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Research Article

Pharmacognostic, Phytochemical and Antioxidant Studies of *Adenanthera pavonina* L.

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ABSTRACT
Pharmacognostic, total phenolic and antioxidant studies of *Adenanthera pavonina* L. (family Mimosaceae) have been carried out. The leaves and bark of this plant are used by the tribal people for curing various ailments and diseases. The parameters like micromorphology, anatomy, phytochemical screening and physical constant have been considered here for pharmacognostic evaluation of different parts of this plant. The stomata are of strictly paracytic type and hypostomatic. Stomatal index is 12.61 and frequency is 158.5/mm². Palisade ratio is 8.2. Non-glandular trichomes are present on both surfaces of the leaf. Xylem axial parenchyma is paratracheal type. Uniseriate, biseriate and multiseriate types of ray structure are found. Perforation plates of the vessel elements are simple and obliquely placed, pits simple, tails present. Fibres are thick walled with pointed tips. Histochemical localization study revealed the presence of tannins, proteins, alkaloids, glycosides, lignin, cellulose, etc. in various tissue zones of the stem like vascular bundles, cortex, phloem, etc. The methanolic extracts of leaf and bark showed positive tests for carbohydrates, proteins, alkaloids, glycosides, saponins, flavonoids, steroids, tannins, etc. Moisture content of leaf and bark drug is 54.6% and 34.5% respectively. Ash value is 15.85% for leaf and it is 21.55% for bark powder. In leaf, percentage of acid soluble and water soluble ash is 81.53% and 42.45%, respectively. Percentage of acid soluble and water soluble ash in bark is 81.33% and 39.65%, respectively. Amount of total phenolics in leaf is 8.53mg/g and in bark, it is 8.51 mg/g. DPPH scavenging activity of methanolic extracts of leaf and bark (200mg/mL methanol) is 32.31% and 30.23% ascorbic acid equivalent, respectively. This study will provide some diagnostic features by which the crude drug of this plant can easily be identified.

Key words: Pharmacognostic study, foliar micromorphology, ash value, phenolic content, antioxidant study, *Adenanthera pavonina* L.

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the alternate healing systems. This is because of the adverse effects associated with synthetic drugs. In this context, herbal drugs play an important role in health care programs in developed as well as in developing countries[1]. The plant is a biosynthetic laboratory for multiple types of compounds. These compounds are responsible for medicinal properties of it. A systematic study of a crude drugs obtained from plants leads to the discovery of new products with pharmaceutical importance. It is highly important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize the adverse side effects. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine[2].

Approximately 2400-3000 medicinal plants species are in use in different Indian systems of medicine and many of them are constantly being screened for their biological activity[3]. Many of important medicinal plants in India have characterised pharmacognostically and documented in standard literature. But a large number of medicinal plants are remaining unattended for their scientific validation through pharmacognostic and other scientific studies. In this context, present study has been undertaken to evaluate the pharmacognostic as well as anatomical, phytochemical and antioxidant properties of *Adenanthera pavonina* L. (Mimosaceae), a lesser known medicinal plant. The important foliar characters like stomatal type and its index, trichomes types and comparative wood anatomy are widely recognised as important parameters in identification and taxonomical characterization of various groups of vascular plants [4-10]. Foliar micromorphology, petiole and stem anatomy are important parameters which are used in identification of a wide range of angiospermic taxa [11-13].

The present investigation has been under taken to study the above mentioned parameters of the investigated plant which will be helpful in proper identification as well as pharmacognostic characterization including anatomical and phytochemical features of the crude drug obtained from *Adenanthera pavonina* L.

Adenanthera pavonina L.

It is a large tree with many branches grown on variety of soils, preferably acidic and it prefers moist and seasonally

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Table 1: Foliar epidermal cell characters

Leaf surface	Epidermal cell shape and outline	Epidermal cell measurement (length × breadth)µm	Epidermal cell frequency (No./mm^2)	Palisade ratio
Upper	Irregular, wavy	50.05 × 32.3	898.0	8.2
Lower	Irregular, wavy	47.73 × 24	1094.8	

Table 2: Stomatal features

Leaf surface	Stomatal type	Measurement (length × breadth) µm	Stomatal index (%)	Stomatal frequency (No./mm^2)
Upper	–	–	–	–
Lower	Paracytic	24.77×18.5	12.61	158.5

