

Pharmacognostical and Physico-Chemical Studies of the Bark of *Baccaurea ramiflora* Lour.

A Choudhury*, N A Patel

Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehsana - 384012, Gujarat, India.

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ABSTRACT

Baccaurea ramiflora Lour., belongs to the family Euphorbiaceae, native to Southeast Asian countries. The present study has been attempted to evaluate the macroscopic and microscopic characteristics, physico-chemical parameters as well as phytochemical analysis of the bark of *Baccaurea ramiflora*. The transverse section of the bark showed presence of pitted stone cells, cork, phelloderm, phloem parenchyma, funnel shaped medullary rays and calcium oxalate crystals. The powder microscopy of the bark showed broken fibers, parenchyma cells, both rosette and prism type of calcium oxalate crystals and stone cells. Physico-chemical constants such as moisture content, ash values and extractive values were established. Phytochemical analysis showed the presence of phytosterols, carbohydrate, gums and mucilage in the bark. These studies will be helpful in identification and authentication of the plant material. Such information can act as reference information for correct identification of particular plant and may be useful in making a monograph of the plant.

Keywords: *Baccaurea ramiflora*, powder microscopy, phytochemical study.

INTRODUCTION

India has about 427 tribal communities and more than 130 tribal communities reside in Northeastern states of India. Northeastern India, comprises of Seven Sister States (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura), and the Himalayan state of Sikkim^{1,2}. The Northeast Himalayan region of India is rich in diversity of wild edible plant species, particularly in Meghalaya. Meghalaya is the wettest state of India. With average annual rainfall as high as 2818mm. Meghalaya has a unique array of vegetation, ranging from tropical and sub-tropical to temperate. This is due to the diverse topography, varied and abundant rainfall and differential climatic and edaphic conditions within the different regions of the state³. *Baccaurea ramiflora* Lour., synonym is *Baccaurea sapida* Muell. Arg and *Baccaurea wrayi* King ex Hook. f. belongs to the family Euphorbiaceae. It is commonly known as Burmese grape. It is an evergreen, dioecious tree 12-15m in height and 0.6-1.5m in girth, native to Southeast Asian countries. In India, the tree is found wild or cultivated in sub-Himalayan tract at an altitude of 900m⁴. It flowers during April-May and fruits ripen during rainy seasons. Trees are cultivated for its edible fruits. Fruits are oval to round in shape and turns yellowish brown when ripens. The juice of the bark is used in constipation^{4,5}. Leaves are elliptical, lanceolate or obovate and flowers are unisexual. Leaves and flowers are reported to be eaten. Leaves and barks yields green dye and are used in dyeing⁴. In Chinese medicine, the whole plant is used to treat the pain in rheumatoid arthritis and injuries⁶. In Thailand it was reported that the nutrient composition

of raw fruit per 100gm was 88.2gm moisture, energy 48kcal, protein 0.7gm, fat 0.3gm, carbohydrate 10.5gm, ash 0.3gm, Ca 2mg, Fe 3mg and vitamin C 55mg⁷. In Mizoram, India, the plant is used for stomach ulcer, stomach ache and colic in traditional ethnomedicine. Seed contains 4.8-6 percent annatto dye which is used for coloring silk, cotton for orange-red colour⁸. In India, seed oil of *Baccaurea ramiflora* was analyzed and found acid value 1.127mg KOH/gm oil, moisture content 0.103% and iodine value 80.32g I2/100gm respectively. The density and refractive index of the oil were found to be 0.8674gm/cm³ and 1.4672 respectively. Saturated fatty acids such as palmitic acid 33.67%, stearic acid 19.38% and arachidic acid 9.69%, oleic acid 24.48, total fatty acids 60%, unsaturated fatty acids 12.75% and trans-11-eicosenoic acid⁹. Leaves of *Baccaurea ramiflora* possess hypoglycemic and hypolipidemic activity, which may be due to presence of flavonoids, tannins, terpenes and steroids¹⁰. Seeds showed to possess analgesic activity¹¹. Seeds are crushed to cure diarrhoea¹².

MATERIALS AND METHOD

Collection and preparation of plant specimen

The fresh barks of *Baccaurea ramiflora* Lour (Euphorbiaceae) were collected in bulk from the Experimental Botanic Garden, Barapani under Botanical Survey of India, Shillong in the month of June-July 2014, and was authenticated by the Botanist Dr. A. A. Mao, Scientist - E, BSI, Eastern Regional Centre, Shillong, India. The voucher specimen (No-AC/004/2014) was preserved for future reference. The herbarium was



A



B

Figure 1 : (A) *Baccaurea ramiflora* – Stem, (B) *Baccaurea ramiflora* – Bark.Table 1: Organoleptic evaluation of the bark of *Baccaurea ramiflora*.

