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Pharmacognostical and preliminary phytochemical evaluation of stem and root bark of *Benkara Malabarica* (Lam.) Tirveng.

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Abstract
Traditional medicinal systems gives much emphasis on the quality of raw drug materials. Pharmacognostical study helped to standardize the identity and preliminary phytochemical analysis revealed the presence of certain phytoconstituents which helped to understand the properties and action of the drug. Different parts of *Benkara malabarica* (Lam.) Tirveng. Family Rubiaceae, known as *phiriki* in Odisha, are used by traditional healers to combat many disease conditions. Its stem and roots are used to treat abdominal pain, throat infection, wound healing, asthma and rheumatic pain.
Material and method: Fresh stem and root bark were collected from Paikamal, Odisha following good collection practice. Pharmacognosy and physicochemical parameters were done as per standard protocol.
Results and Conclusion: The stem and root bark showed similar anatomical structure consisting of outer cork layer, cortical zone, secondary phloem and medullary rays. Powder microscopy reveals presence of prism, cluster crystal and tannin content in both barks. Physicochemical parameters reveals that pH value of both samples are slight acidic in nature. Methanol extract stem bark showed 9 and root bark showed 5 peaks at 254 nm.

Keywords: Anukta dravya, Rubiaceae, *Benkara malabarica*, pharmacognosy, prilimary phytochemical investigation, HPTLC

Introduction
Exploration of medicinal plants is a continuous process and to have new pharmacopeial knowledge, Ayurveda emphasized the need of interaction of the *Vaidya* (physician) with cowherds, forest dwellers, shepherds etc, as they are the treasure of knowledge on medicinal plants [1]. *Benkara malabarica* (Lam.) Tirveng. family Rubiaceae [2] known as *Phiriki*, *Pedda* and *Pedalli* in Odia [3] has been reported for its ethnobotanical uses of its root bark in abdominal pain, throat infection, wound healing, asthma, rheumatic pain and joint pain [5, 6] and stem bark in bronchitis and asthma [7, 8]. It is a small evergreen tree, with simple, opposite, decussate leaves bearing petiole and umbel like corymbs inflorescence. Flowers blooms with white-cream colour. Bark is yellowish colour with short, simple or branched thorns with grey colour, lenticellate [4]. Though stem and root barks are used traditionally their pharmacognostical characters have not been reported to establish the identity purity and strength of the used plant materials. Hence the present study reports the pharmacognostical, Physico-phytochemical analysis and HPTLC study of stem bark and root bark.

Materials and Method
Collection and authentication
The plant *Phiriki*, growing naturally in Lumbini Bhesaj Udyan, Sri Nrusinghnath Ayurveda college & Research Institute, Paikmal, Odisha, was identified by local vaidya and taxonomist and its respective botanical name i.e. *Benkara malabarica* (Lam.) Tirveng. was confirmed by studying the morphological characters comparing them with various characters described in different floras and books [9] Sample was collected in the month of January 2019 and specimen was authenticated by pharmacognosist. Herbarium of the sample was deposited to institute's pharmacognosy laboratory and provided with authentication number (specimen no. phm/6295/19-20)

Pharmacognostical evaluation
Macroscopic characters of the stem bark and root bark such as shape, size, surface, colour etc, were studied by observing them. Thin free hand transverse section of the stem bark and root bark were observed under the microscope to evaluate microscopic characters. Photographs were taken by using canon digital camera attached to Carl zeiss trinocular microscope in pharmacognosy laboratory. Powders (80#) of stem bark and root bark were studied for organoleptic and microscopic characters after proper mounting and staining with different reagents. [10]

Physico-chemical and Phytochemical parameters
Loss on drying, extractive values, Ash value, Acid insoluble ash etc. were carried out as per the guidelines of The Ayurvedic Pharmacopeia of India. [11] Qualitative analysis was performed to detect primary and secondary metabolites in water and alcohol extracts of stem bark and root bark.

