Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 3, 2014

Original Article

PHARMACOGNOSTICAL AND PHYSICOCHEMICAL EVALUATION OF *CROTON BONPLANDIANUM*

NARENDRA KUMAR SINGH[1*], ARKA GHOSH[2], DAMIKI LALOO[2], VIRENDRA PRATAP SINGH[1]

[1]Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, [2]Faculty of Ayurveda, Institute of Medical Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur 231001.
Email: narendra_pharma1982@rediffmail.com

Received: 21 Feb 2014, Revised and Accepted: 13 Mar 2014

ABSTRACT

Objective*:* To evaluate the physicochemical and pharmacognostical standardization parameters for *C. bonplandianum*.

Methods: Physicochemical and pharmacognostical standardization parameters for *C. bonplandianum* was developed as per the methods provided in World Health Organization (WHO) guidelines. Quantitative estimation of flavonoid total phenolics, tannins and total saponines contents were estimated by UV–spectrophotometry. Total alkaloids content was estimated by gravimetric analysis.

Results: Morphological observations revealed that leaves of *C. bonplandianum* are simple, petiolate, alternate, 3–5 cm long, oval to lanceolate in shape, serrated margin with acute apex. The stem is cylindrical, branched, woody, light brown in color, odourless and bitter in taste. Roots are small, dark brown to black in color, cylindrical, odourless and bitter in taste. Histological observations revealed the presence of discontinuous layer of lignified sclerenchymatous cells (stone cells), paracytic stomata, stellate trichomes, xylem with scalariform thickenings and libriform fibres. Qualitative and quantitative study of phytoconstituents revealed the presence of flavonoids (0.57% w/w, equivalent to rutin), phenolics (4.25% w/w, equivalent to tannic acid) tannins (2.15% w/w, equivalent tannic acid) and saponins (7.4 % w/w, equivalent to diosgenin) and gravimetric estimation of alkaloids content was found to be 2.5 % w/w.

Conclusion: The pharmacognostical and phytochemical parameters studied in the present investigation will aid in the identification and will be helpful in maintaining the standard profile of *C. Bonplandianum*.

Keywords: *Croton bonplandianum*; Stellate trichomes; Euphorbiaceae; Alkaloids.

INTRODUCTION

Croton bonplandianum Baill. (Euphorbiaceae), commonly known as "*Ban Tulsi*", is a perennial herb found in waste lands and road side areas in India. Flowering and fruiting time of this plant is from September to December [1]. Traditionally, this plant is used to treat liver and skin disease including ring worm infection and also to cure the swelling of body [2]. Bark and roots of *C. bonplandianum* are alterative and chologogue [3, 4]. Leaves of this plant are medicinally used for the treatment of cuts and wounds, venereal sores and cholera [5]. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [1]. The genus *Croton* is rich in secondary metabolites including alkaloids, terpenoids and also possesses toxic components, phorbol esters [6, 7]. Phytochemically, the plant *C. bonplandianum* has been reported to contain rutin ($C_{18}H_{36}O_{19}$) as main constituent, crotsearinine, crotasparine and its methyl derivatives aphorbol [4, 8].

Literature survey reveals that *C. bonplandianum* is having wide range of therapeutic importance, however, no pharmacognostical and physicochemical parameter has been reported so far. Hence, the present study was aimed to scientifically develop a standard monographs on the basis of physicochemical and pharmacognostical aspects that would be beneficial in the authentication of *C. bonplandianum* for future work and use.

MATERIALS AND METHODS

Plant material

The whole plant of *C. bonplandianum* was collected during the month of October–November, 2012, from the Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh, India. The plant material was authenticated at the Botanical Survey of India, Howrah, West Bengal, India (plant identification letter: CNH/104/2012/Tech. II/950). A voucher specimen (No. PRL–03) of the whole plant has been deposited for the further reference at the Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Preparation of plant extract

The fresh plant material of *C. bonplandianum* was washed thoroughly under running tap water and shade dried for two weeks. The whole plant material was pulverized into coarse powder with the help of mechanical grinder and passed through sieve (20 #). The coarse powdered drug (5 g) was then successively extracted by cold maceration process with 100 ml each of petroleum ether, chloroform, ethyl acetate, methanol and water for 24 h (shaking frequently for 6 h and the allowed to stand for 18 h) [9]. Various extracts obtained were filtered, concentrated under reduced pressure in rotary evaporator (Perfit India, Pvt. Ltd.) below 60°C and stored in a desiccator until it is used.