Organoleptic characters	Observation
Condition	Dry
Shape	Channelled, single quills
Fracture	Splintery, Short
Size	Varies upto 22cm(Length), 3 - 5cm(Breadth), 3 -5mm(Thickness)
Odour	None
Colour	Outer surface – Brownish, Inner surface –Light brown
Taste	No taste at first but at the end slightly bitter.

deposited in Shree S.K Patel College of Pharmaceutical Education and Research, Department of Pharmacognosy, Ganpat University, India. The barks were shade dried at room temperature for 10 days, coarsely powdered and stored in air tight containers for further studies.

Pharmacognostical Studies

Macroscopic evaluation

Macroscopic study included the determination of various organoleptic characters viz., color, odor, taste, texture, fracture, and the shape of bark of *Baccaurea ramiflora*¹³⁻¹⁵ which are reported in Table-1.

Microscopical evaluation

Sections of the bark were taken with the help of sharp scalpel. The sections were cleared using chloral hydrate and they were stained with phloroglucinol and conc. HCl (1:1) finally mounted with glycerin. Photomicrographs of the sections were captured with the help of Scope Photo Image Software, CatCam130, manufactured by Catalyst Biotech (Figure-2).

Powder microscopy was done by staining the crude drug and also without staining. For staining, small amount of powdered drug was heated with chloral hydrate for 2 minutes, this helped to clear the crude drug. Phloroglucinol and concentrated HCl (1:1) were used as staining agent. Finally, glycerin was used to fix the specimen. Whereas in

unstained, the powdered drug was heated using chloral hydrated and glycerin was used to fix the specimen in the slide and covered with a cover slip. Photomicrographs of the microscopical sections were captured with the help of Scope Photo Image Software, CatCam130, manufactured by Catalyst Biotech¹³⁻¹⁵(Figure 3).

Determination of Physico-Chemical Parameters

Moisture content

Weighed powdered bark of *Baccaurea ramiflora* was taken in a china dish. It was kept for 30 minutes in a hot air oven which was adjusted to 105 -110°C. The percentage of moisture content was then calculated with reference to the air dried drug¹⁴.

Ash values

Total ash value

Weighed powdered bark of *Baccaurea ramiflora* was taken in a dried silica crucible. It was incinerated at temperature 450°C until freed from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air dried sample¹⁴.

Acid insoluble ash value

The total ash obtained was boiled for 5 mins with 25 ml of 2N HCl, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible, cooled and the residue obtained was weighed. Finally, the percentage of acid insoluble ash was calculated with reference to the air dried drug¹⁴.

Water soluble ash value

The total ash obtained was boiled with 25 ml of water for few mins. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 mins at temperature not exceeding 450°C. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug¹⁴.

Sulphated ash value

The total ash obtained was cooled and moistened the residue with 1ml of sulphuric acid and heated until all the fumes are no longer evolved and ignited at 800±250°C

