod, thus pod was selected for this study. **Objective:** The purpose of this work was to enrich the research on *B. purpurea* L. Pod in respect of pharmacognostical, physicochemical, qualitative identification of secondary metabolites by HPTLC and ethno-botanical profiling. **Material and Methods:** Entire pod and powder were subject to macro and microscopic study, different physico-chemical parameters including ash, extractive, moisture and pH determined through standard methods with analytical grade chemical and standard equipments. Quantitative estimation of phenolic content was performed by well-known Folin-Ciocalteu method and HPTLC developed through Vision cat software. **Result:** Fresh pod is green, elongated with sub-orbicular seeds. TS show different parenchymatous arrangement with yellow brown tannin content and Ca-oxalate crystals. Alkaloids, tannin, phenolic and flavonoids were reported in the hydro-methanolic extract through phytochemical screening. Consequently, total phenol and flavonoid were found to be 490.62mg/g and 235.28 mg/g, respectively. Moreover, in HPTLC, R_f values of tested extract were reported between 0.2 to 0.72. **Conclusion**: Reported Ethano-botanical values of different parts of this plant were denoted the range of its therapeutic importance in indifferent tribal races. *B. purpurea* L. is a medicinal remedy generally known as Orchid tree. Based on our research pod of this plant has phyto-constituents including alkaloids, phenols and tannins responsible for therapeutic values. Subsequently, HPTLC of hydro-methanolic extract also proved the presence of different compounds which might be support the ethano-botanical utilization of this part of plant. Hence this study would be assist for the identification, standardization of plant pod collected from various geographical area and suppliers in the authentication.

INTRODUCTION: Nature provides a great gift in the form of plants to the human being to have a healthy and disease free life. It plays a tremendous role to our health. India is one of the most popular medico-cultivating countries in the whole world where more than 2000 medicinal plant have been recognized as well cultivated. Consequently, it is noticeable that over 60% of pharma products have been derived from plants, due to their gentle impact on the human and domestic animal body, insignificant adverse effects, cost-effective production, and easy accessibility [1, 2].

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The genus *Bauhinia*, family Leguminosae (Sub-family: Caesalpinioideae), consists 300 species all over the world where around 15 species reported in India as tree, shrubs or few as climber [3]. It is indigenous to South China and India but naturally distributed in Nepal, Bhutan, Pakistan, Shri Lanka, Myanmar and Thailand also. It is mounted at 1300 m altitude in Himalaya but rare in southern region of India [4, 5]. Moreover, it is found in various regions of USA including Hawaii, Coastal California, Southern Texas and Southwest Florida. Orchid tree is a deciduous, tropical climate loving and fast growing tree which is used in abundance for the medicinal purpose by numerous tribes of India [6, 7]. It is name "*Bauhinia*" and bi-lobed leaves exemplified the two brothers Swiss Botanists Jean (1541-1613) and Gaspard (1560-1624) while "*Purpurea*" refers to the purple colour of flower [8].

At present situation, the consumption of herbal medicine is increased either as raw, extract or in the form of formulations have become a great challenge to supply the enough quantity of raw material to the pharma industries to fulfil the demand that aroused the interest for searching new alternative plant or plant parts to overcome this problem [9]. Therefore, ethano-medicinal and folklore utilization drowse the attention of researcher and scholars towards identification of phyto-constituents and their multiple biological responses [10]. Indian tribal and non-tribal people of different regions use the plant pod and seeds as tonic and to overcome the problem of libido. Unripe pod and seed are cooked and eaten by Kathkors and Gondas tribes of India [5, 6]. Moreover, it has been used for different pharmacological activities such as management of gentamicin induced nephrotoxicity and high fat diet induced hyperlipidaemia. The bioactive reported compounds of pod showed anticancer activity by inhibiting the P-288 cancer cell line [3].

MATERIALS AND METHOD:

Materials: All Chemicals, reagents and glasswares used were of analytical grade for extraction, test and for the TLC profiling of plant extract. Trinocular Olympus Microscope with attached camera and drawing tube (Olympus, BX43F), Hot air oven (Yorca, India), Water bath (Sanjay Steel FAB. Works, WBPC60), Muffle Furnace (Narang Scientific Work PVT. LTD, New Delhi, India), Analytical Weighing Balance (Saffron), TLC plate Silica gel60F_{254} (Merck, Germany), Glass capillaries, HPTLC (CAMAG, Muttenz, Switzerland), autosampler (Camag, Linomat-5), TLC chamber (Camag), Visualizer (CAMAG TLC-Visualizer-2).

Collection and Plant Authentication: The unripe fruit pod of *B. purpurea* L. was collected in the end of winter session (February-March, 2022) from Gulmohar city, New Collectrate, Gwalior, Madhya Pradesh. Collected sample was authenticated by the Botanist with standard texts and flora. The collected plant specimen was dried and herbarium was prepared and submitted to EMBS section, RARI with the Boucher no. RARI/PCOG/2022/001.

Method:

Macroscopic Study: The macroscopic study of plant pod was carried out though measurement of various sensorial features including size, shaped, colour, odour, taste, smell, texture and fractures *etc* [1, 11].

Microscopic Study: Free hand, uniform and clear transverse sections of *B. purpurea* L. pod was taken and mounted on slide with the help of 50% glycerine and cover slip and then viewed under 10 X and 40 X. The microphotographs were taken through Olympus microscope attached with Magnus MIPS camera [1, 11, 12].

Powder Analysis: The shade dried powder of *B. purpurea* L. pods was passed through sieve No. 60. Sieved sample was boiled in bleaching agent to remove the colouring matter from the plant tissue for better characterization and viewed under microscope for cellular content and other characters [12].

