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Pharmacognostic study of some important hepatoprotective plants

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Abstract

Pharmacognosy is the study of naturally occurring biological substances, principally those derived from plants that find use in medicine. The word "Pharmacognosy" is derived from the Greek "Pharmakon," "a drug" and "gignosco," to acquire knowledge of. It is closely related to both botany and plant chemistry and both originated from the earlier scientific studies on medicinal plant. In recent years there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by pharmacognostic study is more reliable. The three important hepatoprotective plants *Buchanania lanzan* Spreng (Anacardiaceae), *Artocarpus hirsutus* (Moraceae) and *Terminalia coriacea* (Roxb.) Wight & Arn. (Combretaceae) has been taken for Pharmacognostic study. This information will be of used for further pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and sample identification.

Keywords: Hepatoprotective plants, Morphology, microscopy, powder characteristic and Fluorescence analysis.

Introduction

Medicinal plants are playing very active role in traditional medicines for the treatment of various ailments [1]. However a key obstacle, which has hindered the promotion in use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures. There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of plant to be used as a medicine [2, 3, 4, 5]. In the present studies we have focus our investigations on three important medicinal plants *Buchanania lanzan*, *Artocarpus hirsutus* and *Terminalia coriacea* having hepatoprotective activity. The *Buchanania lanzan* Spreng. (*B. lanzan*) belongs to the Family Anacardiaceae, is commonly known as 'Char' in India. The rhizome of *B. lanzan* finds an important place in indigenous medicine as an expectorant, diuretic and carminative. It is also found to have anticancer, antihypertensive, larvicidal and anti-diabetic activities [6-9]. *Artocarpus hirsutus* belongs to the family Moraceae. *Artocarpus hirsutus* (Wild jack fruit) is been used in antimicrobial activity [10] anti-ulcer activity [11] traditional medicine, food and industry [12]. Plants are vital for the remedies as well as existence for human disease because they contain components of therapeutic value [13]. *Terminalia coriacea* (Roxb.) Wight & Arn. (Combretaceae) have been used traditionally in Indian medicine for the treatment of antifungal, anti-cancer, antibacterial, wound healing, [14] Anti-Epileptic Activity [15], hepatoprotective evidence. [16-17], cardiac stimulant, callous ulcer treatment and atonic diarrhoea. [18]

2. Materials and methods

2.1 Collection of sample

The Fresh bark of *Buchanania lanzan* Spreng.were collected from forest of Basana, Chhattisgarh and authenticated from BSI Attapura, Hyderabad. The voucher specimen No. is-BSI/DRC/2015-16/Tech./552. The leaves of *Artocarpus hirsutus* and *Terminalia coriacea* (Roxb.) Wight & Arn. Collected from Tirumala Hills, Chittoor district (A.P). The plant materials were identified and authenticated by Dr. K. Madhav chetty Assistant professor, S.V. University, Tirupati India. The voucher specimen No.-1116 and 1109 respectively.

2.2 Organoleptic evaluations

Organoleptic evaluations were performed according the colour, odour, taste, size and shape parameters.

2.3 Macroscopic evaluations

Different macroscopic parameters of bark and leaves were noted. Leaves evaluation include absence or presence of petioles and different characters of lamina *i.e.* shape indentations, base, texture, venations, apex. Bark was studied for its size, shape, surface and fracture.

2.4 Microscopic evaluations

Microscopy evaluations were done on both qualitative and quantitative basis. All evaluations were performed on Olympus compound microscope.

2.4.1. Qualitative microscopy

For qualitative microscopic analysis transverse section of bark and leaves were made by using microtome. Staining procedure was performed as per standard procedure. Various identifying characters were studied with staining.

2.4.1.1. Powdered microscopy

Shade dried bark and leaves were finely powdered and studied under microscope. Small quantity of different plant parts powder was placed separately on slides and each slide was mounted 2-3 drops of phloroglucinol and conc. HCl (1:1), each slide was covered with cover slip then examined under microscope. Different cell components *i.e.* cork cells, secondary ploem, lignified fibers, cortex cells, medullary rays, calcium oxalate crystals, mesocarp, endocarp and stomatal cells were noted and photography was done by using digital camera [19].

2.4.1.2. Bark and Leaf microscopy

In this study, transverse sections of Bark and Leaf were studied under photomicrograph. Staining reagents phloroglucinol and HCl (1:1) were applied according to standard method. Different identifying characters were noted with or without staining [20]. The various identifying characters were studied with or without staining and recorded. The Bark and Leaf were fixed in Corney's modified solution. The all above parts were degassed with vacuum pump. The fixed parts were dehydrated in an ascending series of water, ethyl alcohol, tertiary butyl alcohol mixture. The Bark and Leaf were infiltrated with wax for hardening the soft tissues. The infiltrated Bark and Leaf were placed in wax and allowed to cool down, trimmed the edges the cast block and attached on wooden blocks. The sections were cut with rotary microtome and placed on glass slide having egg albumin adhesive. Thickness of the section was 10 μm .

