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Research Article

PHARMACOGNOSTICAL STANDARDISATION OF *POUZOLZIA WIGHTII* BENN, FAMILY: UTRICACEAE

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ABSTRACT

Pouzolzia wightii, Benn*, Utricaceae* is the shrub which is widely distributed in the Tirumala hills, Vijayanagaram and the Mahaboobnagar districts. The different parts of the plant have the various uses like anti inflammatory, wound healing, ulcers, boils. The chemical constituents of *Pouzolzia wightii* Benn include fats, steroidal glycosides, phenol compounds, flavonoids and carbohydrates. Though the plant has pharmacological potentials no standardization has been done pharmacognostically, hence form the basis for performing this work. The transverse section of stem and leaf showed the characters of typical dicot stem with 6 pairs of vascular bundles. The powder microscopy showed the presence of fibers, phloem and xylem vessels with endarch vascular bundles. The proximate analysis, fluorescence analysis and preliminary phytochemical tests were performed and reported. These data would help in the development of the profile of *Pouzolzia wightii* Benn.

Keywords: *Pouzolzia wightii* Benn, Endarch vascular bundles, Physicochemical, Fluorescence analysis.

INTRODUCTION

Pouzolzia wightii Benn is the shrub which is widely distributed in the Tirumala hills, Vijayanagaram, Mahaboobnagar district, etc... This is the dicot plant. This plant is monoecious; Stem type is erect or strong stems, tomentose. Leaf arrangement is opposite, alternate, leaf shape is lanceolate and ovate, Inflorescence is axillary, and flower type is sessile, male flowers are with calyx 1.5 mm across, perianth 4-lobed, free, inflexed, hairy at tip; bud truncate and female flowers are slightly shorter than male, perianth tubular.. Synonyms of the plant include karagada. Mainly leaves of this plant have uses like anti inflammatory, wound healing, ulcers, boils. It is not indigenous to this country but is rarely cultivated. It is also found along hills and valleys. It is drought tolerant but cold sensitive. Propagation is usually done by means of seeds, especially when the seeds are its final position. The flowering and fruiting time of the plant is usually from September – October. The leaves are mainly used for medicinal purposes. *Pouzolzia* species *reports* contain steroids, flavonoids, phenolic compounds and fats. [1, 2, 3]

MATERIALS AND METHODS

The plant *Pouzolzia wightii* Benn was collected in regions of Karnataka, in the months of November to December. The plant material was authenticated by Mr. A. Lakshma Reddy, Retired Professor, Dept. of Botany, and Nagarjuna Govt. College (Autonomous) Nalgonda and was certified under the Voucher No: NCOP-NLG/ph'cog/10-11/037, deposited in the Department of pharmacognosy, Nalanda college of Pharmacy, Nalgonda.

Instruments used

Micro senior precision rotary microtome (latest Spencer 820 types), Sisco muffle furnace (3003137), Rotary vacuum evaporator, Stage micrometer, Eyepiece micrometer.

Chemicals and reagents

All the chemicals and reagents like chloral hydrate, phloroglucinol, hydrochloric acid, nitric acid, potassium hydroxide, picricacid, lead acetate, alcohol, acetone, chloroform, petroleum ether, etc. Used were of analytical grade.

Microscopical studies

Transverse section of stem

Microtome sectioning was done for fresh stem to obtain a thin section. Phuloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. A thin transverse section of *Pouzolzia wightii* Benn stem was taken and studied [4].The descriptions are given as per standard anatomical references. (Figure1)

Transverse section of leaf

Microtome sectioning was done for fresh stem to obtain a thin section. Phuloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. A thin transverse section of *Pouzolzia wightii* Benn stem was taken and studied [4] .The descriptions are given as per standard anatomical references. (Figure 2)

Powder microscopy

Shade dried whole plant were powdered with the help of an electric grinder till a fine powder was obtained[5, 6, 7] .This fine powder was subjected to powder microscopy, as per standard procedures mentioned. (Figure 3)

Determination of Physicochemical properties

Total ash, acid insoluble ash and water soluble ash, sulphated ash of *Pouzolzia wightii* Benn were determined by standard methods and the results are tabulated in table. The crude fiber content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and the petroleum ether soluble extractive values of *Pouzolzia wightii* Benn were determined by standard methods and the results obtained were tabulated in the table[5, 6, 7].