Phyto-chemical Study: The powder sample of plant pods was successively extracted in a Soxhlet apparatus using different solvent as per the increasing polarity index, starting from Pet. ether followed by Benzene, chloroform, ethyl acetate, methanol and aqueous (chloroform:water-1:99). The extracts were filtered, evaporated and concentrated. The concentrated extracts were then subjected to various qualitative tests to determine the presence of different phytochemicals including alkaloids, glycosides, phenolic and tannins,

flavonoids, steroids, saponins, carbohydrate, and fixed oil by using standard procedure [12].

Physicochemical Study: Physicochemical study of *B. purpurea* L. pod such as loss on drying (LOD), total ash, acid insoluble ash, pH of 5% aqueous solution, water and ethanol soluble extractives were carried out as per the standard procedure [12, 13].

Foreign Matter: Raw drug sample was weighed 100g for determining the foreign matter and spread out in thin layer. The foreign matter was detected by inspection with the unaided eye and subsequently by using of a magnifying glass lens (6X), isolated and weighed. The percentage foreign matter present was calculated in respect of raw sample [13].

Moisture Content: The 10g of pod pieces were placed in a tarred dish (accurately weight within 0.01 g) to carry out the moisture content. After placing the drug in dish was dry at 105°C for 5 h and weigh. Drying and weighing was continued at one hour interval until difference between two successive weighing corresponds to not more than 0.25 %.

Constant weight was reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show more than 0.01 g difference [12, 13].

Extractive Value: Accurately, 4 g coarsely powder of pod was placed in a stoppered glass conical flask and macerated with 100 ml of suitable solvents such as water, ethanol and ether (depending upon the fixed oil content), shaking frequently and allowed to stand for 24 hrs., filtered through Whatman No. 42 filter paper and 25 ml filtrate was transferred to the pre-weighted petri-plate and evaporated and dried at 105℃ till constant weight, kept in desiccators and weighed immediately. Percentage extractive value calculated in respect of air dried material [13].

Ash Value: Total ash residue remains after incineration was determined by spreading 4gof the powdered drug in a tarred silica crucible as a fine layer and kept for the incineration at a temperature not exceeding 600 °C until free from carbon content. The crucible was cooled and weighed. This procedure was repeated till constant weight was observed. The percentage of total ash was determined in triplicate with reference to the air dried drug. The similar procedure was performed for acid insoluble ash by dissolving and boiling the total ash into 25 ml of dil. hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper by filtration and it was washed with hot water. Further procedure of incineration, cooling and weighing, calculation of percentage of acid insoluble ash was similar as total ash [12, 13].

pH Value: The pH value of *B. purpurea* L. was performed by dissolving 5g of powdered drug in 100 ml of water and kept the mixture for overnight with vigorously shaking. Filtered through filter paper and pH was determined with help of previously calibrated pH-meter [13].

Determination of Secondary Metabolites:

Phenolic Content: The quantitative estimation of total phenolic content in pod hydro-methanolic extract was done by Folin-Ciocalteu reagent using Gallic acid (100µg/ml) as standard. The calibration curve of standard solutions was developed at 200,300,400,500,600,700,800 µg/ml concentrations respectively. 1ml of hydro-methanolic extract of *B. purpurea* L. pod, with the strength 10 mg/ml, was added to 10 ml volumetric flask containing 3 ml of distilled water, 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min 3ml of 7% sodium carbonate was added to the mixture and the solution was diluted to 10ml with distilled water. After incubation for 30 min at room temperature the absorbance against prepared reagent blank was determined at 765nm. The content of total phenols was calculated as Gallic acid equivalents in mg/g of dry extract [14, 15].

Flavonoid Content: The aluminium trichloride colorimetric method was used for the determination of flavonoid content. 1ml of plant extract (100µg/ml) and 2ml of distilled water were taken in 10ml of volumetric flask. After 5 min 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride were added. After 6 min, 2 ml of 1M sodium hydroxide was added and the volume made up to 10 ml with water shaken vigorously. Absorbance was recorded at 510nm after 30 min of incubation. The standard calibration plot was drawn by using known concentration of quercetin (200-800µg/ml) and total flavonoid content was

expressed as quercetin equivalent in mg/g sample [14, 16].

High Performance Thin Layer Chromatography (HPTLC): HPTLC finger printing of methanolic and hydro-methanolic extract of *B. purpurea* L. pod was performed by using CAMAG (Muttenz, Switzerland) HPTLC system, comprising a Linomat 5 automatic applicator with a 100 µL syringe, a twin trough plate development chamber, Camag TLC scanner and VisionCATs software. Suitable solution 7.5 μL of sample were spotted in the form of band on precoated silica gel 60 F_{254} HPLC plate (10×10 cm, Merck, Mumbai India), 10 mm from the bottom. After application plate was developed vertically ascending ina twin trough glass chamber (Camag, Switzerland) saturated (at room temperature °C) with Hexane: Ethyl acetate: Chloroform: Formic acid as mobile phase in 4:4:1.5:0.2 ratio for phyto-constituents. The chromatographic run length was 90 mm from the bottom edge of the silica gel plate. Sequential to the development, Plate was dried in hot air oven for 5 minutes at 45°C to remove mobile phase. Later on, densitometric scanning was performed with a TLC scanner equipped with VisionCATs software in reflectance absorbance. Developed TLC plate and post-derivatized (derivatizing agent anisaldehyde-sulphuric acid reagent and subsequently band developed at 101°C on hot plate) plate were scanned under 254, 366 nm UV light and white light in Camag TLC scanner and calculate the R_f value. The images captured at the same wave length [17, 18, 19].

