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PHARMACOGNOSTIC AND PHYSICO-CHEMICAL INVESTIGATION OF BARK OF *PUTRANJIVA ROXBURGHII* WALL (EUPHORBIACEAE)

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ABSTRACT

Putranjiva roxburghii Wall (syn. *Drypetes roxburghii* Wall.) of Euphorbiaceae family is an evergreen tree of tropical region often cultivated as avenue tree along the road side. The Objective of the study was to develop various standardization parameters for the evaluation of trunk bark science it is largely used to cure certain ailments. Morphology, histology, powder characteristics and Scanning Electron Microscopy (SEM) of bark were observed and results were recorded. Physico-chemical parameters such as total ash value (13.5%) w/w, moisture content (6%) w/w, foaming index (less than 100), total tannin content (5% w/w), Crude fibres residue (32%) w/w, Fluorescence analysis, extractive value includes pet ether soluble extractive value (3.45%), ethanol (3.90%), ethyl acetate(5.14%) and aqueous extract(3.5%) w/w were analysed by chemical test and shows presence of saponins, tannins, steroids, triterpenoids. So these parameters are usefull in the authentication of *Putranjiva roxburghii* Wall. However the phytochemical analysis of bark of *Putranjiva roxburghii* in the present work is first of its kind used for the pharmacognostic and phytochemical evaluation.

KEY WORDS: ***Putranjiva roxburghii*, Euphorbiaceae, Physico-chemical, Pharmacognostic.**

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INTRODUCTION

Putranjiva roxburghii Wall of Euphorbiaceae is an evergreen tree of moderate size growing up to 9-12m high with pendent branches very often found as an avenue tree along road side in tropical parts of India.[1] The tree has very attractive, pendent branches with glacous leaves which give an alluring avenue shape to the plant. The tree has greyish white, very hard and tough bark and it has manifold local uses. [2] The plant has been used against various ailments for its medicinal properties. It has been reported that the plant is cooling, pungent, aphrodisiac, laxative, diuretic and prevents abortion. The leaves and stones of the fruit are given in decoction for colds and fevers.[3] The bark and seed are used as an antidote in the treatment of snake bite. The oil from seed contains olive oil which is brown or pale yellow containing glycerides with sitosterol (mp 143^0 -145^0.) (Wealth of India) Moreover the leaves of this plant have been reported to cure swollen throats of the cattles. This taxon is also reported to contain phytoconstituents like β-amyrin, stigmasterol, putrol, putrone, putranjivic acid, putranflavavone, amentoflavone, saponins A, B, C, D.[4] The phytochemical analysis of the bark of this plant is not studied so far hence; an attempt has been made to carry out detail pharmacognostic and phytochemical analysis of bark to explore newer phytoconstituents which can be used for further studies.

MATERIALS AND METHODS

Collection of Plant Material

Plant trunk bark material of fully grow tree of *Putranjiva roxburghii* Wall was collected from Tal. Haweli, Pune, Khadaki region of Maharashtra, India in June 2014. The taxon is authenticated from Botanical Survey of India, Pune dated 18/08/2014 with Voucher number BSI/WRC/Cert./2014 and collection no.KKA 01. The herbarium specimen is deposited in the herbarium of Modern college of pharmacy, Nigdi, Pune.

Physicochemical analysis

Ash value [5]

Total ash value

According to the procedure, weigh accurately 2 gm of air dried bark of the plant is weighted and kept in the tarred platinum crucible. It was kept in a muffle furnace for ignition at 450^0C. After ignition white ash was weighed and means results were recorded.

Acid insoluble ash

The ash obtained in the total ash method was boiled with 25 ml of 2N hydrochloric acid for 5 min. Insoluble matter was collected on ashless filter paper (Whatman paper no. 40) and washed with hot water. The material retained on filter paper was ignited and weighed. Percentage of acid insoluble ash was calculated with reference to air dried material.