Table 3: Trichome features

Leaf surface	Type	size (length × width) (µm)	Trichome frequency (No./mm^2)
Upper	Non glandular, unicellular	127.94 × 8.55	14.11
Lower	Non glandular, unicellular	126.8 × 8.29	29

moist tropical climate. The plant is planted as ornamental tree in the gardens, but also occurs as wild. In India, it is known by different vernacular names such as Rakta

Table 4: Xylem elements characters

Structure	Type	Measurements
Vessel elements	Perforation plate	Simple
	Arrangement of perforation plate	Oblique
	Pits	Simple pit
	Tail	Present
	Length (µm)	349.96
	Breadth (µm)	21.8
	Frequency (no./mm^2)	22.2
Tracheids	Wall thickening	Spiral
	Diameter (µm)	27.64
	Frequency (no./mm^2)	26.6
Fibres	Ends	Pointed
	Pits	Absent
	Length(µm)	535.8
	Diameter(µm)	16.01
	Frequency (no./mm^2)	25

Kambal (Bengali), Rakta chandan (Hindi), Kunchandana (Sanskrit), Red sandal wood (English), Bir Mungara (Santali). Different parts of this plant are used in treatment of several types of diseases. Leaves and bark paste is used to treat chronic rheumatism, gout, haemorrhage from the bowels and haematuria[14-15]. The bark powder is applied as antiseptic to prevent microbial infection. The ground bark or its decoction is ingested to cure bloodied urine and stool. Plants used as shampoo for those who suffer from dandruff or scalp psoriasis[16].

MATERIAL AND METHODS

Collection of plant material: Leaf and bark samples were collected from the investigated plant species grown in and around Santiniketan, West Bengal.

Study of foliar micromorphology: Leaf samples were cleared following the Bokhari's method [17]. The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin solution and observed under compound light microscope.

Vegetative anatomy: Free hand sections of petiole and stem wood of the selected plant were cut, stained suitably following safranin-light green staining schedule [18] and observed under compound light microscope.

Xylem elements study: The stem pieces (1 cm) were macerated following the standard method [18]. Boiled wood samples were then washed in distilled water for several times and observed under compound light microscope.

Preliminary microchemical tests: Methanolic extracts of leaf and bark powders were used for different chemical colour reaction tests with the help of different reagents to detect different phytochemical groups present in the powdered samples following standard methods [19-25].

Histochemical study: Transverse sections of the stem were laid out in several glass slides; one to two drops of different reagents (Wagner's, Dragendroff, Mayer's, Lugol's, 1% lead acetate, Phloroglucinol, Ferric chloride, Millon's) were added to the sections and kept for few minutes. Then observed under compound light microscope to detect different phytochemical groups localized in different tissue zones of the sections [13, 19-25].

Moisture content study: 5 gm of leaf sample were weighed and dried for few days. The sample was incubated at the temperature of 80°- 90C for one hour. Then final weight was taken and calculated the percentage of moisture content [26].

Fluorescence analysis: Powdered drug samples treated with different chemical reagents gave characteristic colour when seen under UV light (365 nm) and it was compared with the colour observed under ordinary light [27] .

Estimation of total phenolic content: It was estimated by standard methods [28]. 0.5 g plant sample was ground with pestle and mortar in 10 volume of 80% ethanol. Homogenate was centrifuged at 10,000 rpm for 20 min, supernatant was collected and the pellet was re centrifuged. Both the supernatants were collected and evaporated to dryness. Residue was dissolved in 5 ml distilled water. Then 0.5 ml of aliquot was pipette out and volume was made to 3 ml with distilled water. 0.5 ml of folin- ciocalteau reagent was added to the test tube. After 3 minutes, 2mL of 20% Na_2CO_3 was added to it. After thorough mixing the test tube was placed on boiling water bath for 1 minute, cooled at room temperature and absorbance was measured at 650 nm against a blank.

