Indian Journal of Natural Products and Resources
Vol. 7(1), March 2016, pp. 59-64

Pharmacognostic and preliminary phytochemical evaluation of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet bark

Barnali Gogoi*, Bibhuti Bhusan Kakoti, Nilutpal Sharma and Sudarshana Borah
Department of Pharmaceutical Sciences, Dibrugarh University, Assam - 786 004, India

Received 27 July 2014; Accepted 18 December 2015

Pharmacognostic, physico-chemical and preliminary phytochemical studies of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Family Lauraceae) bark was carried out. Physico-chemical parameters such as total, acid insoluble and water soluble ash value were determined. In microscopic studies, transverse and longitudinal section of bark and its powder characters were studied and characteristic features were established. Different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents, viz. alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds. The present study of *C. bejolghota* bark will be useful in laying down standardization and pharmacopoeia parameters and information obtained can be used as markers in the identification.

Keywords: *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, Microscopy, Pharmacognostical, Phytochemical.

IPC code; Int. cl. (2015.01)–A61K 36/00

Introduction

Pharmacognosy may be defined as an applied science that deals with the biological, biochemical and economic features of natural drugs and their constituents[1]. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained. It deals with medicinal plants as crude herbs or extracts, pure natural compounds and foods having health benefits. The scope of pharmacognosy is defined as the study of physical, chemical, biochemical and biological properties of drugs, drug substances, potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources[2]. With the present search of interest in the phyto-therapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity and standardization of the plant material becomes essential. Phytochemical investigation involves extraction of plant materials, separation and isolation of the constituents of interest, characterization of isolated molecules, investigation of biosynthetic pathways and quantitative evaluation. The classical mode of extraction naturally depends on the texture and water content of the plant material being extracted and the type of substance that is being isolated. The procedure for obtaining drug substances from dried plant tissue (whole plant, root, leaf and dried seeds) is either of the continuous hot percolation, by Soxhlet apparatus or cold percolation with a range of solvents like hexane, petroleum ether, chloroform, ethanol, methanol, etc.

The genus *Cinnamomum* belonging to family Lauraceae comprises of 270 species which occur naturally in Australia and Asia. They are evergreen trees and are mostly aromatic and economically important. About 20 species are found in India[4]. Cinnamon is a common spice used across different cultures around the world for several centuries. *Cinnamomum verum* J. Presl and *C. cassia* (L.) J. Presl barks are among the earliest known spices used by the human kind and were among the spices sought after by most of the 15th and 16th century European explorers[4].

C. bejolghota (Buch.-Ham.) is a medium- to large-sized evergreen tree with aromatic stem, leaf, flower, bark and panicle. The plant is distributed in Eastern part of Himalayas up to an altitude of 2100 m. The plant is found in Jorhat, Sibsagar, Golaghat, Nowgaon and Kamrup districts of Assam[5]. The plant is found in

*Correspondent author
E-mail : barnali.gogoi88@gmail.com
Phone : 09435865691

sparse or dense forests on mountain slopes and in valleys; 600-1800 m (Bangladesh, Bhutan, India, Laos, Myanmar, Nepal, Thailand, Vietnam)[6]. It is known as *Pati-hunda*, *Naga-dal-chini* in Assamese, *Ram tejpat* in Bengali, *Katkaula* in Garhwal, *Chhamejong* in Garo, *Dieng-la-si-sirmor* in Khasi, *Phatgoli* in Kumaon, *Nupsor* in Lepcha, *Thakthingsuak* in Lushai and *Krowai* in Meghalaya[7]. Despite the medicinal value of this plant[8-10], information on the pharmacognostic parameters for identification in whole and powdered form are unavailable. The present study aimed at investigating the macromorphology, pharmacognostic evaluation and phytochemical screening of the bark of *C. bejolghota* towards standardization and monograph development.