HPTLC study
Methanolic extract of stem bark and root bark were exposed to HPTLC study. The solvent system used for the study is toluene: Acetic acid (7: 2: 1) Chromatographic conditions:

Application mode was CamagLinomatV, Development Chamber used was of Camag Twin trough Chamber. Precoated Silica Gel GF254 Plates were used. Chamber saturation was done for 30 min and development time was 30 min. The plate was scanned in Camag Scanner III with Deuterium lamp, Tungstan Lamp as detectors and Wincats software was used for data analysis.

Results and Discussion

Morphology

The details of the characters of stem and root bark of *Benkara malabarica* are described in table 1 & fig.1A and table 2 & fig.2A respectively.

Table 1: Macroscopic characters of stem bark **(**Fig.1A)

Average length of procured stem bark	12cm to 13cm
Touch	Rough.
Colour	Bark is grey and blaze yellowish.
Odour	Aromatic and pungent.
Taste	Bitter, astringent.
Fracture	Fibrous with irregular surface.

Table 2: Macroscopic characters of root bark (Fig. 2A)

Average length of procured root bark	4cm to 5cm
Touch	Rough.
Colour	Externally outer most layers dark greyish brown while inner one reddish brown and internally dark greyish white.
Odour	Aromatic.
Taste	Bitter, astringent.
Fracture	Short
Appearance	Outer layers with shallow cracks.

Microscopic characters

Stem bark: Diagrammatic section of stem bark shows outer cork followed by wide cortex with stone cell Secondary phloem and medullary rays.

Details section shows the outer most cork layer, cells are barrel to rectangular tangentially elongated compactly arranged in several layers can be differentiated into outer and inner cork. Outer cork cells are largely filled with tannin content while inner ones with light brown content. Wide cortex made of parenchyma cells. Most of the parenchyma cells are filled with simple starch grain, oil globules and brown content. Large tenniferous cells widely distributed throughout the cortical region filled with tannin. Discontinues patches of stone cells with wide lumen distributed throughout the section just beneath the cork region. Pericyclic fibres 3 to 5 layers lignified distributed throughout the cortical region. Secondary phloem situated just beneath the lignified pericyclic fibres consist of sieve elements and its fibre. Pericyclic fibres are multiseriated slightly rounded and angular. Some of the medullary rays are filled with starch grain and tannin. (Fig. 1B to 1F)

Root bark: Diagrammatic section of root bark shows outer cork followed by wide cortex, stone cells, secondary phloem and medullary rays.

Detail section shows cork, the outer most layer. Cork cells are angular tangentially elongated compactly arranged several layered cork differentiated into outer cork and inner cork. Outer cork cells are filled with tannin content and are lignified. Inner cork cells are slightly filled with brown contain. Followed by wide cortex, made of parenchyma cells. Most of the parenchyma cells filled with simple and compound starch grain and very few of brown contain. Tanniferous cells few in number as compared to the stem bark. Some of Parenchyma cells filled with cluster crystal also. Discontinues patches of several layer of stone cells largely occupied within the cortical region. Few layer of lignified pericyclic fibres followed after stone cells layer. Some of the secondary phloem just beneath the pericyclic fibres consists sieve elements and fibres. Medullary rays uniseriated to biseriated loaded by simple and compound starch grain and cluster crystals. (Fig. 2B to 2F)

Powder microscopy

Stem bark: Powder colour tortilla (brown); odour, slight oily; taste; texture, fibrous.

Diagnostic powder characters of stem bark shows crystal, cluster crystal, overleaping cork cells, cork in surface view, prismatic crystal, stone cells, sclereids, pitted sclereids, simple and compound starch grain, tannin content, thick walled fibers. (Fig. 3A to 3H),

Root bark: Root powder colour creamish light yellow; odour, slightly woody; texture, fibrous. Diagnostic powder characters of root bark shows prism, cluster crystal, cork in surface view, cork in transvers view, simple and compound starch grains, presence of simple fibres, sclereids, Stone cells, oil globule, tannin content. (Fig. 4A to 4H)

Physico-chemical analysis and phytochemical analysis

Physico-chemical analysis

The detailed results of physicochemical parameters are described in table 3. Both the stem bark and root bark powders of *B. malabarica* were found to be devoid of any foreign matter, which may be due to the good collection and storage practices. Loss on drying more or less similar in both stem bark and root bark. The percentage of ash value was found higher in root bark compared to stem bark. Water soluble extractive represents the percentage of water soluble active constituents such as tannins, sugars, plant acids, mucilage, glycosides and proteins etc., found higher in root bark followed by stem bark. Percentage of Methanol soluble extractive represents the constituents such as alkaloids, steroids, volatile oils etc. found higher in root bark followed by the stem bark. pH was acidic in both samples.