Table 5: Microchemical and Histochemical tests

Test for	Test/ reagents	Nature of changes	Methanolic leaf extract	Methanolic bark extract	Histological location
Alkaloids	Mayer's reagent	White cream ppt.	+	+	Vascular bundle.
	Wagner's reagent	Orange brown ppt.	+	+	Epidermis, sclerenchyma, vascular bundle, pith.
	Dragendroff's reagent	Orange brown ppt.	+	+	Epidermis, cortical zone, sclerenchyma, vascular bundle, pith.
Reducing sugar	Fehling's test	Brick red	+++	+++	-
	Benedict's test	Brick red	+++	+++	-
Carbohydrate	Molish's test	Violet ring	+	++	-
Anthraquinones	Borntrager's test	Pink colour	-	++	-
Saponins	1% Lead acetate test	White ppt.	+++	+++	Vascular bundle.
Proteins	Millon's reagent	White ppt.	++	+++	Epidermis, cortical zone, vascular bundle, sclerenchyma.
	Lugul's reagent	Faint yellow colour	+	+	Epidermis, cortical zone, Sclerenchyma, vascular bundle, pith.
Amino acids	Ninhydrin test	Purple colour	+	-	-
Flavonoids	Shinoda's test	Magenta colour	-	++	-
Tannins	10% Ammonium hydroxide	Yellowish white ppt.	+	+	Intercellular space
	10% Lead acetate	Yellow ppt.	+++	++	Cortex
	5%Ferric chloride	Greenish black	+++	+	Inter cellular space of pith zone.
Steroids	Salkowaski tests	Reddish blue and green fluorescence	+	-	-
Lignin	Phloroglucinol	Red colour	+	+	Phloem, Sclerenchyma, some cells of pith.

- = Absent; + = Present

DPPH radical scavenging activity: It was determined by following standard method [29]. The stock solution was prepared by dissolving 24 mg DPPH in 100mL methanol and then stored at -20 ^{0} C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1± 0.02 units at 515 nm using the spectrophotometer. Plant extracts (150 µL) were allowed to react with 2850 µL of the DPPH solution for 24 h in the dark. Then absorbance was taken at 515 nm. The standard curve for ascorbic acid was prepared. Results were expressed in % of ascorbic acid equivalent fresh mass. Additional dilution was needed when the DPPH value measured was over the linear range of the standard curve.

RESULTS

Foliar micromorphology

General description along with measurement of the epidermal cells, stomata, trichomes are given below.

Epidermis- Epidermal cells are irregular in shape and cell wall outlines are wavy in both leaf surfaces. The size of the epidermal cell is 50.05µm × 32.3µm on the upper surface and it is 42.73µm × 24µm on the lower surface. Epidermal cell frequencies of the upper and lower surfaces are 898/mm² and 1094.8/mm², respectively. The palisade ratio is 8.2 (Table – 1) [Fig –1].

Table 6: Moisture content and Ash value

Crude drug	Moisture content (%)	Total ash (%)	Water soluble ash (%)	Acid soluble ash (%)
Leaf powder	54.6	15.85	42.45	81.53
Bark powder	34.5	21.55	39.65	81.33

Stomatal complex- Stomata are present only on the lower surface of the leaf, i.e. leaves are hypostomatic and stomata are strictly paracytic type. Stomatal size is 24.77µm × 13.77µm. Stomatal frequency on the upper surface is 158.5/mm². Stomatal Index (S.I.) is 12.61 (Table – 2) [Fig -1].

Trichomes- Epidermal trichomes are present on both epidermal layers of the leaves. Trichomes are exclusively