Botanical description of the plant[11]

The plant is a middle sized evergreen tree, 6-8 m tall. Bark brownish-white, brittle, inside creamish-white, turning darker-brown on exposure, 4-8 mm thick. Leaves alternate, sub-opposite or opposite, narrowly oblong to oblong-elliptic-lanceolate or ovate-lanceolate, apex obtusely acute to rarely acute, base cuneat to the decurrently acute, 1.8-5 × 4.5-14 cm, triplinerved rigidly coriaceous, glossy above, glabrous, pale beneath, each epidermal cells on lower surface represented by papillae. Panicle pseudo-terminal, axillary to solitary axillary, slender, pale yellowish-green, minutely pubescent, glabresent, usually equal to the leaves or slightly shorter, upto 13.5 cm long, sub-equal, ovate-elliptic-lanceolate, silky on both surfaces. Stamens 3+3+3, 1.5-1.75 mm long, pale yellowish-green, anther 4-locular, introrse, whorl 111 extrorse, silky pubescent to villous, glands of whorl 111 yellow, attached 1/3 of the base of the filament. Staminode 3, pale yellowish-green, 1.5 mm long, broadly sagittate head, villous, filament greenishwhite. Pistil 2 mm long, ovary globose, pale green, silky puberolous, stigma capitates.

Traditional uses

The bark is used in Assam as a spice in curry. The leaves are used for preparation of a special kind of rice-beer known as *Apong* by *Mishing* tribe of Assam[5,11]. The bark of *C. bejolghota* is used to cure bone fracture and in wound healing by smashing and heating the bark by fire followed by dressing[7]. It has been reported that *C. obtusifolium* root paste is applied to the forehead to cure headache and dizziness[8]. Fresh bark of *C. obtusifolium* after boiling in water is given for stomach disorder[9]. The slurry of woody part is used as lotion for muscle stiffness and pain, tingling and numbness, skin rashes and skin diseases and arthritis[12]. The plant is also used in traditional folklore medicine for the treatment of diarrhoea[13] and bark in the treatment of fever[14]. The *Nyshi* tribe of Papumpare District, Arunachal Pradesh use the plant in liver trouble[15]. In Manipur, *C. bejolghota* bark is used in treatment of urinary stone troubles[16].

Extensive literature search revealed lack of systemic pharmacognostic and phytochemical study on the bark of *C. bejolghota* and hence, present work was undertaken to study macroscopical, microscopical, physico-chemical and chromatographic characteristics which would serve as a standard reference for identification, authentication and for distinguishing the plant from adulterants.

Materials and Methods

Plant material

The bark of *C. bejolghota* (Buch.-Ham.) Sweet was collected from Jorhat district, Assam, India during July 2013. The plant was authenticated by Dr. A A Mao, Botanical Survey of India, Eastern Regional Centre, Shillong and a voucher specimen (No. DU/CB/2014/07, No. BSI/ERC/2014/Plant identification/882) is deposited in the Department of Pharmaceutical Sciences, Dibrugarh University, Assam[5].

Macroscopy

The macroscopy of crude drug includes its visual appearance to the naked eyes and its sensory characteristics i.e. odour, taste and touch.

Microscopy

For microscopic study of fresh bark, collected bark was washed with water. Thin sections were cut both transversely and longitudinally, boiled in 10 % potassium hydroxide solution to remove the fatty matters, colouring substances etc., stained with safranin and observed under light microscope. Photomicrographs were taken with Leica Digi 3 Photo micrographic unit at 40x magnification.

Powder Characteristics

This helps in observation of cells in powdered state and their evaluation. Powdered stem barks were sieved through 60 no. mesh, cleared with chloral hydrate (clearing agent), stained with different staining reagents, mounted in glycerine and then

observed under microscope. Photomicrographs were taken with Leica Digi 3 Photomicrographic unit at 40x magnification.

Quantitative Standards

Percentage of total ash, acid insoluble ash, water soluble ash, extractive values, loss on drying was examined and calculated[17].