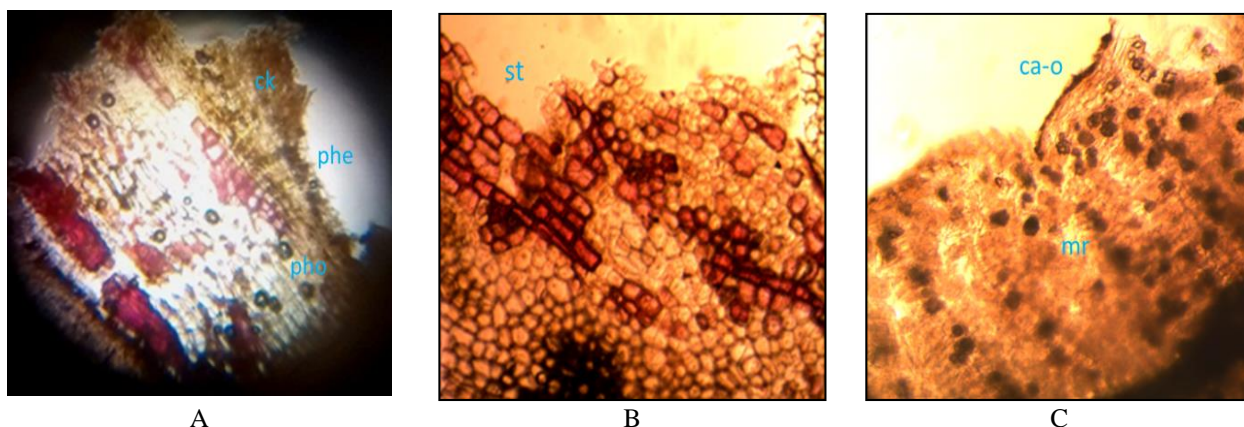


Figure 2: A-Section showing cork(ck), phelloderm(phe) and phloem parenchyma cells(pho), B-Section showing pitted stone cells(st), C-Section showing funnel shaped medullary rays(mr) with ca-oxalate crystals(ca-o).

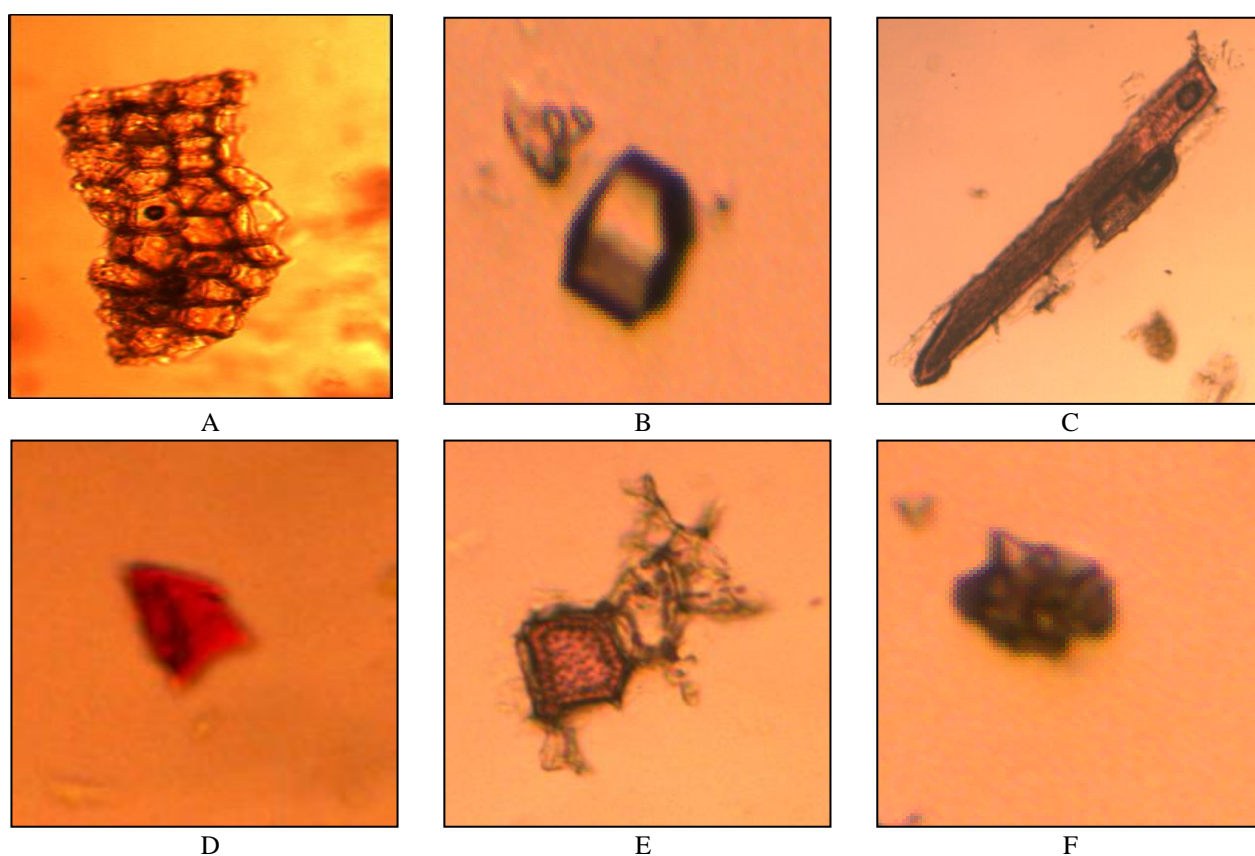


Figure 3: (A) Cork Cells, (B) Unstained Fiber, (C) Prism shaped ca-oxalate crystals, (D) Pitted stone cells, (E) Rosette shaped ca-oxalate crystals, (F) Brown coloring matter.