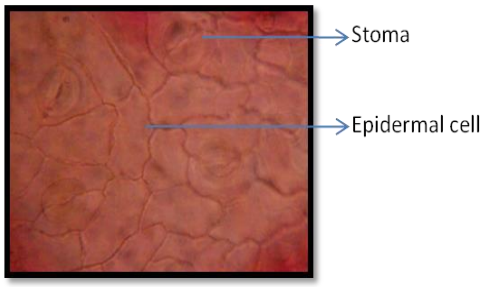

Fig. 1: A portion of lower epidermis with stomata

Fig. 2: Non-glandular trichome

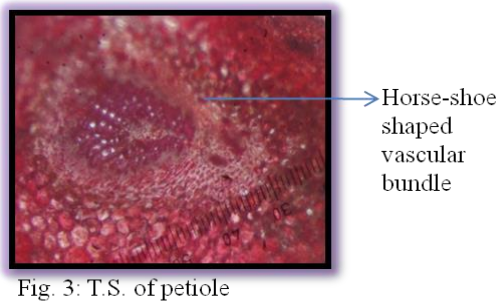

Fig. 3: T.S. of petiole

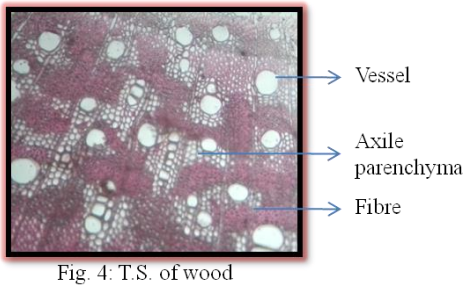

Fig. 4: T.S. of wood

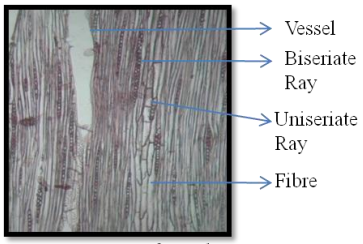

Fig. 5: T.L.S. of wood

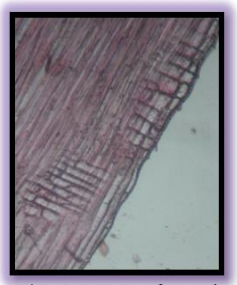

Fig. 6: R.L.S. of wood

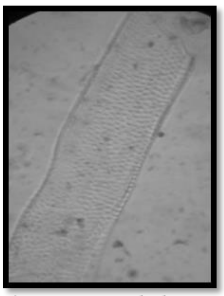

Fig. 7: A vessel element

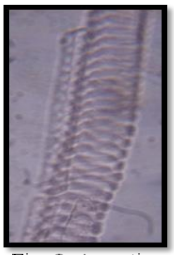

Fig. 8: A portion of tracheid

Fig.9: A portion of fibre

non-glandular type. They are unicellular, tips pointed, size 127.97µm × 8.55µm on upper surface and 126.8µm × 8.29µm on the lower surface. Trichome frequency is 14.11/mm^2 in upper surface and it is 29/mm^2 in lower surface. Trichome index is 2.24 (Table –3) [Fig-2].

Petiole anatomy In transverse section, outline of the petiole is more or less circular. Epidermis is single layered and cells are compactly arranged. Cuticle is thin. 3-4 layers of collenchyma cells are present just below the epidermal layer. A large horse shoe shaped vascular bundle is present. At the centre, large parenchyma cells with intercellular spaces forming the pith (Fig-3).

*Wood anatomy:*T.S. - In T.S. vessels are circular to oval in outline; solitary, twin or radially clamped; diffuse porous wood. Vessel diameter varies from 18µm to 36.4µm. Axial parenchyma cells are living, rectangular and paratracheal confluent in arrangement; few are arranged in paratracheal aliform manner. Ray cells are rectangular, arranged longitudinally and one celled thick [Fig - 4].

T.L.S.-Tangential longitudinal section shows mostly uniseriate and biseriate types of ray structure. Only few multiseriate types are observed. Ray structures are spindle shaped. Height of ray structure varies from 163µm to 327.6 µm (in uniseriate), 254µm to 364µm (in biseriate) and 291.2µm to 436.8µm (in multiseriate). Number of ray cells in a ray structure ranges from 4 to 10 in uniseriate ray, 9 to 12 in biseriate ray and 12 to 16 in multiseriate ray structure. Vessels are longitudinally arranged, unequal in diameter, perforation plate obliquely placed and simple, circular simple pits are on the side wall. Fibres are longitudinally arranged, closely packed on both sides of the ray structure, wall thick, lignified, cell lumen reduced [Fig - 5].

R.L.S.-. Both rhomboidal and rectangular types of ray parenchyma cells are present. They are living and thin walled. Rectangular ray cells are arranged procumbently. Ray structures are heterogeneous in nature. Vessels are longitudinally arranged, unequal in diameter with circular simple pit. Fibres are elongated, thick walled cells, arranged longitudinally [Fig -6].

Xylem elements: General description along with measurements of the xylem elements of stem has been presented below (Table-4).

Vessel element: Vessel elements have simple and obliquely placed perforation plates. Pits are simple and arranged in horizontal lines on the side wall. Tails present in some of the vessel elements. Size of the vessel element is 349.96µm × 21.8µm and frequency is 18.24/mm² [Fig - 7].