RESULT:

Organoleptic and Macroscopic Study: The elongated dehiscent pods were green when fresh and brown after drying; strap shaped, un-separated, glabrous, 29.5-22.2 cm long and up to 2.7-2.3 cm wide, twists as open. Each pod contains about 12-15 sub-orbicular, flattened, nearly 1.1-1.4 cm wide and shiny brown to dark brown seeds. The odour of powdered raw material of pod was faint and characteristic while bitter, astringent with slightly slimy in taste **Fig. 1.**

FIG. 1: PHOTO-MACROSCOPIC IMAGE OF *B. PURPUREA* L. POD A. YOUNG GREEN POD; B. SHADE DRIED FRAGMENTS OF POD, C. POWDER OF POD (RIGHT-LEFT)

Microscopic study: The transverse section of ventral suture of *B. purpurea* L. pod showed perisperm where exocarp consists of epidermis made up straight wall cuboidal, rectangular and brick shaped parenchyma cells with few multicellular, curved covering trichome.

Followed by epidermis 12-14 layered thin-walled, round to oval shaped mesocarp cells are arranged with intracellular space. Mesocarpcells contain yellowish-brown content of tannin and large rosette crystals. Thick, 8-12 layered, compactly arranged lignified polygonal cells of bundle cap separate the mesocarp from the vascular part of endocarp which is thicker at ventral suture and covered the fiber cap cells (FCC), but separate at the lower side through abscission layer consists of collapsed parenchymatic cells that made zone of dehiscence (ZD).

Both mesocarp layer and zone of dehiscence help pod dehiscing due to lignification at the ripening stage. Vascular bundles arranged in a parallel manner just below the thin-walled, without intracellular space polygonal endocarpic cells (EC) filled with tannin content and crystals where meta-xylem facing outward while pro-xylem present toward the centre. Xylem cells also contain the

brown content of tannin. Both cotyledon of seed separated through the thin layer of flat of brick shaped, loosely arranged perispermic parenchyma. Mesospermic parenchyma fused with oval shaped thick walled Collenchymatous endospermic with narrow lumen **Fig. 2.**