Pharmacognostical evaluation

Macroscopical, Microscopic and powder evaluation

The macroscopical evaluation of the whole plant parts *viz.* leaf, stem and roots of *C. bonplandianum* was performed as per the methods of Khandelwal (2007) [10]. Various organoleptic characters such as color, shape, size, odour, taste and texture were studied carefully using simple microscope. For the microscopical evaluation, the fresh samples were cut free handed and immersed in clearing reagent (chloral hydrate). The sections were dehydrated with varying strength of absolute alcohol and then stained with the mixture of phloroglucinol and conc. HCl (1:1, v/v). Finally, the stained sections were permanently mounted with DPX for histological observations [11]. For the study of isolated elements, pieces of roots were macerated with the mixture of concentrated nitric acid and potassium chlorate, washed with distilled water and finally mounted in glycerine for observation [12]. The photographs were taken at different magnifications with the help of Nikon trinocular digital microscope (Eclipse E200).

Determination of physicochemical parameter

Crude powdered drug (whole plant) of *C. bonplandianum* was used for the determination of various physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, loss on drying, foreign matter, extractable matter, foaming index, swelling index, volatile oil content and pesticide contamination. All these parameters were evaluated following the standard methods of W.H.O. guidelines [13].

Fluorescence analysis of powdered drug

Fluorescence powder drug analysis of the crude powder of *C. bonplandianum* was carried out in the day light, short UV (254 nm) and long UV (366 nm) as per the method of Chase and Pratt (1949) [14]. The fluorescence patterns were obtained after the powdered drug was made to react with different chemical reagents.

Phytochemical evaluation

Preliminary phytochemical screening

Preliminary phytochemical screening for the presence of various phytoconstituents such as alkaloids, carbohydrate, glycosides, saponins, steroids, terpenoids, phenolics, flavonoids and protein were analysed in all the tested extracts *viz.* petroleum ether extract, chloroform extract, ethyl acetate extract, methanol extract and aqueous extract of *C. bonplandianum* [10, 15].

Thin layer chromatography

For further confirmation regarding the presence of various components in all the tested extracts, thin layer chromatography (TLC) was performed using silica gel 60 F254 as stationary phase [16]. Mobile phases used for the development of chromatogram were composed of the mixture of different solvents with varying polarity.

Visualizing reagents used for the detection of various phytoconstituents includes Dragendorff's reagent (alkaloids), benzidine and sodium metaperiodate (glycosides/sugars), Liebermann–Burchard reagent (saponins/steroids/terpenoids), 5% ferric chloride (phenolics) and Sinoda reagent (flavonoids).

Quantitative estimation of phytochemicals

Quantitatively, various classes of phytochemicals were estimated depending on the type and nature of phytochemical class as observed from the preliminary testing and TLC results. For the determination of flavonoid content, rutin was used as standard and the results were expressed as % w/w rutin equivalent [17]. Total phenolics and tannins content were determined in methanolic extract using tannic acid as standard and the results were expressed as % w/w tannic acid equivalent [18].

Since saponins are highly soluble in water, hence the estimation of total saponin content was determined in the aqueous extract as per the method of Hiai et al. [19]. Total alkaloid content in chloroform extract of *C. bonplandianum* was determined by the gravimetric analysis [16].

RESULTS

Pharmacognostical evaluation

Morphological evaluation

Leaves of *C. bonplandianum* are simple, petiolate, alternate, 3–5 cm long and oval to lanceolate in shape. The upper surface is glabrous and dark green in color; whereas, the lower surface is light green and slightly pubescent. Leaf margin is serrated and the apex is acute. The odour is aromatic, characteristics and taste is bitter to unpleasant. The stem is cylindrical, branched, woody, light brown in color, bitter in taste and odourless. Roots are small, dark brown to black in color, cylindrical, odourless and bitter in taste. Numerous adventitious roots are also seen to be originating from the main root (Figure 1).

Microscopical evaluation

Leaf: Histologically, transverse section of the leaf showed the isobilateral arrangement. Transverse section through the midrib and lamina region showed the presence of epidermal layer (upper and lower) with thick cuticle. The epidermal layer is made up of single layer of compressed to elongated parenchymatous cells (12–20 µm in length and 5–10 µm in width).