Measurement of cell structure and content

The length and width of phloem fibers and the diameter of the starch grains were measured using a stage micrometer and the eyepiece micrometer by standard procedures[5, 6, and 7].

Extraction

The collected plant was washed and dried under the shade. It was then coarsely powder using an electric grinder. 50 g of the coarsely powdered stem and leaf was packed separately in soxhlet apparatus and extracted with, chloroform, acetone, alcohol, and water after defatting with petroleum ether at 60 -80^{0}c for 72 hours. The extract obtained was concentrated under vacuum using a rotary vacuum evaporator [8, 9].

Preliminary chemical screening

The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books[8, 9].

Determination of Fluorescence analysis

The powdered mass was subjected to analysis under ultraviolet light after treatment with various chemical and organic reagents [10, 11].

RESULTS AND DISCUSSION

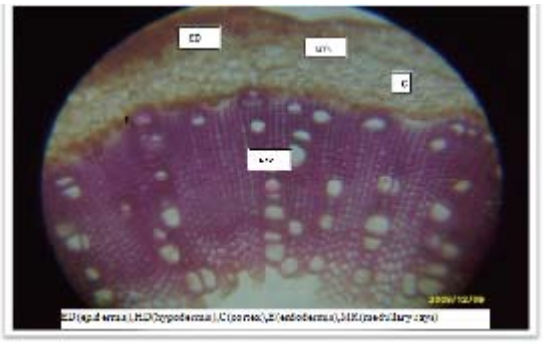

Fig. 1

Microscopical studies

Transverse section of stem

Transverse section of the stem is divided into following parts, Epidermis, Hypodermis Cortex, Phloem, Endodermis, Vascular bundles**.**

Epidermis

The outermost layer of the stem is covered by a layer of cuticle and rectangular single layers of parenchymatous cells which were arranged compactly without intracellular spaces

Hypodermis

The hypodermis consists of a single layer of cholenchymatous cells with intracellular spaces and it is nonlignified.

Cortex

The cortex was extensive and made up of several rows of thin walled parenchyma showing intercellular spaces and striations.

Endodermis

Endodermis formed the innermost layer of the cortex. It was well developed and clearly demarcated as a single layer of barrel shaped cells with thickenings arranged compactly without leaving any intercellular spaces. The cells found in this region were non-lignified.

Vascular bundles

Vascular bundles contained radial polyarch vascular bundles with 6 pairs of xylem and phloem cells. Conjoint and collateral vascular bundles are present. The pith was found to be absent.

Transverse section of leaf

Transverse section of leaf consists of the Upper epidermis, Mesophyll, midrib Lower epidermis.

Upper epidermis

Upper epidermis consists of a single layer of rectangular closely arranged cells covered by a layer of the cuticle.

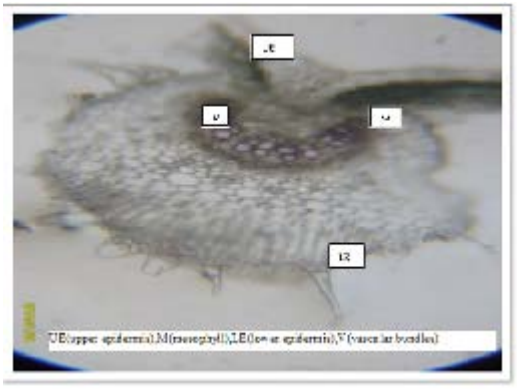

Fig. 2:

Mesophyll

The mesophyll is divided into the palisade parenchyma and spongy parenchyma. Palisade parenchyma consists of compactly arranged cells in 4 rows. Cholenchymatous cells are present in this region which is the seat of photosynthesis. Spongy parenchyma consists of parenchymatous cells with intercellular spaces.