Fig. 10: Chemical colour reaction test of leaf powder

Fig. 11: Chemical colour reaction test of bark powder

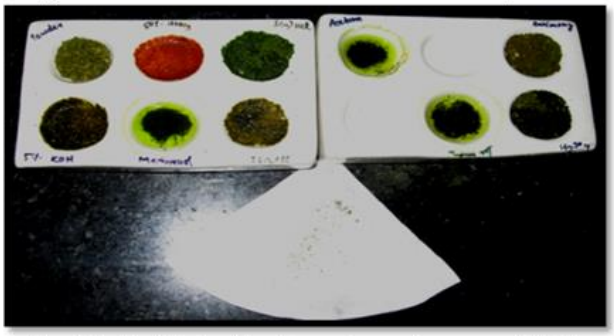

Fig. 12: Spot test of leaf powder under normal light

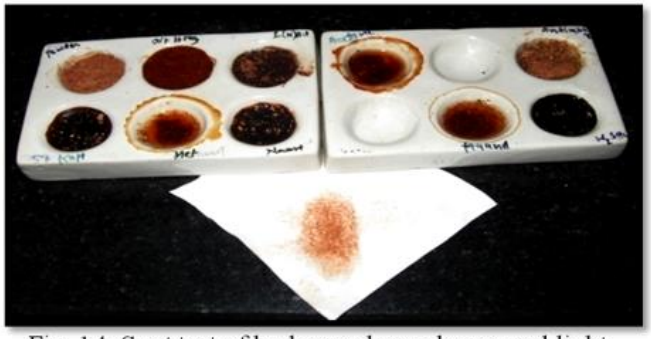

Fig. 14: Spot test of bark powder under normal light

Fig. 13: Spot test of leaf powder under UV light

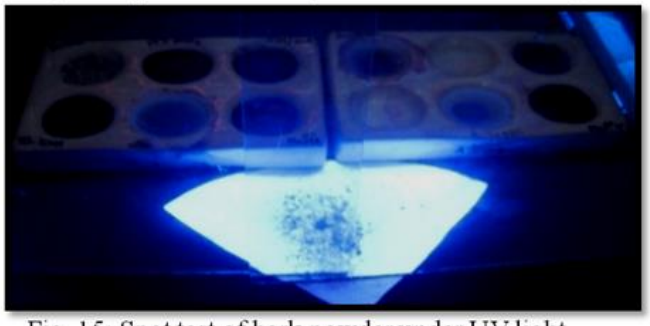

Fig. 15: Spot test of bark powder under UV light

Tracheids: They are very long and with spiral side wall thickening. The diameter of tracheid is 27.64µm and frequency is 16.21/mm² [Fig –8].

Fibres: Fibres are typically libriform type with pointed ends. Septa and pits are totally absent. Size of fibre is 535.8µm × 16.01µm and frequency is 25/mm² [Fig -9].

Microchemical tests of powder drug:

The methanolic extracts of the leaf and bark parts showed presence of some important phytochemical groups which confirms the medicinal properties of this plant. The methanolic extract of the leaf powder has shown positive test for carbohydrates, alkaloids, saponins, proteins, amino

Table 7: UV fluorescence nature

Materials and treatment	Leaf powder		Bark powder	
	In normal light	In UV light	In normal light	In UV light
Powder as such	Greyish green	Fluorescent green	Pale brown	Sienna
Paper with powder	Yellowish green	Dark pink	Pale brown	Magenta
Treated with 50% HNO_3	Orange	Red rose	Reddish brown	Dark red
Treated with Methanol	Olive green	Fluorescent orange	Pinkish brown	Turquoise
Treated with 5% KOH	Dark chocolate	Saddle brown	Dark coffee	Dark maroon
Treated with Ethanol	Olive green	Fluorescent reddish orange	Pinkish brown	Deep sky blue
Treated with Acetone	Olive green	Reddish orange	Pinkish brown	Pinkish red
Treated with 1N HCl	Dark green	Dark coffee	Chocolate brown	Dark maroon
Treated with 1N NaOH	Yellowish brown	Dark reddish maroon	Coffee colour	Reddish maroon
Treated with Antimony trichloride	Greenish brown	Red rose	Pinkish grey	Yellowish red
Treated with 80% H_2SO_4	Coffee colour	Dark green	Dark coffee	Fire brick colour