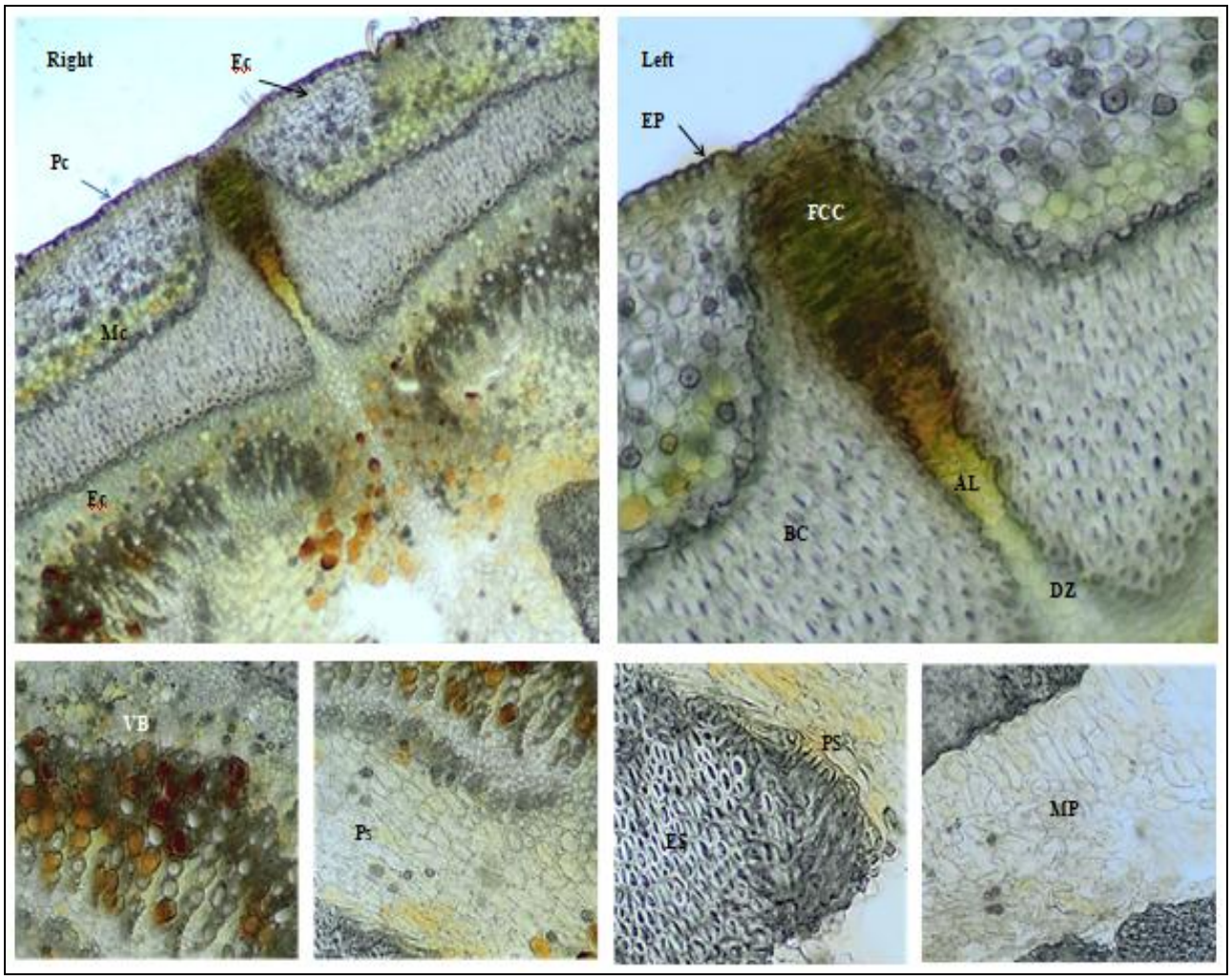

FIG. 2: SEMI-THIN TRANSVERSE SECTIONS OF VENTRAL SUTURE OF A *B. PURPUREA* L. POD, SHOWING LAYER OF PERICARP (PC) FOLLOWED BY EXOCARP CELLS, THICK AND TIGHTLY ARRANGED MESOCARP (MC), ENDOCARP (EC) CONSISTS OF PARENCHYMA CELLS (PC) AND VASCULAR BUNDLE (VB) IN THE RIGHT IMAGE. SINGLE LAYER OF EPIDERMIS (EP), HEAVILY THICKENED SECONDARY WALLS (ON OUTER PORTION) OF THE FIBER CAP CELLS (FCC), ABSCISSION LAYER (AL), DEHISCENCE ZONE (DZ) AND BUNDLE CAP (BP), PERISPERM (PS), FUSED MESOSPERM (MS), ENDOSPERM (ES) AND INTER COTYLEDON THIN WALLED BRICK SHAPED MEMBRANOUS PARENCHYMA CELLS (MP)

Powder Microscopic Study: Pod powder was yellowish to light green in color with un-identifiable odor and slightly warm mucilaginous in taste. It revealed the powder characteristics of plant tissues such as thin walled parenchyma, thick-walled lignified parenchyma stored yellow colored food substance in the form of aleurone grains, loose as well as compactly arranged thin wavy walled or thick straight walled parenchyma cells and membranous, thin-walled with vertical fine lines irregular or somehow rectangular parenchyma cells and straight, thick-wall compactly arranged polygonal, irregular shaped sclerenchymatous cells and thick walled collenchyma cells which are angular shaped. Fragments of epidermal multicellular trichome, spiral vessels, pitted vessels, both straight and pitted lignified thick-walled tracheids, thick short and thin long tapered fiber, sclereids, Starch grain, group of aleurone grains and oil drops, yellow tannin content were present. Different types crystals such as rosette crystals which are present abundantly, prismatic and rod shaped crystal were also observed **Fig. 3.**

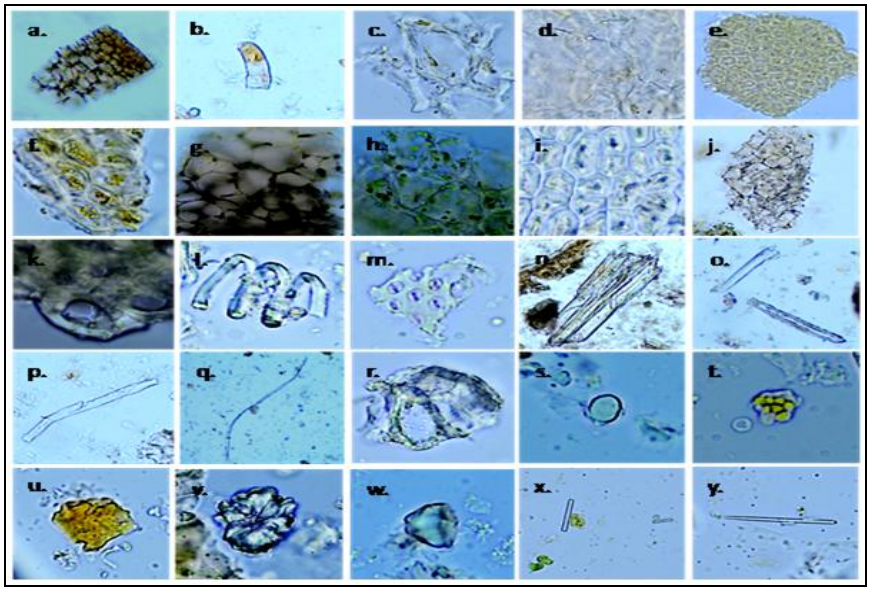

FIG. 3: POWDER MICROSCOPIC IMAGES OF PLANT *B. PURPUREA* L. POD, A. EPIDERMIS WITH EXOCARPIC CELLS; B. FRAGMENT OF MULTICELLULAR TRICHOME; C. LIGNIFIED ANGULAR THICK-WALL PARENCHYMA CELLS WITH INTRACELLULAR SPACE; D. MEMBRANOUS, THIN-WALLED WITH VERTICAL FINE LINES IRREGULAR OR SOMEHOW RECTANGULAR PARENCHYMA CELLS; E. STRAIGHT, THICK-WALL COMPACTLY ARRANGED ANGULAR AND IRREGULAR SHAPED SCLERENCHYMATOUS CELLS; F. LIGNIFIED CELLS FILLED WITH YELLOW CONTENT; G. LARGE ROUND OR ANGULAR, SLIGHTLY THICK WALLED WHERE ROD SHAPED CRYSTAL EMBEDDED PARENCHYMA CELLS; H. WAVY WALLED LARGE AND LOOSELY ARRANGED CHLORENCHYMATOUS MESOCARP CELLS; I. LIGNIFIED STRAIGHT ANGULAR PARENCHYMA CELLS; J. THIN WALLED STORAGE PARENCHYMA CELLS; K. FRAGMENT OF ANGULAR THICK WALLED COLLENCHYMA CELLS; L. LOOSE SPIRAL VESSELS; M. FRAGMENT OF PITTED VESSELS; N. LIGNIFIED THICK-WALLED TRACHEIDS; O. SINGLE PITTED TRACHEID; P. PIECE OF THICK FIBER; Q. THIN LONG TAPERED FIBER; R. SCLEREIDS; S. STARCH GRAIN; T. GROUP OF ALEURONE GRAINS AND OIL DROPS; U. YELLOW TANNIN CONTENT; V. ROSETTE CRYSTAL; W. IRREGULAR SHAPED PRISMATIC CRYSTAL; X&Y. ROD SHAPED OR SOMEHOW NEEDLE SHAPED CRYSTAL.