Vascular bundles

Vascular bundles are present in the midrib region. Arch type of vascular bundles is present with xylem towards the upper epidermis and phloem towards the lower epidermis. These types of vascular bundles are called as conjoint collateral vascular bundles. These are covered by a bundle sheath.

Lower epidermis

Lower epidermis consists of a single layer of parenchymatous cells interrupted by the stomata. Anisocytic stomata are present in lower epidermis. Uniseriate trichomes are present in the lower side.

Powder microscopy

Sclerified parenchyma was found scattered in the powder.

Fibers of phloem were found to lignified with lumen in it.

Calcium oxalates are absent.

Starch grains present were circular to oval in shape.

Lignified cells are present.

Unicellular trichome is present.

Spiral vessels are present.

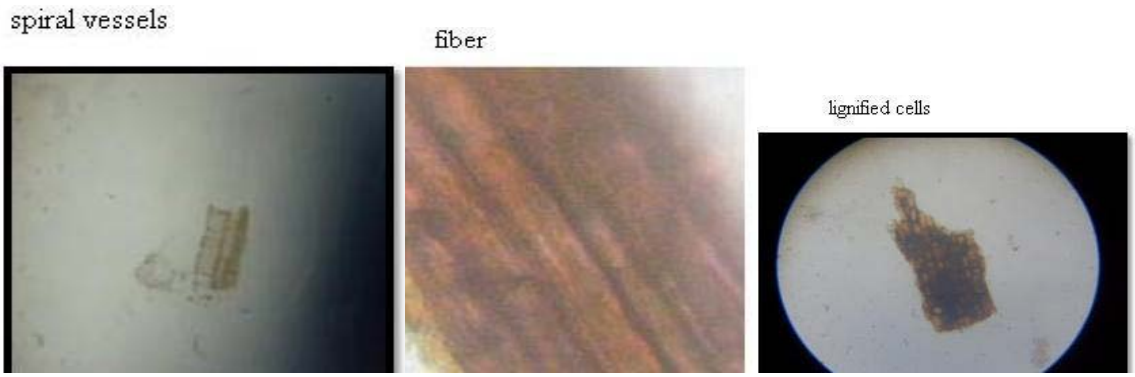

Fig. 3:

Determination of physico chemical properties

The physico chemical properties will help to estimate the amount of impurities like soil and particles present in the drug. It also helps to assess the calcium salts present in the drug sample. The results obtained for the ash values, extractive values, moisture content and crude fiber content, leaf constants, length and width of the fibers, and phloem vessels, diameter of starch grains, yield and physical characters are tabulated in Table1, 2,3,4.

Preliminary chemical screening

The extracts obtained after successive solvent extraction with petroleum ether, chloroform, acetone, ethanol, and water were analyzed for the color, odor and percentage yield. These extracts were subjected to qualitative chemical test for the identification of various chemical constituents.

The chemical test helps in the confirmation of the chemical nature of the active principles present in the plant extract .The results of the chemical tests are tabulated in table 5, 6 7, 8.

Determination of Fluorescence analysis

Fluorescence analysis is a tool to determine the kind of the chemical nature of the drug. The fluorescence obtained in short wavelength, long wave length and daylight after treatment with different chemicals and reagents are tabulated in table 9 and 10.

Table 1: Physicochemical properties

Parameter	Stem (%w/w)	Leaf (%w/w)
Total ash	0.147%w/w	0.395%w/w
Acid insoluble ash	0.131%w/w	0.258%w/w
Water soluble ash	8.2%w/w	9.1%w/w
Moisture content	0.456%w/w	0.540%w/w
Crude fiber content	0.98%w/w	0.87%w/w
Alcohol soluble extract value	7.1%w/w	5.6%w/w
Water soluble extract value	11.03%w/w	9.3%w/w
Chloroform extract value	5.6%w/w	4.2%w/w
Petroleum ether extract value	2.3%w/w	4.2%w/w
Sulphated ash	0.456%w/w	0.536%w/w
Loss of drying at 105°C, % w/w	7.8%	9.1%w/w