2.4.2. Quantitative analysis

2.4.2.1. Stomatal number

It is an average number of stomata present per square millimeter of epidermis of leaf. Stomatal index is the percentage in which the number of stomata forms to the total number of epidermal cells. Stomatal index is $S \times 100 / (E + S)$. Where S is the stomata per unit area, E is the number of epidermal cells in the same unit area. For calculating stomatal index a washed and cleaned piece of leaf was taken and both upper and lower epidermises were peeled with the help of forceps. Stomatal index was calculated by using above given formula.

2.4.2.2. Determination of vein

Small vascular bundle surrounded by many conducting tissues is called vein islet. The end terminal of the vein is the total

number of veinlet termination points present per millimeter on the surface of leaf. A small piece of leaf was treated with chloral hydrate in boiling form then with the help of camera lucida, drawing was made. A square was drawn and slide was placed on it. The completing islets which are overlapping two adjacent sides of square were marked to get the value of one square millimeter area. The number of small vascular bundle terminal points was counted within that square to get the value known as vein termination number [21].

2.5. Fluorescence analysis

Fluorescence analysis in *B. lanzan* bark, *A. hirsutus* and *T. coriacea* leaves powder was carried out using standard method [22]. The analysis was done by treating the plant powder with different solvents including both acidic and basic. After treatment they were exposed to UV light (short wave length and long wave length) as well as were observed in day light [23, 24]. Fluorescence analysis is an important tool for the screening of those compounds which have the property of exhibiting different colours under UV light. Some compounds are not fluorescent themselves but when they are treated with solvents are converted into fluorescent derivatives. During this analysis the change in colour was noted [25].

Fluorescence analysis is a very important and useful tool for the identification of different constituents present in natural products. These constituents exhibited fluorescence under UV light but not show any type of fluorescence when observed in day light. This phenomenon may be due to a particular fluorescent substance or due to some fluorescent derivative formed after treatment with reagents. Still many crude drugs are assessed qualitatively by using this parameter. Powdered bark and leaves were analysed under ordinary light, short ultraviolet wavelength and long ultraviolet wavelength simultaneously after treatment with following organic and inorganic reagent like 50% H_2SO_4 , 10% NaOH, 50% NHO_3 , FeCl_3 , distilled water, aniline, potassium hydroxide and chloroform [22].

3. Results

3.1. Organoleptic evaluation

The bark was herbaceous brown in nature having light brown colour from the basal side. The leaves showed the green appearance from both side having trichomes called as stellate hairs. The leaf powder was green in colour, rough in texture, slightly aromatic with pleasant odour and slimy taste. The bark powder is in pinkish brown in colour.

3.2. Macroscopic evaluation

B. lanzan barks are 10-12 mm thick, surface black or dark brown, rough, tessellate the cracks being deep and narrow, somewhat resembling crocodile hide; blaze red. The *A. hirsutus* grows in altitudes ranging from sea level to an elevation of 1000 m in places with an annual rainfall of 1500 mm or more. Its leaves are simple, alternate and it will ooze latex if broken. *T. coriacea* leaves are large, 15-25 cm (5.9-9.8 in) long and 10-14 cm (3.9-5.5 in) broad, ovoid, glossy dark green, and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown.

3.3. Microscopic evaluation

3.3.1. Qualitative microscopy

3.3.1.1. Bark and Leaf microscopy

In the *B. lanzan* barks the outer most layers is cork. Cork is 5-9 layers, thin walled rectangular cells, some with yellowish matter. Phellogens are two layers of colourless rectangular cells. Phelloderms are 5-10 layers, thin walled somewhat rectangular cells, at times arranged in radial rows. The parenchymatous cells contain rhomboidal crystals and a few starch grains. Cortex is wide, interspersed with groups of lignified, pitted, stone cells of large lumen and of various shapes (rectangular to elongate) and sizes. The cortical parenchyma surrounding the stone cells and as well the stone cells themselves contain rhomboidal crystals. Starch grains are present in cortical parenchyma. One or two groups of non-lignified pericyclic fibres are seen in the cortex. Secondary phloem consists of phloem parenchyma, medullary rays and groups of stone cells arranged in tangential rows separated by medullary rays. The stone cells in the secondary phloem are encircled by a sheath of parenchyma containing rhomboidal crystals of calcium oxalate. Medullary rays are 1-3 seriate, wide towards the outside and consist of thin walled radially elongated parenchymatous cells. Phloem parenchyma and medullary ray cells contain starch grains (Fig. no.1).