Collenchymatous cells were observed to be absent both in the upper and lower region of the leaf. A section through the central midrib showed the arrangement of cortical region which is made up of round to oval shaped parenchymatous cells (25–85 µm in length and 20–60 µm in width) bearing intercellular spaces. The central portion of the midrib was represented by the U–shape collateral vascular bundle which is made up phloem (outer) and xylem (inner). The arrangement of xylem is normally exarch and is lignified (Figure 2).

Fig. 1: Morphology of *C. bonplandianum* leaf (a), stem (b) and root (c).

Stem: The transverse section of the stem showed the presence of a single celled elongated epidermal layer (20–25 µm in length and 8–12 µm in width) covered with cuticle. Cortical region which is made up of 5–8 layers of parenchymatous cells (30–45 µm in length and 18–30 µm in width) was observed lying below the epidermis. A discontinuous layer of lignified sclerenchymatous cells (20–65 µm in length and 15–35 µm in width) was also observed to be situated in the cortical region. Beneath the cortical layer is the vascular bundle which is bicollateral in nature bearing the outer and inner phloem surrounding the xylem. Xylem is lignified, continuous, bears scalariform thickenings and composed of libriform fibers**.** Medullary rays running throughout the whole length of the xylem layer are of uniseriate type. The central portion of the stem is made up of wide pith comprising of round to oval shape thick parenchymatous layer without intercellular spaces (50–125 µm in length and 40–90 µm in width) (Figure 2).

Root: Transverse section of the root is composed of the outer epidermis which is made up of a few layers of irregular and compressed shape cork cells. The cortical region comprising of randomly arranged parenchymatous cells is lying below the epidermal. Sclerenchymatous cells (10–20 µm in length and 8–15 µm in width) were also found to be scattered throughout the cortex and are lignified in nature. The vascular bundle is collateral in nature with phloem arranged outwards and the xylem (lignified) situated inwards occupying the whole area of the section. Medullary rays (uniseriate) were found to be penetrating throughout the whole length of the xylem. The central pith was found to be absent (Figure 2).

UEp: Upper epidermis; LEp: Lower epidermis; Ep: epidermis; UPl: Upper palisade cell; LPi: Lower palisade cell; SP: Spongy mesophyll cell; Cor: cortex; SC: sclerenchymatous cell; Ph: Phloem; Xy: xylem; MR: medullary ray; Pt: pith.

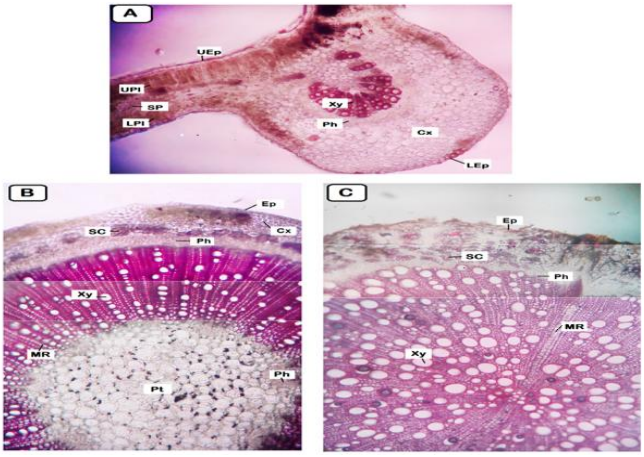

Fig. 2: Microscopical evaluation of leaf (A), stem (B) and root (C) of *C. bonplandianum*

Note- Abbreviations (UEp: Upper epidermis; LEp: Lower epidermis; Ep: Epidermis; UPl: Upper palisade cell; LPi: Lower palisade cell; SP: Spongy mesophyll cell; Cor: Cortex; SC: Sclerenchymatous cell; Ph: Phloem; Xy: Xylem; MR: Medullary ray; Pt: Pith.)

Macerated and powdered drug study: The crude powdered drug of the whole plant of *C. bonplandianum* appears to be greenish to brownish in colour. The powdered drug when made to react with phloroglucinol and HCl mixture showed the strong lignifications of xylem vessels and sclerenchymatous cells. Presence of stellate trichomes was also observed in the powdered drug of *C. bonplandianum* which is the major characteristic feature of the family Euphorbiaceae. Maceration of the whole plant material showed the presence of numerous slender shaped fibres (200–725 µm in length and 10–18 µm in width), vessel elements with scalariform thickenings (100–300 µm in length and 40–80 µm in width) and tracheid elements (80–160 µm in length and 20–70 µm in width) (Figure 3).