Preliminary qualitative analysis

Preliminary phytochemical analysis was done for the identification of different chemical constituents in the samples. The results of phytochemical analysis of stem bark and root bark of *B. malabarica* are compiled in table 4. Protein, amino acids and alkaloids were absent in Methanol soluble extracts of both samples. Carbohydrates, steroids, glycosides, saponins, tannins and flavonoids were present in stem bark and root bark of *B. malabarica*.

HPTLC study

The chromatograms were recorded as densitographic profile under UV radiation at short UV (254 nm) and long UV (366 nm). The methanol extract *B. malabarica* stem bark showed 9 and 6 peaks at 254 and 366 nm respectively, whereas root bark showed 5 and 6 peaks at 254 and 366 nm respectively. At 254 nm, 0.03, 0.22, and 0.90 R_f value were common

whereas 0.03, 0.22, and 0.97 R_f value were common at 366 nm wavelength. 0.03, 0.22 and 0.54 R_f values were common in both sample. The 3D graphs, peak display at UV ranges and spectral comparison are depicted in figure 5. The common Rf values indicates the presence of same chemical components.

Table 3: Physicochemical parameters of powder of *B. malabarica* stem bark and root bark.

Sr. No.	Physico-chemical parameters	Stem bark	Root bark
1.	Loss on drying(%w/w)	2.63	0.95
2.	Ash value(%w/w)	3.64	8.13
3.	Water Soluble Extract(%w/w)	27.36	30.15
4.	Methanol Soluble Extract(%w/w)	20.13	24.12
5.	pH	6.0	5.5

Table 4: Result of Qualitative Tests of methanolic extract of *B. malabarica* root and stem bark

Sr. No.	Active constituent	Test	Stem bark	Root bark
1.	Carbohydrates	Molisch's test	+	+
2.	Protein	Biuret test	-	-
3.	Amino acids	Ninhydrin test	-	-
4.	Steroids	Salkowaski reaction	+	+
5.	Glycosides	Keller Killiani test	+	+
6.	Saponins	Foam test	+	+
7.	Alkaloids	Dragendorff's test	-	-
8.	Tannins	Ferric chloride	+	+
9.	Flavanoids	Lead acetate	+	+

"+": Positive, "-": Negative

Table 5: HPTLC Studies of methanolic extract of stem bark and root bark of *B. malabarica.*

Sample	Solvent system	254 nm (Short UV)		366 nm (Long UV)	
		No. of spots	Rf values	No. of spots	Rf values
Stem bark	Toluene: Ethyl acetate: Acetic acid (7: 2: 1)	9	0.03, 0.22, 0.33, 0.39, 0.49, 0.53, 0.69, 0.88, 0.91	6	0.03, 0.11, 0.22, 0.41, 0.52, 0.97
Root bark		5	0.03, 0.13, 0.22, 0.54, 0.90	6	0.03, 0.23, 0.42, 0.54, 0.87, 0.96,