Table 2: Physico-chemical parameters of the bark of *Baccaurea ramiflora*.

Physicochemical parameters	Values % w/w
Moisture Content	1.4±0.2
Total ash value	21.5±0.5
Acid insoluble ash value	1.5±0.01
Water soluble ash value	1.95±0.01
Sulphated ash value	20±2.4
Methanol soluble Extractive value	4±0.1
Water soluble Extractive value	8±0.5

until all the black particles have disappeared. Ignition is

conducted in a place protected from air currents. The crucible is allowed to cool, added a few drops of sulphuric acid and heated. Ignited as before, allowed to cool and weighed. The difference in weight represents the sulphated ash¹⁴. The results are reported in Table 2.

Extractive value

Weighed bark of *Baccaurea ramiflora* was first coarsely powdered and macerated with 100 ml of alcohol of specified strength in a closed flask for 24 hours. The flask was shaken frequently for 6 hours and allowed to stand for 18 hours. The extract was filtered rapidly and was evaporated up to 25ml rapidly to dryness in a china dish. Further, it was dried to obtain a constant weight. The

Table 3: Behavior of powdered bark of *Baccaurea ramiflora* towards some chemicals.

DRUG + CHEMICALS	COLOR
Powder + Picric acid	Yellowish
Powder + Conc. HNO ₃	Reddish Brown
Powder + Conc. HCl	Dark Brown
Powder + Conc. H ₂ SO ₄	Blackish
Powder + Glacial acetic acid	Light Brown
Powder + 5% FeCl ₃	Yellowish
Powder + KOH	Light Brown
Powder + Iodine	Reddish Brown

Table 4: Phytochemical constituents of the bark of *Baccaurea ramiflora*.

Phytochemical constituents	Acetone extract	Aqueous extract
Alkaloids	-	-
Glycosides	-	-
Saponins	-	-
Phytosterols	+	-
Phenolics and tannins	-	-
Gums and mucilage	+	+

“+” Present, “-“ Absent

percentage yield of alcohol-soluble extractive with reference to air-dried drug was finally calculated. Similar procedures were followed with water to find out respective extractive value¹⁵. The results are reported in Table 2.

Phytochemical Screening

The powdered drug was subjected to systematic phytochemical screening by successively extracting them in different solvents in the increasing order of the polarity i.e. Petroleum ether (60 - 80°C), benzene, chloroform, acetone, methanol and chloroform-water and testing for the presence of chemical constituents such as glycosides, phytosterols, gums and mucilage¹⁶. The results are reported in Table 4.

RESULTS

Histology of the bark

Section of the bark consists of 2 to 3 layers of cork cells followed by cortex. Cortex consist of 2 to 3 layers of phelloderm followed by thick walled sclerenchyma layers, followed by 8 to 10 layers of parenchyma. Bundles of pericyclic fibers were seen in this layer. Next 2 to 3 layers consist of thick walled sclerenchyma which is followed by medullary rays. Funnel shaped medullary rays showing ca-oxalate crystals, rosette type. Prismatic crystals were seen below the medullary rays (Figure 2A-C).

Powder Microscopy

Diagnostic characters of powder microscopy of bark are (Figure 3A-F)

Fibres: Long, thickened, broken lignified phloem fibres are seen.

Parenchyma cells: Group of lignified parenchyma cells are present.

Coloring matter: Brownish coloring matter are seen throughout.

Cork: Cork cells appear reddish brown in color.

Calcium oxalate crystals: Both rosette and prism type of calcium oxalate crystals are present.

Stone cells: Thick walled pitted and non-pitted stone cells are present.