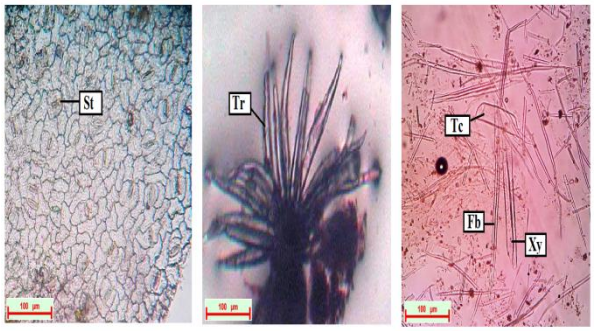

Fig. 3: Macerated and powder evaluation of isolated elements from *C. bonplandianum* whole plant. St: Paracytic stomata; Tr: Stellate trichome: Tc: Tracheid; Fb: Fibre; Xy Xylem elements.

Physicochemical characteristics

Results of various physicochemical parameters evaluated in the present study are represented in Table 1. All the data were taken in triplicates and were expressed in terms of mean ± S.E.M.

Table 1: Physicochemical parameters for whole plant of *C. bonplandianuma*

S.N	Physicochemical parameters	Results
1.	Foreign matter	Not more than 1.0 % (w/w)
2.	Loss on drying.	Not more than 0.5 % (w/w)
3.	Ash value	
	Total ash value	Not more than 6.0 % (w/w)
	Acid insoluble ash value	Not more than 1.8 % (w/w)
	Water soluble ash value	Not more than 4.2 % (w/w)
4.	Extractive value	
	Water soluble extractive value	Not less than 4.20 % (w/w)
	Alcohol soluble extractive value	Not less than 2.10 % (w/w)
	Ethyl acetate soluble extractive value	Not less than 1.93 % (w/w)
	Chloroform soluble extractive value	Not less than 4.65 % (w/w)
	Petroleum ether soluble extractive value	Not less than 16.2 % (w/w)
5.	Foaming index	Not less than 500
6.	Swelling index	Not less than 0.5
7.	Pesticide residue	
	Chlorinated pesticide residue	
	TS1 (First elute)	Not more than 0.008 mg/kg
	TS 2 (Second elute)	Not more than 0.012 mg/kg
	Phosphated pesticide residue	
	TS1 (First elute)	Not more than 0.020 mg/kg
	TS 2 (Second elute)	Not more than 0.009 mg/kg
	TS 3 (third elute)	Not more than 0.008 mg/kg
8.	Volatile oil content	Not less than 0.08 % (v/w)
9.	Heavy metals	
	Lead (Pb)	Not more than 0.010 ppm
	Cadmium (Cd)	Not more than 0.0002 ppm
	Zinc (Zn)	Not more than 0.105 ppm
	Mercury (Hg)	Not more than 0.160 ppm

Fluorescence powder drug analysis

Table 2 represent the results of the fluorescence characteristics of *C. bonplandianum* powdered drug in day light, short UV light (λ_{max} 254 nm) and long UV light (λ_{max} 365 nm). The identification and comparison of the colors was done using the standard colour index chart.

Preliminary phytochemical screening and thin layer chromatography

Preliminary phytochemical screening of different extracts from *C. bonplandianum* indicates the presence of various phytoconstituents such as alkaloids, steroids and terpenes, saponins, phenolics, flavonoids, carbohydrate, amino acids and proteins (Table 3).

Table 2: Fluorescence powder drug analysis of whole plant of *C. bonplandianum*

Powder + Reagent	Fluorescence in day light	Fluorescence in (254 nm)	Fluorescence in (365 nm)
Powder as such	Light green	NF	NF
Powder + 1 N NaOH in water	Mustard oil colour	NF	Yellow
Powder + 1 N NaOH in methanol	Light yellow	NF	Red
Powder + 1 N HCl in water	Colourless	NF	Yellowish green
Powder + 1 N HCl in methanol	Colourless	NF	Strong Red
Powder + 1 N HNO_3 in methanol	Reddish brown	NF	Colourless
Powder + 1 N HNO_3 in water	Deep yellowish brown	NF	Colourless
Powder + I_2 (5%)	Yellow	NF	Light yellowish
Powder + $FeCl_3$ (5%)	Light yellow	NF	Light white
Powder + KOH	Light yellow	NF	Light white
Powder+ NH_3 (25%)	Yellow	NF	White
Powder+ acetic acid	Colourless	NF	Light yellowish pink