Phyto-chemical Screening: The preliminary qualitative analysis of phyto-constituents was tested in the plant pod extract. The hydro-methanolic extract of plant pod achieved through the cold maceration of pod powder at room temperature and confirms the presence of alkaloids, tannin, phenolic compounds, fixed oil and primary metabolites as carbohydrate and protein **Table 1.** Presence of bioactive secondary metabolites compounds exhibits the medicinal value of the flora. Mostly, tannins and phenolics emulate the antioxidant and anti-microbial effects of extract. These properties of plants grave the great interest in the field of neutraceutical technology and research activity instead of processed or synthetic antioxidant and antimicrobials [20].

TABLE 1: RESULTS OF PHYTOCHEMICAL SCREENING OF HYDRO-METHANOLIC EXTRACT OF *B. PURPUREA* L. POD

S. no.	Test	Observation	Result
1.	Alkaloids		
	Hager's test	Yellow color ppt.	Reveals alkaloids
2.	Glycosides		
	Sodium hydroxide test	-	Absence of glycosides
3.	Saponin Glycosides Foam test	-	Absence of saponins glycosides
4.	Tannins and Phenolic compounds		
	Neutral ferric chloride test	Bluish-black color	Indicates tannin and Phenolic compounds
5.	Flavonoids		
	i)Shinoda test	Magenta color	Flavonoid present
	ii) Lead acetate test	Yellowish-white ppt.	Indicates flavonoids
6.	Steroids and tri-terpenoids		
	Liberman test	Reddish-brown	Reveals steroids

7.	Coumarins		
8.	Fixed oil and fats Spot test	Left a spot on filter paper	Present
9.	Carbohydrates Molisch's test Reducing sugar test Benedicts test	Purple color ring Bluish-green ppt.	Carbohydrate confirm Indicates reducing sugar
10.	Gums and Mucilage's		
11.	Protein and amino acids Biuret test.	Purple color	Protein confirmed

Chemical Analysis of *B. purpurea* L. Pod Powder: The result reveals the powder behavior of *B. purpurea* L. pod with different chemicals and their inference in **Table 2.**

TABLE 2: BEHAVIOR OF POD POWDER WITH DIFFERENT CHEMICALS WITH COMPOUND IDENTIFICATION

Test	Observation	Inference
Powder + Picric acid	Yellow color	Presence of alkaloids
Powder + Con. H_2SO_4	Reddish brown color	Reported steroids
Powder + Aqueous $Fecl_3$	Green inflorescence	Flavonoid confirmed
Powder + Iodine solution	Blue color	Presence of starch
Powder + NaOH	Light Yellow color	Flavonoids confirmed
Powder + aqua. $AgNO_3$	White ppt	Presence of protein

Physicochemical Study: The physicochemical analysis of pod powder of *B. purpurea* L. showed loss on drying with the value 4.48% w/w (shade dried pod), total and acid insoluble ash content 3.62 and 0.19% w/w. The extractive range of methanol and water were found 16.03 and 13.75, respectively. However, the pH value of 10% aqueous was reported with the value 6.83 at 26.3°C.

TABLE 3: PHYSICOCHEMICAL STUDY OF *B. PURPUREA* L. POD POWDER

S. no.	Name of the parameters	Value (%) w/w
1	Description	Light creamy green
2	Foreign matter	Less than 1.0
3	pH(10% w/v aq. solution)	6.83 at 26.3°C
4	Loss on drying at 105°C (Shade dried pod)	4.48
5	Total ash	3.62
6	Acid insoluble ash	0.187
7	Ethanol soluble extractive	16.03
8	Water soluble extractive	13.75

Phenolic and Flavonoid Content: The total phenolic and flavonoid contents for hydro-methanolic extracts of *B. purpurea* L. pod were found to be 490.62 mg/g equivalent to Gallicand235.28mg/g equivalent to quercetin, respectively **Fig 4.**

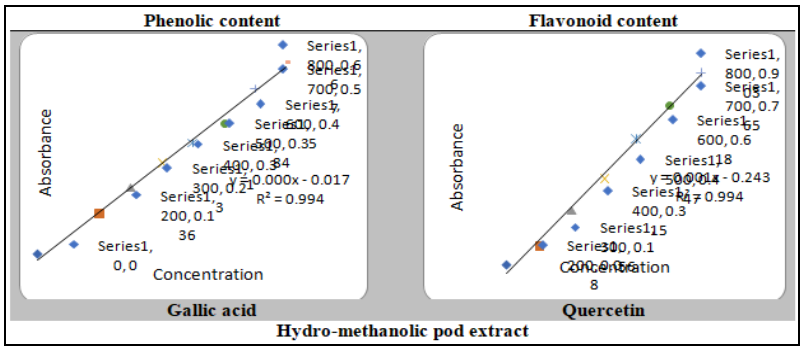

FIG. 4: STANDARD CURVE OF GALLIC ACID (LEFT) AND QUERCETIN (RIGHT) WITH REGRESSION COEFFICIENT WERE DEVELOPED FOR TOTAL PHENOLIC AND FLAVONOID CONTENT OF HYDRO-METHANOLIC *B. PURPUREA* L. POD EXTRACT

Thin Layer Chromatography finger printing: The chromatographic fingerprinting of hydro-methanolic extract has shown better in the developed mobile phase with the R_f value 0.07, 0.3, 0.34, 0.71 at 254 nm and 0.05 (blue), 0.22 (light purple), 0.31(purple), 0.34 (orange), 0.45 (brown), 0.55, 0.62 (reddish orange) at 366 nm, respectively. Subsequently, TLC plate was sprayed with anisaldehyde- sulphuric reagent followed by heating and then visualized in white light which showed 3 and 5 prominent bands in methanolic and hydro-methanolic extracts, respectively with the R_f value 0.06, 0.09 (light yellowish green), 0.2, 0.3 (grey), 0.37 (pink), 0.46 (blue), 0.56 (grey), 0.65 (red), 0.72 (light blue) in white light, respectively **Fig. 5.**