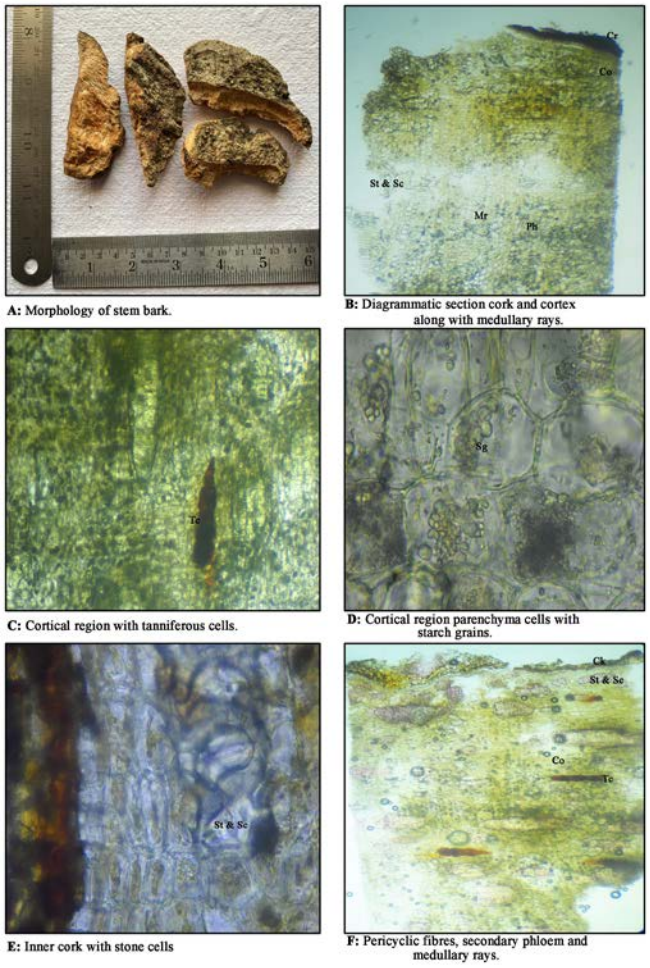

Fig 1: Morphology and microscopic key characters of *B. malabarica* stem bark

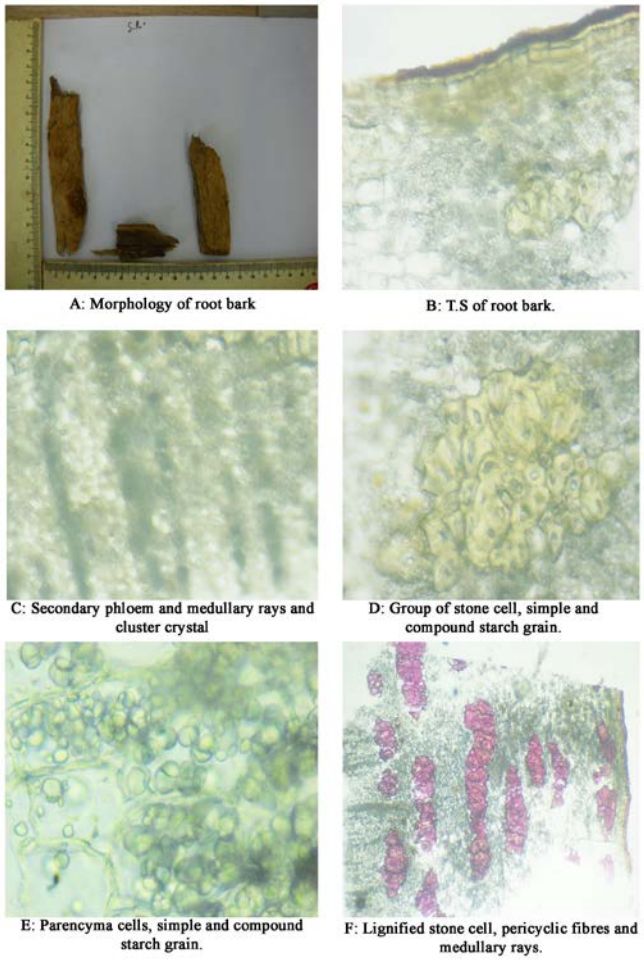

Fig 2: Morphology and microscopic of *B. malabracia* root bark

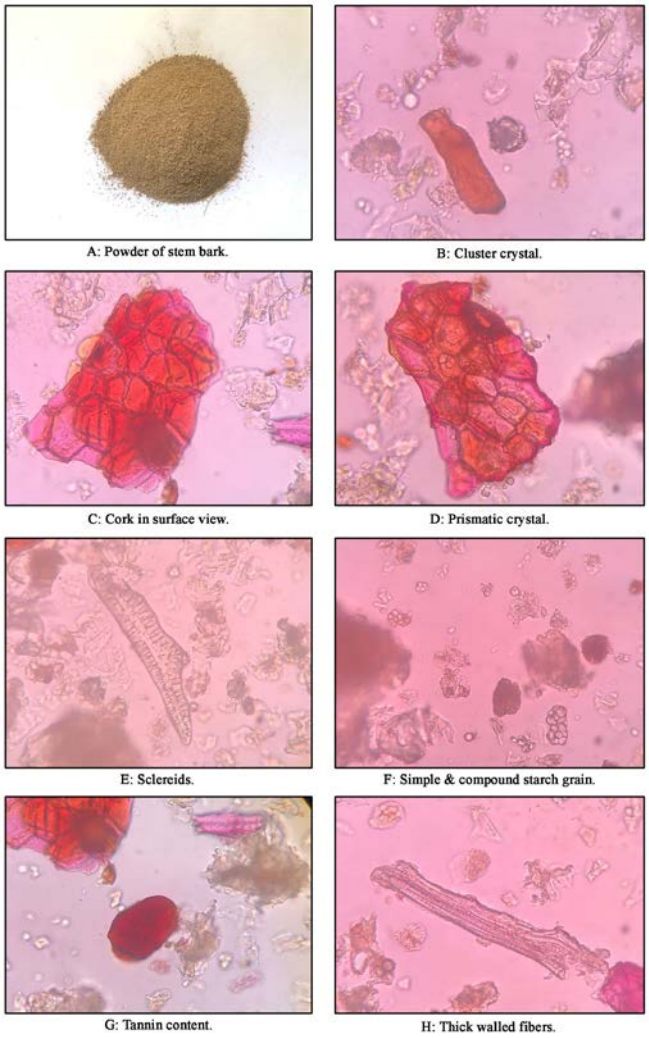

Fig 3: powder microscopy of *B. malabarica* stem bark

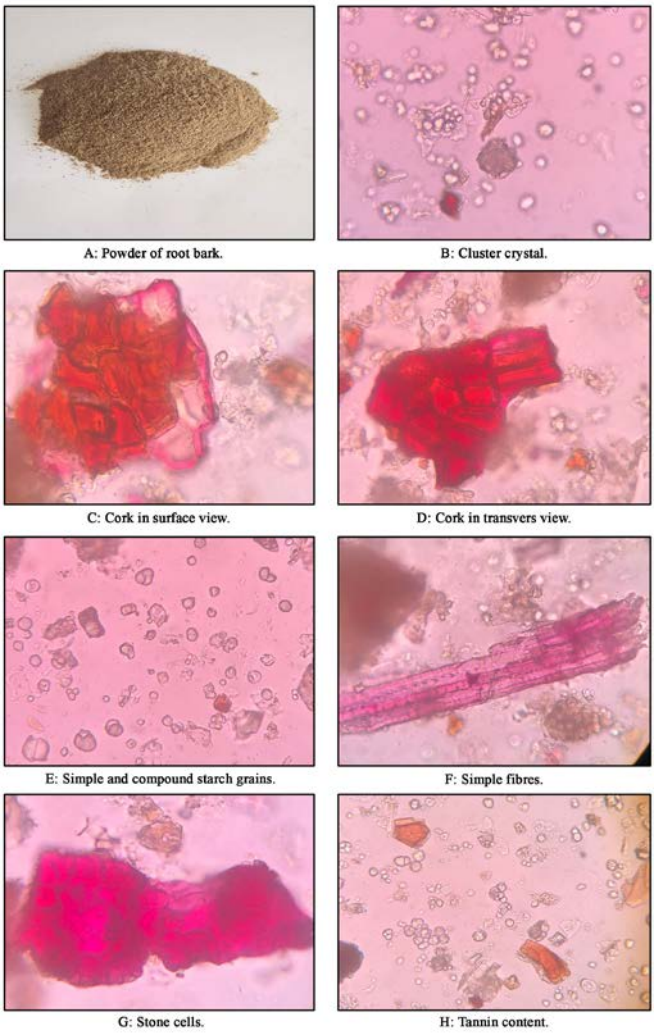