NF: No fluorescence

Table 3: Preliminary phytochemical investigation of *C. bonplandianum*

Phytoconstituents	*C. bonplandianum* extracts				
	WE	ME	EA	CE	PE
Alkaloids	-	+	-	+	-
Steroids/terpenes	-	+	-	+	+
Anthrauqinone glycoside	-	-	-	-	-
Saponin	+	+	-	-	-
Phenolic compound	-	+	+	-	-
Carbohydrate	+	-	-	-	-
Flavonoid	-	+	+	-	-
Protein	+	-	-	-	-
Amino acid	+	-	-	-	-

(+) indicate the presence of phytoconstituents, (–) indicate the absence of phytoconstituents.

WE: water extract, ME: methanol extract, EA: ethyl acetate extract, CE: chloroform extract, PE: petroleum ether extract.

Quantitative estimation of phytochemicals

Quantitative estimation of total content of phenols, tannins and flavonoids were determined from the ethyl acetate extract of *C. bonplandianum* by UV spectrophotometer. Total phenols, tannin and flavonoid content in the ethyl acetate extract of *C. bonplandianum* were found to be 4.25 % w/w, 2.15 % w/w and 0.57% w/w respectively equivalent to standard tannic acid and rutin. Alkaloid content in the chloroform extract of *C. bonplandianum* was found to be 2.5 %. Saponin content in the aqueous extract of *C. bonplandianum* was found to be 7.4 % w/w equivalent to standard diosgenin.

DISCUSSION

Over the last few years medicinal plants have played a significant role in the healthcare system. Medicinal plants have important contribution for the therapeutic effectiveness as well as they serve as a source for the development of lead compounds [20]. According to the World Health Organization (WHO) about 70–80% of the world's population in developing countries depend on plants for their primary healthcare due to deficiency and lack of admittance and adverse effects of the modern medicines [21]. Approximately 25% of the modern medicines are directly or indirectly derived from plants sources. Indian medicinal plants are recommended as a valuable source of several pharmacologically active principles which are used for the treatment of various diseases [22, 23]. The main benefit behind the use of plant–derived medicines is mainly attributed to their safety margin, good therapeutic activity and less cost as compared to that of modern medicines [21]. For these reasons medicinal plants are blooming globally with an enormous demand worldwide. However, the main problem arises with the chances of adulteration and replacement of authentic drugs with substandard plant drugs [24]. Furthermore, it is not possible to assume that all plants are safe for consuming as there is a possibility of contamination with various contaminants like pesticides, heavy metals, microbes and inorganic salts [12]. Therefore, the standardization of medicinal plants is required to maintain the quality as well as therapeutic effectiveness [25, 26]. In the present study an attempt has been made to standardize the whole plant of *C. bonplandianum* and to develop a standard monograph that will be helpful for the authentication of the plant for future studies. Pharmacognostical evaluation of the whole part of *C. bonplandianum* revealed the presence of various important diagnostic characters of the family Euphorbiaceae *viz.* presence of discontinuous layer of lignified sclerenchymatous cells (stone cells), paracytic stomata, stellate trichomes, xylem with scalariform thickenings and libriform fibres [27]. The presence of such characters can serve as useful parameters for the identification of the drug on the basis of microscopic aspects. Phytochemical estimation revealed the presence of high quantity of phenolics, alkaloids and saponins. Heavy metals content and pesticide residue estimated in *C. bonplandianum* were found to be below the permissible limit of WHO standard which depicts that the plant is safe for use [13].

CONCLUSION

In conclusion, the overall studies on pharmacognostic and phytochemical features may serve as an important tool for the identification and purity of this plant. Moreover, the parameters estimated in the present study will also aid in maintaining the genuine nature of this plant thereby help in preventing the possible steps of adulteration/substitution with other *Croton* species.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Subir Bandyopadhyay, Botanical Survey of India, Howrah, West Bengal, India for proper identification of plant material.