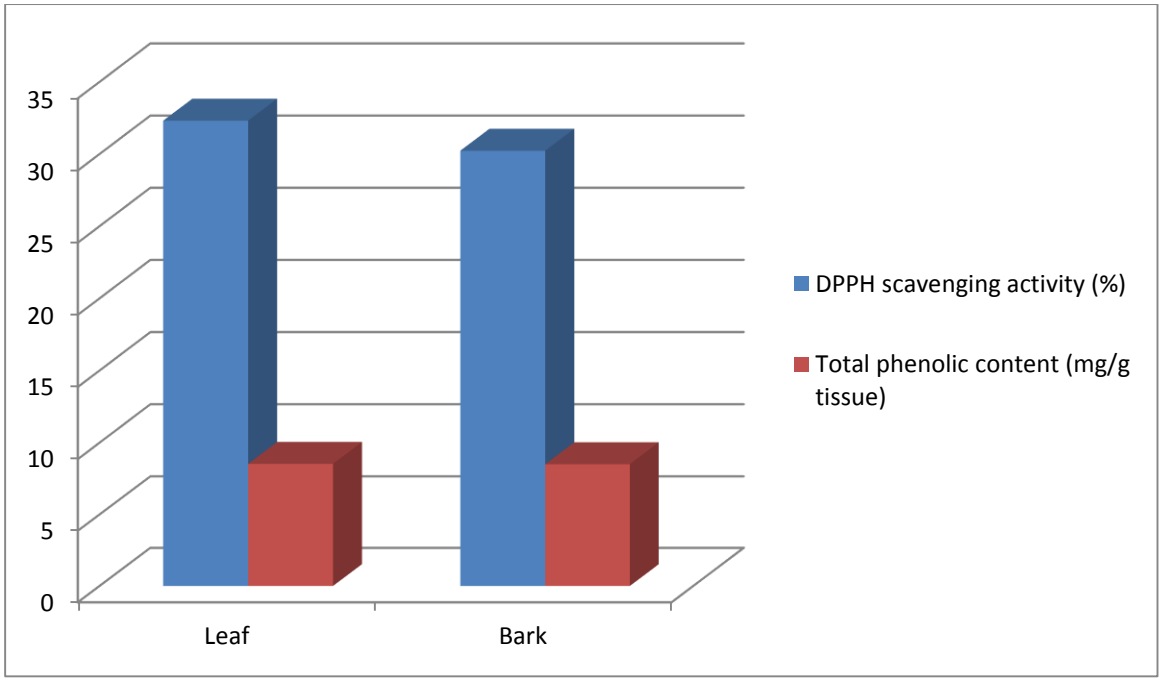

Fig. 16: Antioxidant activity and total phenolic content of the leaf and bark

acids, tannins, steroids and lignin, but showed negative result for anthraquinones and flavonoids. Methanolic extract of the bark powder drug showed positive results for carbohydrates, alkaloids, anthraquinones, saponins, proteins, amino acids, flavonoids, tannins and lignin, where as it gave negative results for steroids and amino acids (Table -5) [Figure –10, 11].

Histochemical study: Histochemical study has been carried out to detect various phytochemicals groups localized in different tissue zones of the stem. Different phytochemical groups like tannins, proteins, alkaloids, lignin, saponins etc. have found localised in different tissue zones of the stem. It has been found that vascular bundles and cortical zone are main active sites for synthesis of high content of different phytochemical groups (Table-5).

Moisture content & Ash value : Moisture content, ash value of the leaf and bark powder drugs are given in tabular form (Table-6). Moisture content of leaf and bark drug is 54.6% and 34.5% , respectively. Ash value is 15.85% for leaf and it is 21.55% for bark powder. In leaf, percentage of acid soluble and water soluble ash is 81.53% and 42.45%, respectively. Percentage of acid soluble and water soluble ash in bark is 81.33% and 39.65%, respectively.

Fluorescence analysis: The drug powders of the both plant parts treated with different chemical reagents gave characteristic colour when seen under UV light (365 nm) and it is compared with colour observed under ordinary light. In some cases, there are marked differences in colour have been observed when different solvent treated powder drugs seen under UV light (365 nm) (Table - 7) [Fig.-12-15].

*Antioxidant study:*Total phenolic content- Amount of total phenolics in leaf tissue is 8.53mg/g tissue and in bark, it is 8.51mg/g tissue. Phenolic content of both the parts is more or less same.