Fluorescence analysis[18]

Some chemical constituents of plants show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are also assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. The behaviour of the powdered barks with different chemical reagents and their fluorescent characteristics were observed under ultraviolet (254 and 366 nm) and visible light using UV-Viewer Ultraviolet Fluorescence analysis cabinet, MAC® Macro Scientific Work.

Preliminary phytochemical studies

For preliminary phytochemical studies; powdered bark was extracted successively with Petroleum ether (40-60 °C), chloroform, ethyl acetate and methanol using a hot Sohxlet extractor. The different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents, viz. alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds[1]. Essential oil was analyzed by adding alcoholic solution of Sudan III to a thin section of powdered fresh bark[2]. The TLC of the extracts was done using Silica Gel G as adsorbent. Glass plates of 100 cm^2 size were coated with silica gel G (Himedia Labotratory, Mumbai, India) with the help of spreader to a layer of 0.25 mm thickness. After spreading, the plates were first air dried and then activated in hot air oven at 110 ºC for 30 min. After cooling, the plates were kept in a dessicator until further analysis. Mobile phases selected were benzene : methanol (80 : 1), hexane : dichloromethane (1 : 9), toluene : ethyl acetate : formic Acid (7 : 2 : 1), ethyl acetate : methanol : water (7 : 2 : 1) and chloroform : ethyl acetate (4 : 1).

Results

Macroscopy

The Colour of outer surface of the bark is brownish-white, turning darker-brown on exposure (Plate 1). Colour of inner surface is dark yellowish brown. Odour is aromatic and pleasant. Taste is sweet followed by warm sensation. Bark is found in the form of compound quills.

Microscopic studies

The transverse section of the bark shows the presence of Phloem parenchyma, Phelloderm and cork cells (Plate 2). The longitudinal section shows oil globules, medullary ray cell containing starch, soft bast cell and bast fibre (Plate 3). Power microscopy shows the presence of oil globule, fibres, sclereids and starch grains (Plate 4).