REFERENCES

1. Reddy KR. Folk medicines from Chittor District, Andra Pradesh, used in treatment of jaundice. Pharm Biol 1995; 26(3): 137–40.
2. Bapuji WJ, Ratnam VS. Leaflets. Ethnobotany 2009; 13: 388–89.

3. Das AJ, Dutta BK, Sharma GD. Medicinal plants use by different tribes of Cachar District, Assam. Indian J Tradit Knowl 2008; 7(3): 446–54.
4. Chandel KPS, Shukla G, Sharma N. Biodiversity in medicinal and aromatic plants in India: Conservation and utilization. New Delhi: National Bureau of Plant Genetic Resources; 1996. p.361.
5. Rajakaruna N, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharm Biol 2002; 40(3): 235–44.
6. Rizk AM. The chemical constituents and economic plants of Euphorbiaceae. Bot J Linn Soc 1987; 94(1–2): 293–326.
7. Phillipson JD. A matter of some sensitivity. Phytochemistry 1995; 38(6): 1319–43.
8. Dosuma OO, Nwosu FO, Nwogu CD. Antimicrobial studies and phytochemical screening of extracts of *Hyphaene thebaica* (Linn) Mart fruits. Int J Trop Med 2006; 1(4): 186–22.
9. Anonymous. The Ayurvedic Pharmacopoeia of India, Part–II, Volume–I, 1st ed. New Delhi: Ministry of Health & Family Welfare, Govt. of India; 2007. p.191.
10. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. India: 17th ed.; 2007.
11. Johansen DA. Plant Microtechnique. New York: McGraw Hill; 1940. p.182.
12. Laloo D, Kumar M, Prasad SK, Hemalatha S. Quality control standardization of the roots of *Potentilla fulgens* Wall.: A potent medicinal plant of the Western Himalayas and North–Eastern India. Phcog J 2013; 5(3): 97–03.
13. Anonymous. Quality control methods for medicinal plant materials (World Health Organization), Geneva. Delhi: A.I.T.B.S. Publishers and Distributors; 2002.
14. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharmacol Asso 1949; 38(6): 24–31.
15. Harbone JB. Phytochemical Methods. 3rd ed. London: Chapman Hall; 1984.
16. Wagner H, Bladt S. Plant drug analysis: A Thin Layer Chromatography Atlas. New York: Springer–Verlag Berlin Heidelberg, 2nd ed.; 1984.
17. Kumaran A, Karunakaran J. *In–vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. Lwt–food Sci Tech 2007; 40(2): 344–52.
18. Makkar HPS. Quantification of tannins in tree foliage: A laboratory manual for the FAO/IAEA co–ordinated research project on use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants of tanniniferous tree foliage. Working document. Vienna: FAO/IAEA; 2000. p. 3–5.
19. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulphuric acid. Planta Med 1976; 29(2): 116–221.
20. Tantiado, RG. Survey on ethnopharmacology of medicinal plants in Iloilo, Philippines. Int J Biosci Biotech 2012; 4(4): 11–26.
21. Muniappan A, Savarimuthu I. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. J Ethnopharmacol 2011; 134(3): 851–864.
22. Mohd D, Singh P, Mishra G, Srivastava S, Jha KK, Khosa RL. *Cassia fistula* Linn. (Amulthus)–An important medicinal plant: A review of its traditional uses, phytochemistry and pharmacological properties. J Nat Prod Plant Resour 2011; 1(1): 101–18.
23. Majumder P, Paridhavi M. An ethno-phytochemical and pharmacological review on novel indian medicinal plants used in herbal formulations. Int J Pharm Pharm Sci 2013; 5(4): 74–83
24. Singh NK, Kumar P, Gupta DK, Singh S, Singh VK. UV-spectrophotometric method development for estimation of piperine in Chitrakadi Vati. Der Pharmacia Lettre 2011; 3(3): 178–182.
25. Laloo D, Sahu AN, Hemalatha S, Dubey SD. Pharmacognostical and phytochemical evaluation of *Cinnamomum wightii* Meissn. flowers. Indian J Nat Prod Resour 2012; 3(1): 33–9.
26. Ghosh A, Laloo D, Singh NK. Comparative estimation and chemical standardization of new and old sample of Chyawanprash. Int J Pharm Pharm Sci 2013; 5 Suppl 3: 801–804.
27. Metcalfe CR. Chalk L. Anatomy of dicotyledons Vol. II. England: Clarendon Press, Oxford; 1950.