Water soluble ash

The ash obtained from total ash was boiled with 25 ml water for 5 min. All insoluble matter was collected on ashless filter paper, washed with hot water and ignited for 15 min at the temperature not exceeding 450 ^{0}C. The percentage of water soluble ash was calculated by subtracting weight of insoluble matter from weight of total ash. The difference between weights represents water soluble ash. Percentage of water soluble ash was calculated with reference to air dried drug.

Moisture content[5]

LOD was determined as per procedure given in WHO, Guidelines. In brief, 2 g of air dried drug powder was placed in a silica crucible. Before that weight of empty crucible was noted. The powder was spread in a thin uniform layer. The crucible was then placed in the oven at 105 ^{o}C. The powder was dried for 4 h and cooled in desiccators at room temperature and drying was continued till the constant weight is achieved and subsequently the weight of crucible with powder is noted. Result is given in table 1.

Foaming index [5]

Weigh 1 gm powder of bark is transferred to 500ml conical flask containing 100ml boiling water. Maintain moderate boiling for 30min.Cool and filter into 100ml volumetric flask and add sufficient water to maintain the required volume. After that pour the decoction into 10 stopper test tubes in successive portions of 1ml, 2ml, 3ml etc up to 10ml. Adjust the volume with distilled water and stopper the tubes and shakes them in lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15min and measure the height of the foam. The results are noted as per the length of the foam.

Crude Fibers by Dutch method [6]

Crude fibers are the residue of resistant tissues which can be obtained after giving treatment to the bark powder with dilute acid and alkali. (Table 1)

Extractive value [7]

Extracts of Powder of bark were prepared by successive extraction methodology by using Soxhlet apparatus. Color of extracts and yields mentioned in (Table 2)

Fluorescence analysis of powder [8]

Fluorescence analysis was carried out as per Trease and Evans. Powder was treated with different solvents and chemical regents. (Table 4)

Total tannin content [8]

Total tannin content was determined by using Hide powder as per WHO Guidelines. (Table 1.)

Preliminary chemical analysis [6, 10, 11]

Extracts were further analyzed by performing chemical tests for identification of chemical constituents. (Table 3)

Microscopy [6]

Fresh barks of the trunk were collected and transverse sections were prepared by conventional method. The section was dehydrated and stained with conventional stains for observation of various tissues. The slides were observed under Digital microscope (Motic) by using different magnifications.

Powder analysis of plant [6]

Powder of bark has been observed with different stains for specific characters. such as phloem fibers, prisms of calcium oxalate in medullary rays, parenchyma cells of cortex region. So observing all these characters it helps in identification of Putranjiva bark specifically helps in addition of data into a monograph of plant. The microscopic analysis of powder of Putranjiva bark reveals various anatomical characters of phloem fibers, calcium oxalate crystals in medullary rays etc to substantiate the authentication of *Putranjiva roxburghii.*

SEM Microscopy

Powder of bark of putranjiva was observed on FEI (Field Emission Ion) -Quanta 200 SEM with x microscope Control Software., LFD – Large Field Detector, Light source is electron beam by tungsten filament. During SEM study some parameters were adjusted such as high Voltage range in between 200 V – 30 KV, Magnification range variably use in between 30X – 1,00,000x, Pressure Range is in between 10 Pa – 130 Pa (Low vacuum) generally used is 65 Pa. All mention parameters were adjusted depending upon the type of plant tissue.

RESULTS AND DISCUSSIONS

Physicochemical analysis

Physicochemical analysis of bark of *Putranjiva roxburghii* has total ash value which indicates presence of inorganic radicals. Fluorescence analysis helps in analysis of chemical constituents and produce supportive data for chemical test (Table 3). Foaming index have been performed on aqueous extracts which shows the presence of adequate saponin content, aqueous extracts also shows presence of tannin, it can be determined by total tannin content, Crude fibers determination again help in detailed analysis of Phloem Fiber tissue of the plant. (Table 1)

Preliminary chemical analysis

Various chemical tests of the extracts show presence of tannins, flavonoids, steroids, triterpenoids. (Table 2 & 3).