DPPH assay: DPPH scavenging activity for methanolic extracts of leaf and bark (200mg/ mL methanol) is 32.31 % and 30.23% ascorbic acid equivalent, respectively (Fig.- 16). Here, in case of leaf extract the antioxidant activity is little higher than the bark extract.

DISCUSSION

The present study reveals that foliar epidermal features, vegetative anatomy (stem wood and petiole), stem xylem elements characters, primary phytochemical screening and physical evaluation of this selected plant species are of some important taxonomic value in identification of this investigated plant species in its fresh as well as dried form. Some of the general anatomical characters of this investigated plant confirmed to the features identified earlier by different workers in other members of Leguminosae [10,13, 30]. Epidermal cell shape and size are sometimes found as very distinctive ones which help in proper identification of plants. From this study it has been observed that epidermal cells are irregular in shape but cell wall out line is strictly wavy. Studies of stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants [4,5,30-38]. Here, strictly paracytic type of stomata are found only in lower surface of the leaf. Leaves are hypostomatic in nature that confirms the observation of earlier worker [11]. Stomatal index is used as marker character for taxonomic identification of plant species. In this investigation, stomatal index is 12.61which is very specific to this plant. Stomatal size and frequency are also considered in many cases as distinct features for identification of the plant species. Here, stomatal size is 24.77µm × 18.5µm and stomatal frequency is 158.5/mm^2. These two features are also found very distinct for this plant. The palisade ratio is 8.2 which is specific for this species.

Trichome features are very important in proper identification of the plants and considered as one of the valuable taxonomic marker now [6, 36]. Epidermal trichomes of the investigated plant are non-glandular and unicellular type, present on both surfaces of the epidermis. It confirms the observation made by other workers[11]. The size of the trichomes is 127.94µm ×8.55µm in the upper surface and it is 126.8µm ×8.29µm in the lower surface.

Chemical analyses and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants [25]. Chemical test of methanolic extracts of leaf and bark revealed that the important phytochemical groups like alkaloids, steroids, tannins, saponins, anthraquinones, flavonoids, etc. are present in the plant which confirm the therapeutic potential of it. Leaf extract gave negative result for the test of anthraquinones and flavonoids , though bark of it showed very positive result for these two phytochemical groups. In leaf, steroid has been detected which has not been found in bark. So, it has clearly been found that both parts of this plant are medicinally important. From histochemical localization test, it is observed that alkaloids, saponins, proteins, tannin and lignin are present in detectable amount in different tissue zones of the stem. This test highlights that vascular bundles and cortical zone are the main sites for the synthesis or storage of different phytochemical groups.

Moisture content, ash value and UV fluorescence characters of the powder drugs are the two established parameters which are used for crude drug identification. In this study, it has been observed that moisture content is higher (54.6%) in leaf powder than the bark powder (34.5%). Similarly, difference in ash value was observed between leaf and bark powder of the plant. Ash value is higher in bark (21.55%) than the leaf powder (15.85%). Ash values of leaf and bark of the investigated plant will help in proper identification of crude drugs obtained from respective parts of this plant. Here, leaf powder treated with methanol and acetone showed olive green colour under normal light. It turns fluorescent orange when seen under UV light. Again ethanol treated leaf powder showed olive green in normal light and it turns fluorescent reddish orange in UV light. These differences in colour change are very much marked one which can be employed as authentic tool for proper identification of the leaf drug of this medicinal plant. Bark powder treated with ethanol showed pinkish brown colour under normal light. It turns deep sky blue colour when seen under UV light. Again methanol treated bark powder showed pinkish brown in normal light and it turns into turquoise colour under UV light. These distinct colour changes can be used as specific characters for proper identification of bark drug obtained from this medicinal plant.

It has been observed that the total phenolic content in leaf is 8.53 mg/g tissue which is slightly higher than the bark (8.31 mg/g). DPPH radicals scavenging activity for methanolic extract of leaves is 32.31 % that is higher than the bark (30.23%). It clearly indicates that the leaf part of the plant is more active in respect of its antioxidant activity than bark, though leaf part is lacking flavonoids, the important phytochemical group with good antioxidant properties. Qualitative phytochemical tests showed that concentration of tannins is higher in leaves which may also be the factor for its higher level of antioxidant activity.

So, it can be said that *Adenanthera pavonina* is a very promising medicinal plant with its good antioxidant potential and phenolic content. Further scientific studies of this plant are to be needed for proper validation of these preliminary findings made here in this study.

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