Plate 1—Bark of *Cinnamomum bejolghota*

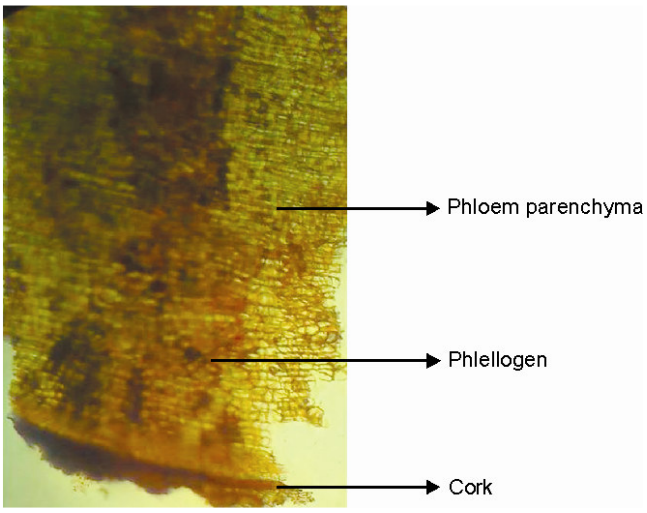

Plate 2—Transverse section of bark

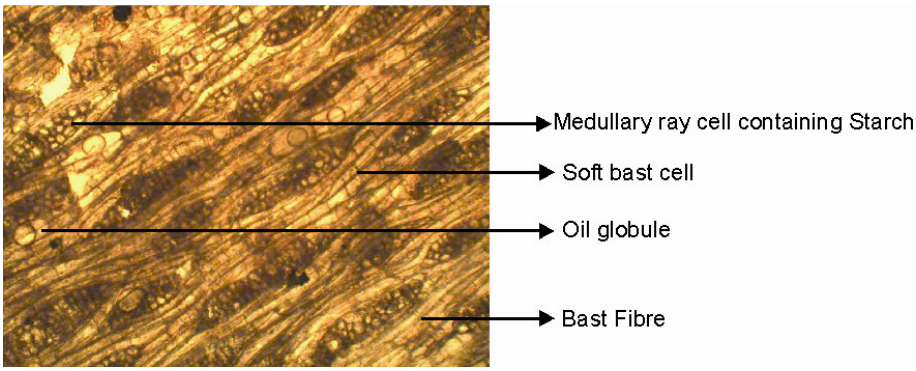

Plate 3—Longitudinal section of bark

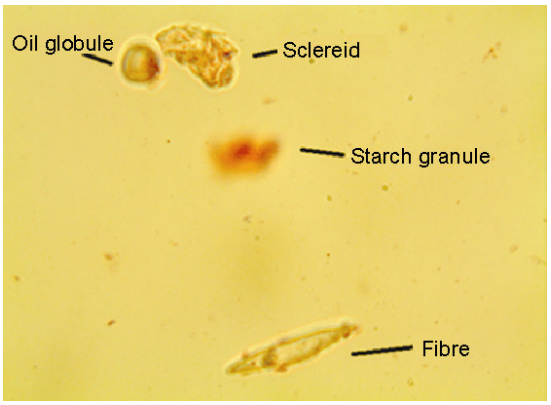

Plate 4—Powder microscopy of stem barks

Determination of quantitative standards

The purpose of standardization of medicinal plant product is to ensure therapeutic efficacy. Standardization is essentially a measure to ensure the quality control of herbal drugs[2]. Quantitative standards are a number of standards numerical in nature, which can be applied for evaluation of crude drug either in the whole or powdered form. These are the standards for identity, purity and quality of drugs. Purity depends upon the absence of foreign matter, while quality refers essentially to the concentration of active constituents in the drugs that make it valuable as medicine[16].

Table 1 reveal that total, acid insoluble and water soluble ash values are 11.70, 3.76 and 3.43 %, respectively. Water and alcohol soluble extractive values are 14.3 and 4.10 %, respectively. Moisture content was 11.80 %.

Fluorescence analysis

Powdered drug was treated with different reagents and examined under daylight and UV light (254 and 366 nm) and the results are shown in Table 2.

Table 1—Ash values, extractive values and loss on drying of *C. bejolghota* bark

S. No.	Parameters	Mean ± S.E.M
1	Total ash	11.70± 0.74
2	Acid insoluble ash	3.76± 0.3561
3	Water soluble ash	3.43± 0.69
4	Water soluble extractive	14.33± 0.80
5	Alcohol soluble extractive	4.10± 1.02
6	Loss on drying	11.80 ± 0.28

Preliminary phytochemical tests

The results of the different phytochemical tests are cited in Table 3. Presence of volatile oil was confirmed by red colour obtained by globules in Sudan- III Test.

TLC finger print profile

The TLC study of the petroleum ether and methanolic extract (Plate 5, 6) was done by selection of five different solvent systems via a series of trial and error methods. The retention factors (R_f) values for the different extracts are presented in Table 4.

Discussion

An earlier study has reported that the colour of outer surface of *C. bejolghota* is brownish-white while colour of inner bark is dark yellowish brown and that of *C. zeylenicum* collected from Hisar, Haryana is dull yellowish brown on the outer surface while inner bark is darker compared to outer surface. Odour is aromatic and pleasant for the both[19]. In microcopy, the bark of *C. bejolghota* shows the presence of phloem parenchyma, phelloderm, cork cells, oil globules but devoid of calcium oxalate crystals where as bark of *C. cortex* contain calcium oxalate crystal[20]. Total ash, acid insoluble ash values of *C. bejolghota* are 11.70, 3.76 % while that of *C. zeylenicum* is 2.5, 1.8 %, respectively. Water and alcohol soluble extractive values are 14.3 and 4.10 %