DISCUSSION

Proper authentication and identification of plants play a significant role in the field of research and health care system. Detailed pharmacognostical study of plant drug is very necessary before its use in the field of research and also in pharmaceutical formulation. It helps to identify other allied species and adulterants from the authentic drug. The present study has been attempted to evaluate the macroscopic and microscopic characteristics, physico-chemical parameters as well as phytochemical analysis of the bark of *Baccaurea ramiflora*. *Baccaurea ramiflora* Lour. (Euphorbiaceae), found wild or cultivated in the North Eastern parts of India. The bark of the plant was traditionally used to treat constipation, flowers and leaves used in dyeing^{2,3}. Microscopic approach utilizes techniques such as light microscopy to analyze different characteristics of *Baccaurea ramiflora*. Section treated with iodine gave negative result for starch granules. Behavior of powdered bark of *Baccaurea ramiflora* towards some chemicals like picric acid, Conc. HNO₃, Conc. HCl, Conc. H₂SO₄, Glacial acetic acid, 5% FeCl₃, KOH and Iodine solution were observed (Table 3). Total ash value, acid insoluble ash, water soluble ash and sulphated ash were determined and found to be 21.5%, 1.5%, 1.95% and 20% w/w respectively. Extractive values of methanol and aqueous was found to be 4% and 8% respectively. The chemical constituents such as phytosterols, gums and mucilage in acetone and aqueous extracts were found to be present. This information may be useful for further studies which are in progress.

CONCLUSION

In the present study, pharmacognostical, physico-chemical and phytochemical analysis of *Baccaurea ramiflora* were investigated. This study showed botanical characteristics that can differentiate the plant from other plants. The macroscopic description will be helpful in identification of the plant. Microscopical study in entire and powdered form of the drug is one of the aspects of histological evaluation. These results may be useful to supplement information regarding the identification of *Baccaurea ramiflora* bark. Further studies on this species are in progress.

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REFERENCE

1. Kala C.P. Ethnomedicinal botany of the Apatani in the eastern Himalayan region of India. J Ethnobi Ethnomed 2005; 1: 11.

2. Dutta B.K and Dutta P.K. Potential of ethnobotanical studies in Northeast India: an overview. *Indian J Traditional Knowledge* 2005; 4: 7-14.
3. Jeeva S. Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalaya. *Journal of Horticulture and Forestry* 2009; 1(9): 182-192.
4. Anonymous. *The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials. Volume 2-B*, CSIR, New Delhi, 1988, 1.
5. Asolkar L.V, Kakkar K.K, Chakre O.J. *Glossary of Indian Medicinal Plants with active principles. Volume-I (A-K)*, CSIR, New Delhi, 1965-1981, 112.
6. Lin Y.F, Yi Z and Zhao Y.H. *Chinese Dai Medicines Colorful illustrations*. Yunnan Nationality Press. 1st ed; 2003.
7. Puwastein P, Burlingame B, Raroengwicht M and Sungpuag P. *ASEAN food composition tables*. Institute of nutrition, Mahidol University. Salaya, 2000, 157.
8. Abdullah A.T.M, Hossain M.A and M.K. Bhuiyan. Propagation of Latkan (*Baccaurea sapida* Muell.Arg.) by Mature Stem Cutting. Institute of Forestry and Environmental Sciences, University of Chittagong. *Research Journal of Agriculture and Biological Sciences* 2005; 1(2): 129-134.
9. Dutta K, Basumatary S and Deka D.C. Physicochemical properties of *Baccaurea ramiflora* (Letuk) seed oil. *Journal of Chemical and Pharmaceutical Research* 2013; 5(9): 210-218.
10. Ullah M.O, Urmi K.F, Howlader M.A, Hossain M.K, Ahmed M.T and Hamid K. Hypoglycemic, Hypolipidemic and Antioxidant effects of leaves methanolic extract of *Baccaurea ramiflora*. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4(3): 266-269.
11. Akter S, Majumder T, Karim R, Ferdous Z, Sikder M. Analgesic activities of *Geodorum densiflorum*, *Diospyros blancoi*, *Baccaurea ramiflora* and *Trichosanthes dioica*. *Journal of Pharmacognosy and Phytochemistry* 2015; 4(3): 209-214.
12. Rout J, Sajem A.L, Nath M. Traditional Medicinal Knowledge of the Zeme (Naga) tribe of North Cachar Hills District, Assam on the Treatment of Diarrhoea. *Assam University Journal of Science & Technology: Biological and Environmental Sciences* 2010; 51: 63-69.
13. Khandelwal KR. *Practical pharmacognosy techniques and experiments*. Nirali Prakashan. New Delhi, 2002, 15-163.
14. Anonymous. *Quality Control Methods for Medicinal Plant Materials*. Geneva: Office of the Publications, World Health Organization, 1988, 8-46.
15. Kokate CK. *Practical Pharmacognosy*. 1st ed. Vallabh Prakashan, New Delhi, 2005, 111.
16. Kokate CK. Plant constituents. In *Practical Pharmacognosy*. 3rd ed. Vallabh Prakashan, New Delhi, 1991, 107-125.