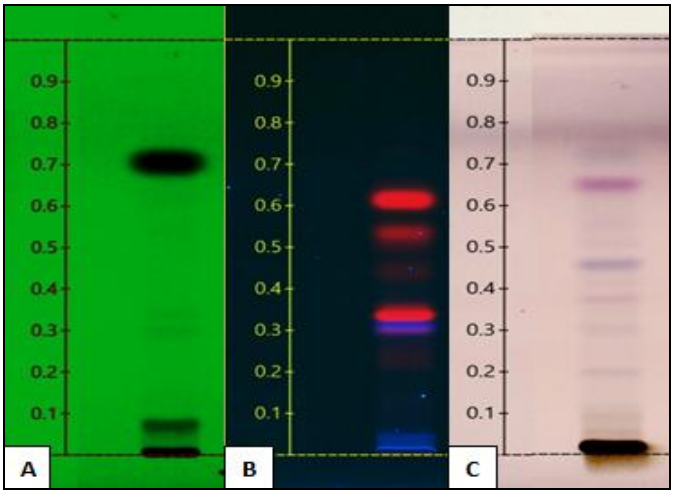

FIG 5: TLC PROFILE OF THE HYDRO-METHANOLIC POD EXTRACT OF *B. PURPUREA* L. POWDER; A). PLATE DEVELOPED UNDER UV-254 NM; B). PLATE DEVELOPED UNDER UV-366 NM; C). DERIVATIZED PLATE UNDER WHITE LIGHT

Ethano-medicinal uses of Different Parts: The tribal people are belief that disease and death caused by certain spirits or supernatural power and this belief affect their attitude and persona about the ailments.

In general, this plant species used as folk medicine by various Indian tribal communities for the management and treatment of different health related issues. *B. purpurea* L. is known by various names by different tribes such as Bhil (*Kanchana*), Santhals (*Baper*), Lodhas (*Kochner* or *Sing-ara*) and Mundas (*Sapidanka*) *etc* [21].

Although, it is used as folklore medicine in various country but there is no folk use as well traditional use data available in Malaysian region [5] (Kumar *et al.* 2011). Few researchers have been observed that unripe pod ethanolic extract showed protective effect against Gentamicin (GM)-induced nephrotoxicity at a dose 300 mg/kg/d. It also normalized the GM-induced increase level of serum creatinine, and uric acid level in rats [22].

Similarly, unripe pod extract also found effective against Cisplatin-induced nephrotoxicity in rats at 200-400 mg/kg in dose dependent manner. Moreover, it also increased CAT and GSH and decreased the MDA level in the Cisplatin-induced nephrotoxic group which depicts its antioxidant property [23]. Different Ethano-medicinal importance of *B. purpurea* L. has mentioned in the given **Table 4.**

TABLE 4:

Plant Parts	Ethno-medicinal uses
Whole plant	In south-East Asia various parts of are used as poultice to reduce swelling, bruises and to ripen the ulcerations and boils.[6] Plant decoction taken orally for the treatment of fever, diarrhea and dysentery.[24] The whole plant is preferred for the management in dropsy, pain, rheumatism, convulsions, delirium and septicemia.[3] Naga tribes are preferred this plant as an antidote to certain toxins and poisons.[21, 25]
Root	Root is work as carminative while infusion of small pieces of root is given in the management of white spot on skin [26, 27]. Dried root powder is given with water by Oraons tribes to treat rheumatism while Mundas tribes topically used dried root powder with mustered oil in equal ration as balm on cuts and wounds[28]. Bhoxa use the bark as an astringent to treat diarrhea.[21]
Root bark	Root bark past mixed with rice water (3:1) for ripening of boil is preferred by Lodhas tribes[21]. Root bark with curd is preferred for hemorrhoids while its paste with dried ginger given internally to treat goiter.[5]
Stem	In Assam region, Khasi tribes and non-tribal are used its stem for healing of bone fracture.[21]
Stem bark	The pounded stem bark is given by Lodhas people to cure the rheumatism problem[21]. Mundas tribe is used stem bark past to heal the bone fracture[28]. (Pal et al. 1998). Stem bark decoction is given orally twice in a day to cure the asthma or other respiratory disorders by overcome the inflammation of respiratory tract.[5]
	Bark is used topically for the management of skin diseases[24]. Various Indian folk people are used its

Bark	bark as antidote and applied in glandular diseases[5].Strong decoction of bark is used by tribal people of Jalgon district to treat lymph swelling.[29] Raw bark juice is given to overcome the problem of menstrual trouble while with honey orally given against leucorrhoea [30]. Bark is used by Khasi tribes and non-tribal people of Assam region to cure small pox.[21]
Leaves	Malays people preferred the plant leaves for the treatment of sores and boils[24]. In India, plant leaves are given as cough remedy[5]. The plant leaves are recommended in south India, Sikkim, Bengal, Bihar and Orissa in the treatment of jaundice, wound and in stomach tumor.[21]
Flower	Recommended as laxative by the Malays people to treat constipation[5, 24]. Flower jam also called "*PushpaGulakanda*" is recommended for the treatment of constipation[3]. Flower bud and ghee fried flower are given to the patient those are suffering from dysentery. However, flower bud also used as laxative[5]. In Assam region, Khasi tribes and non-tribal people are used its flower in case of indigestion.[21]
Pod and Seed	In Michilka LDA, Adamawa State Nigeria, the pods are preferred as plaster for the old and fresh wound healing[31]. Indian tribal and non-tribal people of different regions use the plant pod and seeds as tonic and to overcome the problem of libido.[6]

DISCUSSION: Medicinal plants play an important role in human health and have potential to treat various diseases [32]. Available scientific research on *B. purpurea* L. is suggested a huge biological potential of this plant leaf, flower and bark but there is very limited data available on pod (fruit) [5]. In this present study demonstrated *B. purpurea* L. pod pharmacognostic, physicochemical and Ethano-botanical values as it traditionally used in various countries for different ailments.