Table 2—Fluorescence analysis of powdered bark drug of *Cinnamomum bejolghota*

S. No.	Treatment	Daylight	UV Light	
			254nm	366nm
1	Powder as such	Light Brown	Light Green	Greenish brown
2	Powder + Acetic acid	Brown	Dark brown	Blackish brown
3	Powder + Ferric chloride 5 % $Fecl_3$	Yellowish brown	Brown	Black
4	Powder + Conc. Hydrochloric acid Hcl (5N)	Brownish yellow	Brown	Dark brown
5	Powder + Conc. Nitric acid (HNO_3)	Yellowish brown	Brown	Dark brown
6	Powder + Conc. Sulphuric acid (H_2SO_4)	Reddish Black	Black	Bluish
7	Powder + Iodine Solution (1 %)	Brownish Black	Brown	Brownish Black
8	Powder + Methanol	Yellowish Brown	Dark Brown	Blackish Brown
9	Powder + Picric acid	Yellowish Brown	Greenish Yellow	Black
10	Powder + NaOH Solution (1N)	Reddish Yellow	Yellow	Blackish Yellow
11	Powder + Distilled water	Deep brown	Brown	Greenish
12	Powder + Liquid Ammonia (NH_3)	Blackish Yellow	Yellow	Black
13	Powder + Conc. HNO_3 + NH_3	Yellow	Reddish	Yellowish Black
14	Powder + Dil. HNO_3	Reddish Brown	Brown	Blackish
15	Powder + 10 % Potassium dichromate solution	Deep Yellow	Yellowish brown	Black
16	Powder + Benedict's Reagent	Yellowish Blue	Yellowish	Greenish Black
17	Powder + Acetone	Brown	Light Brown	Blue

Table 3—Preliminary phytochemical analysis of *Cinnamomum bejolghota* bark extracts

Plant Constituents	Petroleum-ether extract	Chloroform extract	Ethyl acetate	Methanolic Extract
Alkaloids	-	-	-	-
Carbohydrates	-	-	+	+
Fats & Oils	+	+	-	-
Flavonoids	-	-	+	+
Glycosides	-	-	+	+
Gums	-	-	-	-
Steroids	+	-	-	-
Proteins	-	-	-	+
Saponins	-	-	+	+
Tannins	-	-	+	+
Lignin	-	-	-	+
Triterpenoids	+	+	-	-

(+) means present and (-) means absent.