Table 1
Physicochemical parameters

Parameters	Mean (% w/w)
Total ash	13.5
Water soluble ash	12.5
Acid- insoluble ash	9
Loss on drying (LOD)	6
Crude Fibers by Dutch Method	32
Foaming Index	Less than 100
Total tannin Content	5

Table 2
Extractive values and colour of extract

Sr.No.	Extractive value	Colour of extract	Extractive value % w/w
1	Petroleum ether soluble	Brownish colour	3.45%
2	Ethanol soluble	Greenish Brown colour	3.90%
3	Ethyl acetate	Yellowish Green	5.14%
4	Water soluble	Yellowish Green	3.5%

Table 3
Preliminary phytochemical investigation of bark of Putranjiva roxburghii Wall

Qualitative chemical analysis						
Extracts	**Carbohydrates**	**Proteins**	**Glycosides**	**Tannins**	**Flavonoids**	**Steroids & triterpenoids**
Pet. ether	-	-	-	-	-	**+**
Ethanol	-	-	-	++	++	**-**
Ethyl acetate	-	-	-	-	+	**+++**
Aqueous	+	+	+	+	-	**-**

Presence of constituents (+), absence of constituents (-)

Table 4
Fluorescence analysis of powder of bark of Putranjiva roxburghii Wall

Reagents used	Day light	Shorter wavelength (254nm)	Longer wavelength (366nm)
Powder + 1N HCL	Pale yellow	Pale green	Orange
Powder + 1N NaOH	Pale yellow	Pale Green	Milky Green
Powder + 50% HCL	Light Brown	Pale Green	Pale orange
Powder + 50% H_2SO_4	Brown	Brown	Brown
Powder + 50% HNO_3	Pale yellow	Pale Green	Orange
Powder + Methanol	Light Green	Pale green	Light Green
Powder + Methanol+ 1N NaOH	Light green	Green	Light Green

Microscopical study

Transverse section of the bark shows cork composed of uniformly arranged 10-15 layers of small elongated cells covered with loosely packed cells of lenticels. Below cork is a zone of cortex, composed of 2-5 layers of stone cells followed by 8-10 layers of parenchymatous cells. Pericycle of 3-5 layers of sclerenchymatous cells or sclereids. Secondary phloem is composed of parenchyma, fibers and medullary rays. Phloem parenchymatous cells contain prism like calcium oxalate crystals. Medullary rays are composed of cells, uni- or bi- seriate near cambium and widening as they approach pericycle. (Fig. 2 and 3)

Powder analysis of bark

The powder analysis of the bark shows presence of multiseriate medullary rays,

phloem fibers tapering at both ends with narrow lumen and well marked calcium oxalate crystals where observed these characters verily help in the identification of this plant. Cork in surface view shows distinct lenticels and in sectional view exhibits Calcium oxalates crystals, lignified fibres, medullary ray cells etc. (Fig 4)

Scanning electron microscopy

Powder of bark observed on FEI (Field Emission Ion) -Quanta 200 SEM with microscope Control Software., LFD – Large Field Detector, Light source is electron beam by tungsten filament. During SEM study some parameters were adjusted such as high Voltage range in between 200 V – 30 KV, Magnification range variably use in between 30X – 1,00,000x, Pressure Range is in between 10 Pa – 130 Pa (Low vacuum) generally used is 65 Pa. All mention parameters were adjusted depending upon type of cell.(Fig 5).

Figure 1
Photograph of Trunk Bark and Herbarium of Putranjiva roxburghii

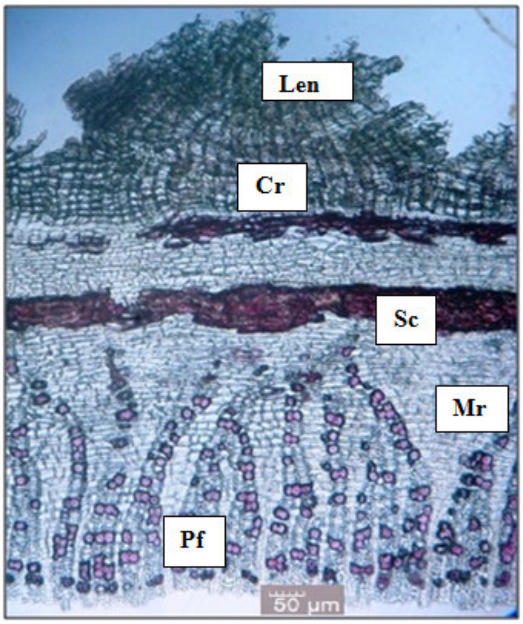

Figure 2
T. S. of bark Putranjiva roxburghii Wall. Len- Lenticel, Cr- Cork cell, Sc- Stone cells, Pf-Phloem Fibers, Mr-Medullary rays