Fig 4: Powder microscopy of *B. malabarica* root bark

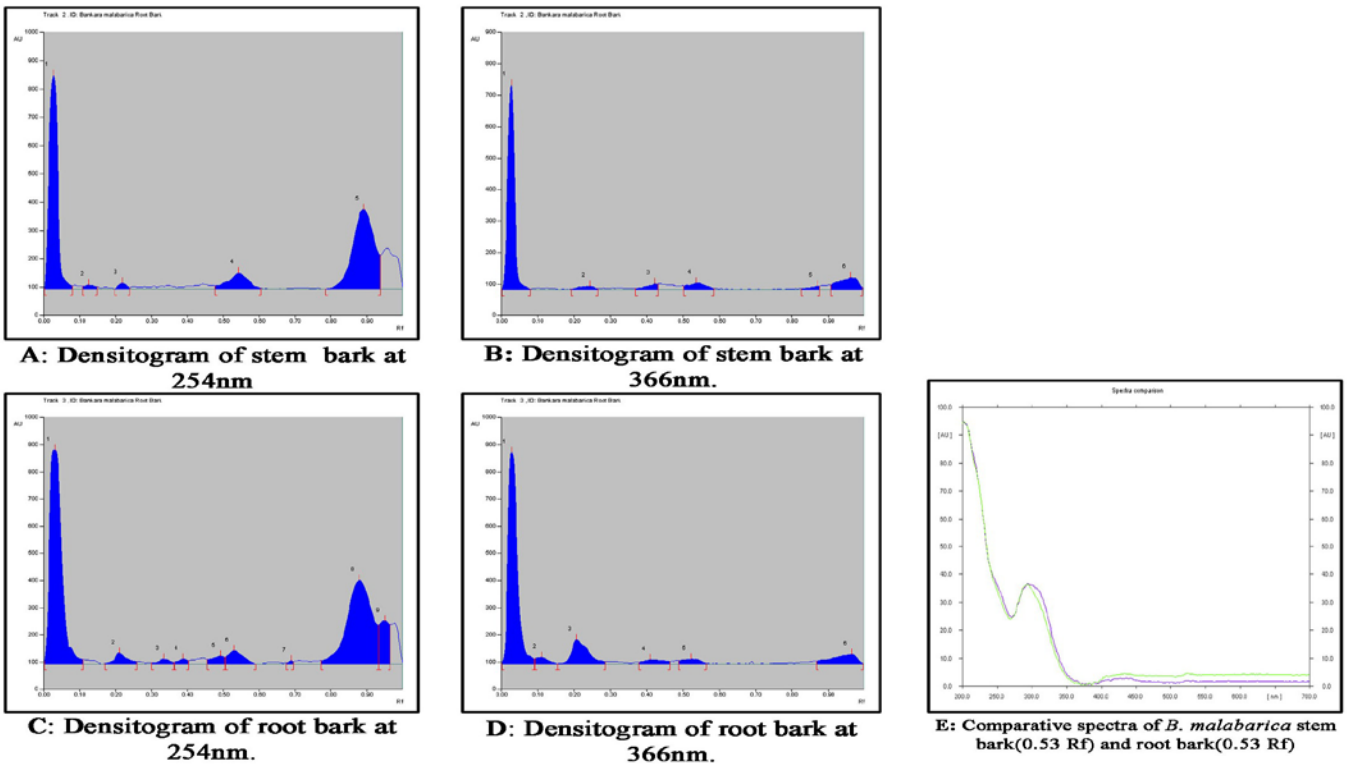

Fig 5: HPTCL profile of *B. malabarica* stem bark and root bark.

Conclusion

Benkara malabarica (Lam.) Tirveng. stem bark contains multiseriated medullary rays filled with starch grain and tannin. Whereas in root bark medullary rays are uniseriated to biseriated loaded by simple and compound starch grain and cluster crystals In HPTLC study, 0.03, 0.22 and 0.54 R_f values were common in both sample which indicates the presence of same chemical components. All these parameters can act as diagnostic tool for identification and authentication of the drug.

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