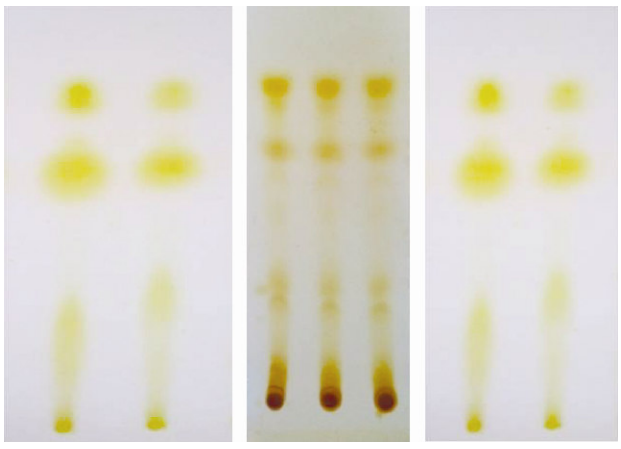

Plate 5—TLC chromatogram of methanolic extract

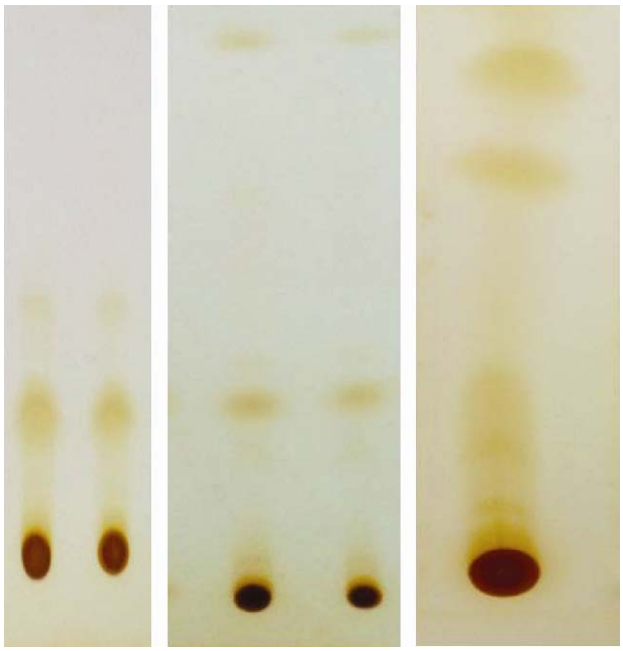

Plate 6—TLC chromatogram of petroleum extracts extract

for present study while the values are 10.8 and 6.5 %, respectively for *C. zeylenicum*. Moisture content was found to be 11.80 and 5.1 % for *C. bejolghota* and *C. zeylenicum*, respectively[19]. Methanolic extract of *C. bejolghota* contains carbohydrate, flavanoid, glycoside, lignin, tannin and phenolic compounds while methanolic extract of *C. zeylenicum contains* glycoside, tannin, alkaloid, terpenoid and saponin[21]. Phytochemical analysis of *C. bejolghota* appears to be comparable to other species of Cinnamon. Morphological

Table 4—Thin layer chromatography of *Cinnamomum bejolghota* bark extracts

Extract	Chromatography solvents	Number of spots	Rf Values	Visualizing agents used
Petroleum ether	Benzene: Methanol (80:1)	2	0.32, 0.45	5 % vanillin in Ethanolic H_2SO_4
	Hexane: Dichloromethane (1:9)	2	0.56, 0.66	Iodine
Methanol extract	Ethylacetate:Methanol:H_2O (7:2:1)	3	0.28, 0.76,0.24	Iodine
	Chloroform: Ethyl acetate(4 : 1) Acetate (4.5:0.5) acid =7:2:1	3	0.28, 0.76,0.24	Iodine
	Toluene: Ethyl acetate : Formic acid=7:2:1	4	0.23,0.56,0.74,0.88	Iodine

characters, quantitative standard, moisture content are important parameters of drug standardization.

Conclusion

Different pharmacognostic evaluations serve as standard reference for identification, authentication and for distinguishing a plant from its adulterants. Preliminary phytochemical tests showed that methanolic extract of *C. bejolghota* has greater amount of phyto-constituents compared to other three extracts i.e., petroleum ether, ethyl acetate and chloroform extracts. The powder drug exhibited different fluorescence characters due to presence of different functional groups. TLC could be helpful in identification and authentication of the drug.

Acknowledgement

The authors are thankful to Department of Pharmaceutical Sciences, Dibrugarh University, India for providing the research facilities for this work and All India Council of Technical Education, Research Promotion Scheme-2011 for providing financial assistance (Grant no. 8023/RIB/RPS-10/ (NER)/2011-12).

References

1 Evans W C, Trease and Evans Pharmacognosy, 15th Edn, W B Saunders, USA, 2005.

2 Kokate C K, Practical Pharmacognosy, 4th Edn, Vallabh Prakashan, Delhi, 1999, 107-113.

3 Sharma G and Nautiyal A R, *Cinnamomum tamala*: a valuable tree from Himalayas, *Int J Med Aromat Plants*, 2011, **1**(1), 1-4.

4 Dao N K, Hop T and Siemonsma J S, *Cinnamomum* Schaeffer., *In* Plant Resources of South-East Asia, de Guzman C C and Siemonsma J S, Eds, Backhuys Publishers, Leiden, 1999, 13, 94–99.