In the *A. hirsutus* the upper most epidermal layer comprises of small polygonal cells which have irregular margins and beneath the epidermis photosynthetic tissue mesophyll present. The palisade has doubled layer while spongy mesophyll cells were 2 to 3 layered. The cortical region consisted of parenchymatous cells in the central vascular bundle having 4 to 5 layers. The vascular bundle having xylem and Phloem (Fig. no.2).

In *T. coriacea* the stellate multicellular trichomes with glandular base are present on both surfaces of leaf, the epidermal cells are isodiametric. The trichomes present outside show single layer of cells, and star shape with blunt tip and smooth walls. The upper most epidermal layer comprises of small cells which have irregular margins and beneath the epidermis photosynthetic tissue mesophyll present. The vascular bundle having xylem and Phloem (Fig. no.3).

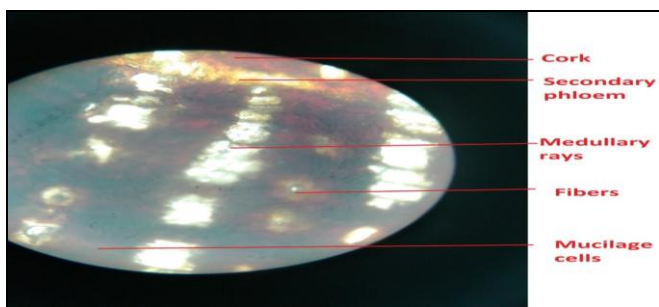


Fig 1: Transfer section of *Buchanania lanzan* Bark.

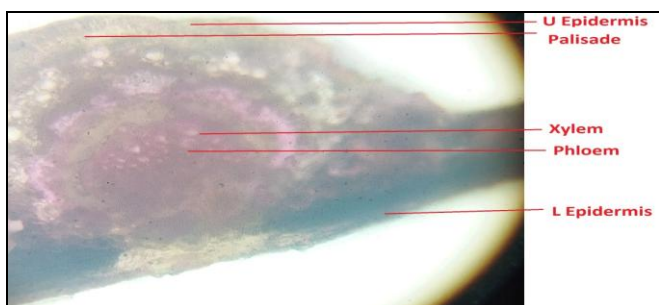


Fig 2: Transfer section of *Artocarpus hirsutus* leaves.

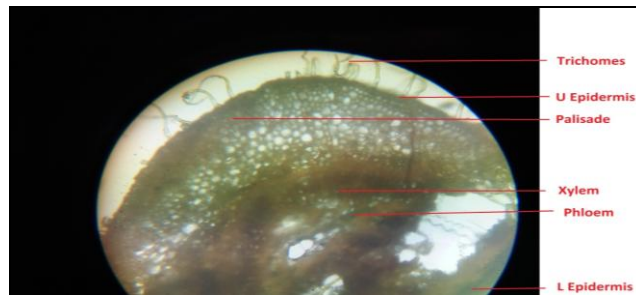


Fig 3: Transfer section of *Terminalia coriacea* leaves.

3.3.1.1. Powder microscopy

In the powder characteristics of *B. Lanzan* bark powder it was observed that the fibers are showing pink colour with phloroglucinol and conc. HCl. Cork cells are thin walled, few colourless and few with yellowish brown matters. Stone cells are rectangular to oval in shape, walls striated, pitted and lignified surrounded by sheath of parenchymatous cells containing calcium oxalate prism. Medullary rays at right angle. Few simple starch grains are observed.

In *A. hirsutus* leaf powder it was observed that the epidermal cells are polygonal, straight walled, epidermal cells with stomata. Trichomes are unicellular, thick wall. Xylem vessels are angular, thickening, lignified. Phloem fibers are pink in colour.

The powder characteristics of *T. coriacea* leaves powder it was observed that the trichomes are covering trichomes which are unicellular, thick watery wall, acute apex, bulbous base, narrow lumen conical shape. Xylem vessels are lignified yellowish brown colored cylindrical. Phloem fibers are pink in colored in cylindrical shape only.

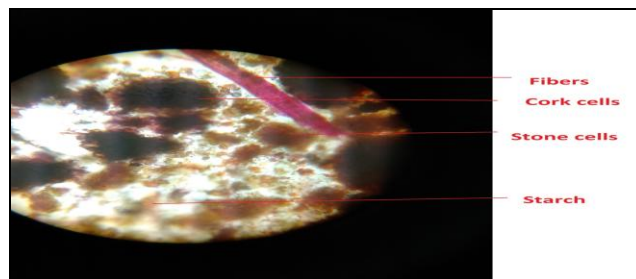


Fig 4: Powder characteristic of *Buchanania lanzan* Bark.