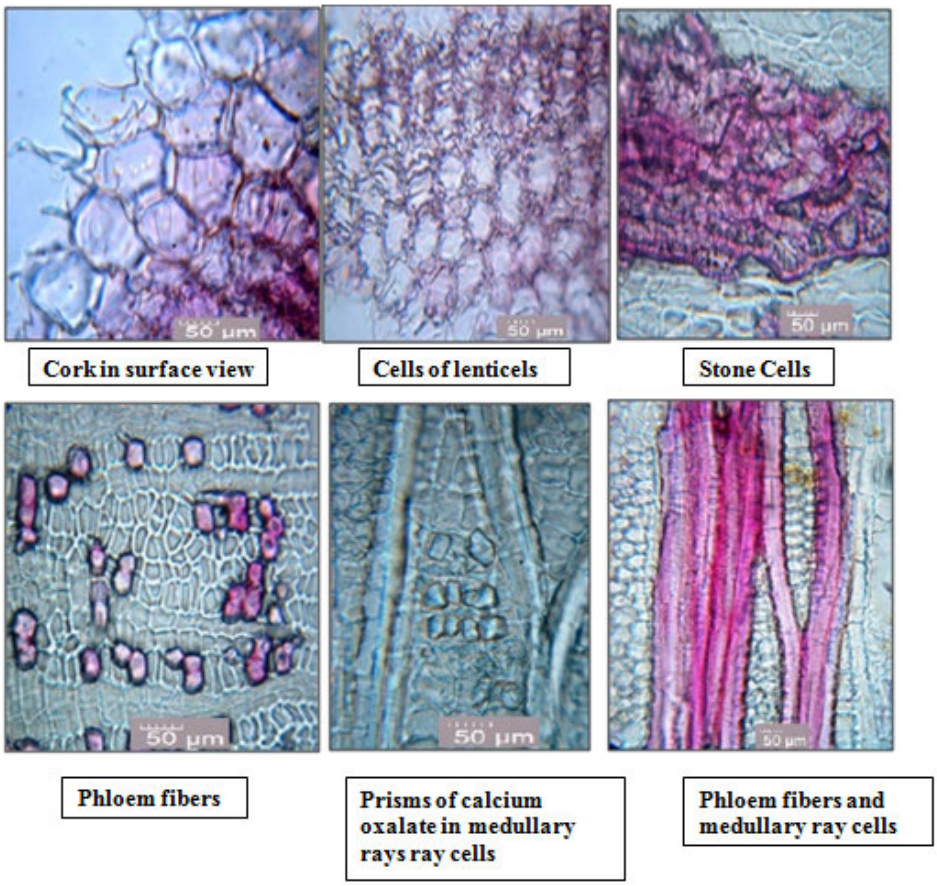

Figure 3
Microscopy of Bark of Putranjiva roxburghii Wall

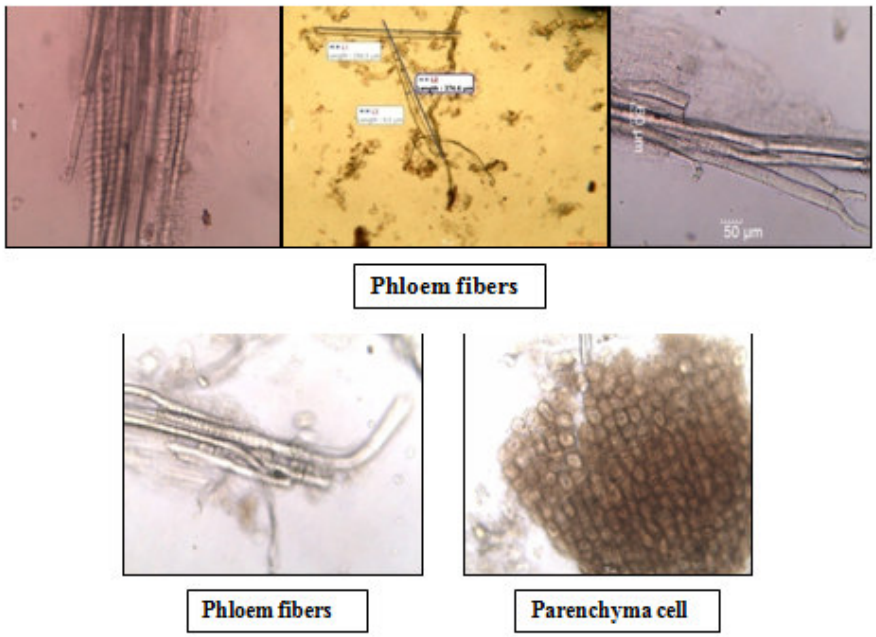

Figure 4
Powder analysis of Bark of Putranjiva roxburghii Wall

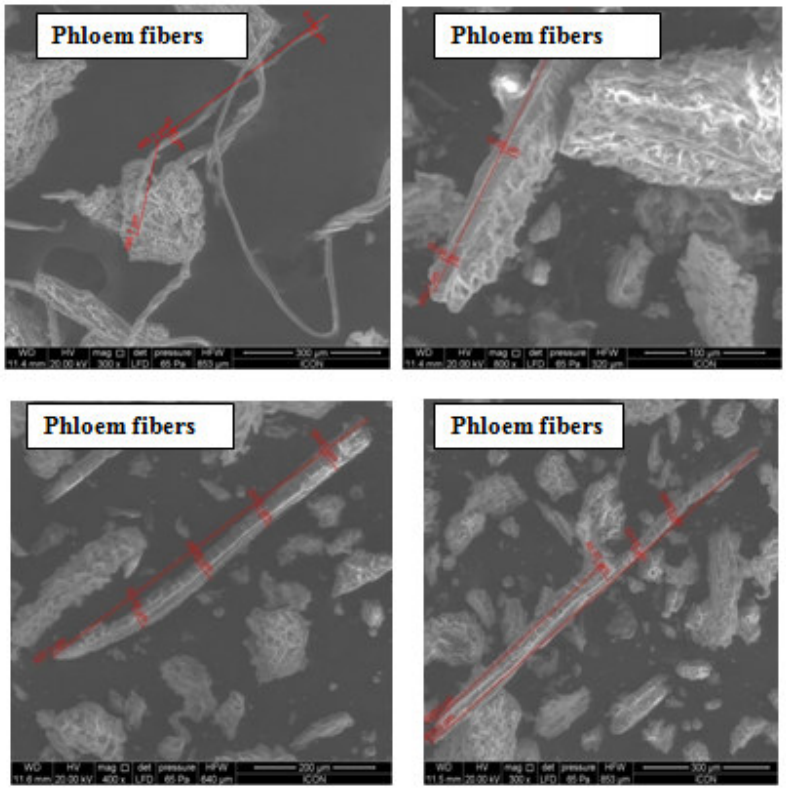

Figure 5
SEM Images of powder of Bark showing parenchyma cells and phloem fibers

CONCLUSION

Putranjiva roxburghii of Euphorbiaceae is commonly found tree of tropical region. The taxon is reported to have shown various therapeutic properties and it has been traditional used to cure different ailments. The bark of this taxon has not been studied so far. In the present work, the pharmacognostic and phytochemical study of the bark of Putranjiva has been carried out. The detailed phytochemical analysis of the bark reveals that it contains adequate amount of tannins, saponins, steroids and terpenoids, which shows tremendous pharmacological potential of further studies. The morphoanatomical and pharmacognostic study of this plant however, help in the identification and authentification of taxon. Furthermore, the quantification of above mention phytoconstituents merits further studies.

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CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest.

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