Macroscopic studies are the oldest method of raw drug identification based on human sense organs where the actual characters of drug varies with identifier but still used due to fast and easier way of standardization. However, Microscopy is a traditional Pharmacopoeial method used for plant identification to authenticate herbal medicines, neutraceutical and food supplements commercially available on the global market [33]. This method is potentially useful for identifying species with similar morphological characters. It is a unique, valuable, rapid and cost-effective assessment tool which plays an important role in the authentication and assessment of medicinal plants. An especial emphasis on the powder microscopy of the herbal plants was given by American Herbal Pharmacopoeia (AHP) [34].

Phytochemical screenings of methanolic extract of plant pod achieved through cold maceration method pivotal onset point for qualitative evaluation of their secondary metabolites such as alkaloids, tannin, steroids, flavonoids and phenolic compounds. These secondary metabolites have been shown to have therapeutic activities of plants and function in a synergistic orantagonistic fashion for the treatment of diseases [35].

Alkaloids are known for its protective effect and mostly recommended as analgesic, anti-inflammatory and help to alleviate pain develop resistance against diseases and endurance against stress [31, 36]. However, steroid works as signaling molecules and decreasing the membrane fluidity. Generally, they are immunosuppressant and associate with the formulation of sex hormones in various pharmaceutical industries.

Similarly in the same manner, glycosides also being used as active ingredients for many drugs related with various cardiac issues including heart attack, hypertension and cardiac insufficiency [37]. In the latest study**,** researcher has been proved the pod ethanolic extract achieved through Soxhlet (hot extraction) shown the moderate antimicrobial activity against most pathogenic bacteria namely Cyst *Entamoebahis tolitica* (highest among the tested organism), *Staphylococcus aureus*, *Escherichia coli*, and *Helicobacter pylori* [31]. Phenolic components namely phenolic acids, tannins, flavonoids, etc. are believe the high substantial phyto-constituents produced by plant species. In fact, these compounds reported in various parts of the plant and their amount significantly based on the type of plant organ, geographical conditions, variety and climate.

In this research study phenolic and flavonoid quantitative estimation in pod was conducted by most authentic Folin-Ciocalteu and $AlCl_3$ method where phenolic compound react with reagent and gives colorimetric absorbance at fixed wavelength for the respective standard used for estimation [14, 38]. HPTLC fingerprinting is a valuable analytical technique provides to be a linear, precise and accurate method for phyto-constituents separation

and identification which accomplish for the quality control of herbal products and detection of adulterant. Thus, it is useful for the evaluation of various secondary metabolites and its presence in marketed herbal formulations which support the traditional therapeutic uses of this plant species [17, 39, 40].

CONCLUSION: At this planate plants have tremendous opportunities to offer alternative medicine and play a vital role in the field of health care sector and Pharmaceutical industries. The present research work reveal the pharmacognostical feature as abundance of rosette crystals, excess of tannin content and thick lignified bundle cap as of its characteristic feature responsible for strength and prevents from microbial attach and premature seed dispersion. Aqueous solution of pod found with the acceptable pH value 6.83 at 26.3 °C as similar as human gut environment, about three times high total ash than the acid insoluble values. There was no major variation has observed in alcoholic and aqueous extractive value. Total phenolic and flavonoids were calculated in respect of Gallic acid and Quercetin i. e. 490 mg/g and 235 mg/g, respectively. HPTLC profiling concluded that the hydro-methanolic extract has high range of mid polar and polar compounds with the R_f value ranging from 0.2 to 0.72. These compounds might be reason for its better healing and overcome the problem of libido.

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REFERENCES:

1. Htay, TM, Sann, KK and Haini H: A Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of *Bauhinia purpurea*. Bioactivities 2023; 1(1): 24-31.
2. Pahwa S, Mazumder R, Bhattacharya S, Mazumder A and Kumari S: *Bauhinia purpurea*-A phyto pharmacological review. Adv Pharmacol 2010; 11(3): 115-118.
3. Arora SK, Hussain M, Yende SR, Moharir K, Pande V and Ittadwar A: *Bauhinia purpurea*: An Updated Pharmacological Profile. J Ayu Herb Med 2020; 6(2): 81-85.
4. Sharma R, Sarswat A, Pittman CU and Mohan D: Cadmium and lead remediation using magnetic and non-magnetic sustainable biosorbents derived from *Bauhinia purpurea* pods. RSC Advances 2017; 7(14): 8606-24.
5. Kumar T, Alexander A, Dewangan D and Nagori K: Anthelmintic activity of the whole plant of *Bauhinia purpurea* (Linn.). Asian J Pharm Clin Res 2011; 4(3): 110-111.
6. Kumar T and Chandrashekar KS: *Bauhinia purpurea* Linn.: A review of its ethnobotany, phytochemical and pharmacological profile. Res J Med Plant 2011; 5(4): 420-431.
7. Krishnaveni M, Lavanya K, Dhanalakshmi R, Kalimuthu R, Ponraj K and Magesh P: A preliminary study on phytochemical screening and antioxidant potential of *Bauhinia purpurea* Linn flower. Int J Pharm Sci Rev Res 2014; 28(1): 172-175.
8. Orwa C, Mutua A, Kindt R, Jamnadass R and Anthony S: Agroforestry Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya 2009; 15: 5.
9. Shamala T, Surendra BS, Chethana MV and Shanmukhappa S: Extraction and isolation of Isoflavonoids from stem bark of *Bauhinia purpurea* (L): Its biological antipsychotic and analgesic activities. Smart Materials in Medicine 2022; 3: 179-187.
10. Rashed K: *Bauhinia purpurea*: Phytochemical and Biological Review. Int J Innov Pharm Sci Res 2020; 8(02):33-40.
11. Dutta P and Chaurasia B: Microscopic characterization and analysis of ayurvedic herbal products using light microscopy. International Journal of Ayurvedic Medicine 2022; 13(4): 859-66.
12. Eruygur N, Akyol Y and Tekin MA: Preliminary phytochemical, pharmacognostic and physicochemical evaluation of endemic *Thymus spathulifolius* (Lamiaceae). Kastamonu University Journal of Forestry Faculty 2023; 23(1): 11-21.
13. Anonymous, The Ayurvedic Pharmacopoeia of India (API), Part II New Delhi: Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy 2008; 5(1): 227-228.
14. Sudheer WN and Nagella P: Production of Boeravinone-B, total phenolic, flavonoid content and antioxidant activity from callus cultures of Punarnava (*Boerhavia diffusa* L.). Plant Science Today 2023; 10(2): 354-65.
15. Singleton VL and Rossi JA: Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagent. Am J Enol Vitic 1965; 16: 144-158.
16. Chang CC, Yang MH, Wen HM and Chern JC: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis (JFDA) 2002; 10(3): 1-6.
17. Champati BB, Padhiari BM, Ray A, Jena S, Sahoo A, Sahoo T, Panda PC and Nayak S: Quality control of marketed herbal products of *Asparagus racemosus* Willd. through high performance thin layer chromatography (HPTLC) analysis. Plant Science Today 2023; 10(2): 345-53.
18. Bhatkande AP and Teklae P: HPTLC-densitometric and HPTLC-MS Methods for Analysis of Triterpenoids from Cucurbita maxima Peel. Journal of Plant Science Research 2022; 38(2): 717-724.
19. Wagner H and Bladt S: Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media 1996.
20. Harborne JB: Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd, London 1973.

21. Sharma S and Kumar A: Tribal uses of medicinal plants of Rajashthan: Kachnar. IJLSPR 2012; 2(4): 69-76.
22. Lakshmi BVS, Neelima N, Kasthuri N, Umarani V and Sudhakar M: Protective effect of *Bauhinia purpurea* on gentamicin-induced nephrotoxicity in rats. Indian J Pharm. Sci 2009; 71(5): 551-554.
23. Rana MA, Khan RA, Nasiruddin M and Khan AA: Amelioration of cisplatin-induced nephrotoxicity by ethanolic extract of *Bauhinia purpurea*: An *in-vivo* study in rats. Saudi J Kidney Dis Transplant 2016; 27(1): 41-48.
24. Flora and Funa: *Bauhinia purpurea* L. https://www.nparks.gov.sg/florafaunaweb/flora/2/7/2750.
25. Rao RR and Jamir NS: Ethanobotanical study in Nagaland-1: Medicinal plants. Economy Botany. Bronx, New York 1982.
26. Kamble SY, Patil SR, Sawant PS, Sawant S, Pawar SG and Singh EA: Studies on plants used in traditional medicine by Bhilla tribe of Maharashtra. Indian J Tradit Knowl 2010; 9: 591-598.
27. Chatterjee A and Pakrashi SC: The Treaties of Indian Medicine Plants, Publications and Information Directorate, New Delhi, India 1992.
28. Pal DC, Jain SK. Tribal medicine. Calcutta, India 1998.
29. Pawar S and Patil DA: Ethnomedicinal uses of barks in Jalgaon district. Nat. Prod. Radiance 2007; 6: 341-346.
30. Das AK, Dutta BK and Sharma GD: Medicinal plants used by different tribes of Cachar district, Assam, Indian J Tradit Knowl 2008; 7: 446-454.
31. Khan ME, Anyam JV and Okwudili EE: Elemental, phytochemical and anti-microbial analysis of the pods extract of *Bauhinia purpurea*from Michika Nigeria. ADSUJSR 2015; 3(2): 252-260.
32. Lianah L, Khasanah RAN, Pranatami DA and Krisantini K: Phytochemical screening and cytotoxic evaluation of *Bauhinia scandens* leaf extracts using HeLa and T47D cell lines. Biodiversitas Journal of Biological Diversity 2021; 22(2): 913-919.
33. Ichim MC, Häser A and Nick P: Microscopic authentication of commercial herbal products in the globalized market: Potential and limitations. Front Pharmacol 2020; 11: 876-884.
34. Aeri V, Narayana DBA and Dharya Singh D: Powdered crude drug microscopy of leaves and bark. Elsevier 2019.
35. Trease GE and Evans WC: Textbook of Pharmacognosy, 15th ed. Tindall, London 2002.
36. Ahamed T, Rahman SM and Shohael AM: Thin layer chromatographic profiling and phytochemical screening of six medicinal plants in Bangladesh. Int J Biosci 2017; 11(1): 131-140.
37. Kren V and Martínková L: Glycosides in medicine: "The role of glycosidic residue in biological activity". Curr Med Chem 2001; 8(11): 1303-1328.
38. Milan SS: Total Phenolic Content, Flavonoid Concentration and Antioxidant Activity of *Marrubium peregrinum* L. Extracts. Kragujevac J Sci 2011; 33: 63-72.
39. Pachouri K and Yadav S: Physicochemical and phytochemical analysis of *Bauhinia variegata* modern analytical HPTLC fingerprinting. Int J Sci Eng 2015; 6(8): 2009-2018.
40. Senguttuvan J and Subramaniam P: HPTLC Fingerprints of Various Secondary Metabolites in the Traditional Medicinal Herb *Hypochaeris radicata* L. J Bot 2016. https://doi.org/10.1155/2016/5429625

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