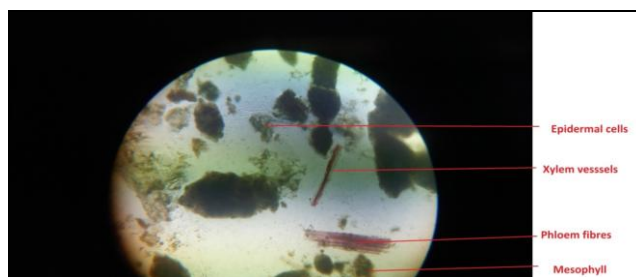


Fig 5: Powder characteristic of *Artocarpus hirsutus* leaves

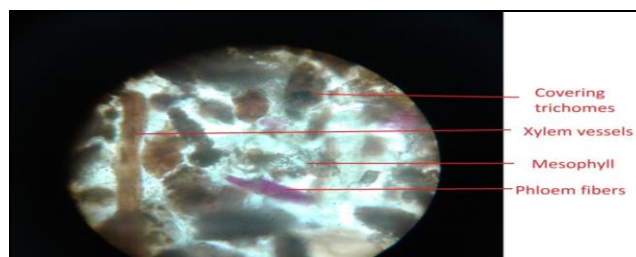


Fig 6: Powder characteristic of *Terminalia coriacea* leaves

3.3.3. Quantitative microscopy

The stomatal number on the epidermis surface was found for *A. hirsutus* and *T. coriacea* as 17 and 19, respectively. The

stomatal indexes were found 39.3 and 41.5, respectively. The vein islet and vein termination were calculated as 20 and 16.

3.4. Fluorescence analysis

Table 1: The results of fluorescence analysis are given in

Plants	<i>Buchanania lanzan</i>			<i>Artocarpus hirsutus</i>			<i>Terminalia coriacea</i>		
	Ordinary light	Short wavelength (254 nm)	Long wavelength (265 nm)	Ordinary light	Short wavelength (254 nm)	Long wavelength (265 nm)	Ordinary light	Short wavelength (254 nm)	Long wavelength (265 nm)
5% NaOH	Reddish brown to black	Black	Dull brown	Greyish green to brown to black	Green	Dull brown	Greenish brown to black	Greenish to brown	Dull brown
50% H ₂ SO ₄	Reddish pink	Brownish black	Dull brown	Green	Red pink	Dull pink	Brownish green	Green	Dull brown
50% HNO ₃	brown	Brownish black	Dull brown	Yellow	Brown	green	Yellow	Brown	green
5% FeCl ₃	Yellowish brown	Dark brown	Dark brown	Yellowish brown	Dark brown	Dark brown	Yellowish brown	Dark brown	Dark brown
Water	Pinkish brown	Green	Dull green	Greyish green	Green	Dull brown	Reddish brown	Green	Dull brown
Aniline	Brown	Brownish black	brown	Red	Brown	Green	Red	Brown	Green
Conc. KOH	Reddish brown to black	Reddish pink	Dull brown	Greenish green to brown	Green	Dull Green	Brown	Black	Dull brown
66% H ₂ SO ₄	Brownish black	Brownish black	Dull brown	Reddish black	Greenish pink	Dull pink	Brownish green to black	Greenish pink	Dull brown
Powder	Reddish brown	Dark brown	Dull brown	Light green	Brown	Green	Light green	Brown	Green
Chloroform	Brownish black	Pinkish brown to black	Dull brown	Green	Red to pinkish brown	Dull pink	Greenish to brown	Brownish pink	Dull brown

4. Discussion

The standardization of the herbal medicines is also necessary to assure the quality of the drug like the allopathic medicine quality control. This analysis will help to ensure the identity, quality, purity and safety of drug for the hepatoprotective active plants. Various parameters studied are microscopic analysis, macroscopic analysis and fluorescence analysis. Microscopic analysis is one of the cheapest methods to correctly identify the particular drug and the surety of raw material. Morphological and microscopical studies of bark and leaf will be helpful in the identification of these three plants. Quantitative analysis of some pharmacognostic characters is helpful to establish quality standards of the plant. Different parameters used for identification of different plant parts are so important for drug evaluation. The results of all type analysis are helping in establishing quality control standards and purity assurance of drugs.

5. Conclusion

In the present study three medicinal plants has been taken which have potent hepatoprotective activity. Thus liver diseases are one of the fatal diseases in the world today. They pose a serious challenge to international public health. Many polyherbal formulations are being used for liver protection, in that scenario the correct data about the plant materials are very useful. This information will be of used for further hepatoprotective activity, pharmacological and therapeutical evaluation and will assist in standardization for quality, purity and sample identification.

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