5 Gogoi B, Kakoti B B, Borah S and Borah N S, Antihyperglycemic and *in vivo* antioxidative activity evaluation of *Cinnamomum bejolghota* (Buch.-Ham.) in streptozotocin induced diabetic rats: an ethnomedicinal plant in Assam, *Asian Pac J Trop Med*, 2014, **7**(suppl 1), S427-34.

6 Anonymous, The Wealth of India- A Dictionary of Indian Raw Materials and Industrial Products, Vol 3 Ca-Ci, Council of Scientific and Industrial Research, New Delhi,1992.

7 Flora of China, Vol 7, Wu Z, Raven P H and Deyuan H, Eds, Missouri Botanical Garden Press, 170.

8 Ghorbani A, Langenberger G, Feng L and Sauerborn J, Ethnobotanical study of medicinal plants utilised by Hani ethnicity in Naban River Watershed National Nature Reserve, Yunnan, China, *J Ethnopharmacol*, 2011, **134**(2), 651-667.

9 Hossan Md S, Hanif A, Khan M, Bari S, Jahan R and Rahmatullah M, Ethnobotanical survey of the Tripura tribe of Bangladesh, *Am Eurasian J Sustain Agric*, 2009, **3**, 253-261.

10 Majumdar K and Dutta B K, A study on ethnomedicinal usage of plants among the folklore herbalists and Tripuri medical practitioners: Part-II, *Nat Prod Rad*, 2007, **6**(1), 66-73.

11 Baruah A and Nath S C, Taxonomic status and composition of stem bark oil of a variant of *Cinnamomum bejolghota* (Lauraceae) from Northeast India, *Nord J Bot*, 2002, **21**, 571-57.

12 Medicinal plants of Myanmar, Ministry of Health Department of Traditional Medicine, 41.

13 Chopra R N, Nayar S L and Chopra I C, Glossary of Indian Medicinal Plants: Council of Scientific and Industrial Research, New Delhi, 1956.

14 Sajem A L , Rout J and Nath M, Traditional Tribal knowledge and Status of some Rare and Endemic Medicinal Plants of North Cachar Hills District of Assam, Northeast India, *Ethonobot Leaflets*, 12, 2008, 261-267.

15 Doley B, Gajurel P R, Rethy P and Saikia B**,** A Check list of commonly used Species by the Nyshi tribes of Papumpare District, Arunachal Pradesh**,** *J Bio Sci Re*s, 2010, **1**(1), 9-12.

16 Lokendrajit N, Swapana N, Singh Ch D and Singh C B, Herbal folk medicines used for urinary and calculi/stone cases complaints in Manipur, *NeBIO*, 2011, **2**(3), 1-5.

17 Indian Pharmacopoeia, Vol 3, The Indian Pharmacopoeia commission, Central Indian Pharmacopoeia Laboratory Govt. of India, Ministry of Health & Family Welfare, Raj Nagar, Ghaziabad, 2007, 830-831.

18 Chase Jr C R and Pratt R, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J Pharm Sci*, 1949, **38**(6), 324-331.

19 Setia A and Goyal N, Comparative evaluation of different samples of Cinnamon, *Int J Res Ayurv Pharm,* 2010, **1**(2), 606-610.

20 Xiao X and Satake M, Calcium oxalate crystals in several kinds of Cinnamon bark, *Zhongguo Zhong Yao Za Zhi*, 1998, **23**(9), 515-8, 574.

21 Ramya B S and Ganesh P, Phytochemical Analysis and Comparative Effect of *Cinnamomum zeylanicum*, *Piper nigrum* and *Pimpinella anisum* with Selected Antibiotics and Its Antibacterial Activity against Enterobacteriaceae Family, *Int J Pharma Biol Arch*, 2012, **3**(4), 914-917.