Table 2: Leaf constants Measurements

Upper epidermis	4.5 - 5.8
Lower epidermis	12 - 13
The Stomatal index for lower surface	18.5
The Stomatal index for upper surface	15.6
Vein islet number	16-18mm2
Vein termination number	10-12mm2

Table 3: Cell structure and content

Parameters	Length	Width
Fibers	1.25 to 2mm	8µm
Phloem vessels	270 to 400µm	50-60µm

Table 4: Measurement of starch grains

Parameter	Diameter
Starch grains	8µm

Table 5: Stem extract

Extracts	Color	Consistency	%yield
Petroleum ether	Yellow	Gummy mass.	0.9%
Chloroform	Black	Gummy mass.	1.5%
Acetone	Green	Gummy mass.	3.5%
Alcohol	Green	Oily mass.	4.1%
Water	Brown	Powdery mass.	3.1%

Table 6: Leaf extract

Extracts	Color	Consistency	%yield
Petroleum ether	Yellowish brown	Gummy mass.	1.9%
Chloroform	Greenish black	Sticky mass.	0.5%
Acetone	Brownish black	Gummy mass.	2.5%
Alcohol	Green	Oily mass.	3.1%
Water	Brown	Powdery mass.	4.1%

Table 7: Preliminary Phytochemical screening of leaf extract

Compounds	Pet ether	Chloroform	Acetone	Alcohol	Water
Saponins	-ve	-ve	-ve	-ve	+ve
Carbohydrates	-ve	+ve	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve	+ve	-ve
Steroids	+ve	-ve	+ve	-ve	+ve
Proteins	+ve	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	-ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve	-ve	-ve
Fats and oils	-ve	-ve	-ve	+ve	+ve

Table 8: Preliminary Phytochemical screening of stem extract

Compounds	Pet ether:	Chloroform:	Acetone	Alcohol	Water
Carbohydrates	-ve	+ve	+ve	+ve	+ve
Glycosides	+ve	+ve	-ve	+ve	-ve
Steroids	-ve	-ve	+ve	-ve	-ve
Proteins	-ve	-ve	-ve	+ve	-ve
Phenolic compounds	-ve	-ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve	-ve	-ve
Fats and oils	-ve	-ve	-ve	-ve	-ve
Saponin glycosides.	-ve	-ve	-ve	-ve	+ve

Table 9: Fluorescence analysis of leaf:

Chemicals	Daylight	Short wavelength	Long wavelength
Drug+50%H_2SO_4	Green	Green	Black
Drug +50%HNO_3	Red	Green	Black
Drug+1N methanolic NAOH	Greenish white	Green	Reddish black.
Drug +1N KOH	Greenish yellow	Green	Black
Drug +5%KOH	Brownish yellow	Green	Black
Drug +5%$FECL_3$	Black	Green	Yellowish red
Drug + methanol	Yellowish green	Green	Red
Drug+conc H_2SO_4	Brownish black	Greenish black	Reddish black
Drug+ ammonia	Blackish brown	Greenish black	Black
Drug+conc HNO_3	Reddish brown	Greenish black	Black

Table 10: Fluorescence analysis of stem

Chemicals	Daylight	Short wavelength	Long wavelength
Drug+50%H_2SO_4	Yellow	Yellow	Black
Drug +50%HNO_3	Red	Green	Black
Drug+1N methanolic NAOH	Brown	Yellow	Blackish yellow.
Drug +1N KOH	Yellowish brown	Green	Black
Drug +5%KOH	Brownish red	Yellowish green	Black
Drug +5%$FECL_3$	Yellow	Green	Black
Drug + methanol	Brown	Brownish black.	Black.
Drug+conc H_2SO_4	Blackish red.	Greenish black.	Black.
Drug+ ammonia	Brownish black.	Green	Black.
Drug+conc HNO_3	Yellowish brown.	Yellowish black.	Black.

CONCLUSION

The macro and microscopical characters along with physicochemical and fluorescence characters of powder of *Pouzolzia wightii Benn*, is used to establish the Pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guidelines. Preliminary phytochemical screening of different plant extracts revealed the presence of phenols, flavonoids, saponins, glycosides and carbohydrates.

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