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SEASONAL ALTERATIONS IN THE CARBOHYDRATE STATUS AND SECONDARY METABOLITE CONTENTS OF STEM BARK OF *CRATAEVA RELIGIOSA*

Patil Udaysing Hari*[1] and Gaikwad Dattatraya K[2]
[1]Department of Botany, Bhogawati Mahavidyalaya, Dist- Kolhapur, Maharashtra, India
[2]Department of Botany, Shivaji University Kolhapur, Maharashtra, India

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ABSTRACT
Crataeva religiosa, a medicinal plant devoted in the treatment of kidney related disorder. The present study evaluates seasonal variations in the reducing sugars, total sugars, amylose, amylopectin, starch, crude fibers and crude proteins, total polyphenols, water soluble tannins, total flavonoids, total alkaloids, nitrate, total oxalate and total ash value in the three bark samples (apical bark, middle bark and mature inner bark). Except oxalate and total ash, the concentration of other contents was found higher in the apical stem bark and middle bark than mature inner bark. The oxalate and total ash content in the apical stem bark and middle bark were lower than the mature inner bark.
KEY WORDS: *Crataeva religiosa,* carbohydrate moieties, phytochemicals, antinutritional factors.

***Correspondence author**
Dr. Udaysing Hari Patil, Assistant Professor, Department of Botany, Bhogawati Mahavidyalaya, Kurukali. Tal-Karveer Dist- Kolhapur [MS] India-416001 Email: superoxide2311@gmail.com

INTRODUCTION

Crataeva religiosa (Hook and Forst) a medium sized, much branched deciduous tree belongs to the family Capparaceae. It is commonly known as Varun[1]. The trade name given for this tree is three leaved capper[2]. *Crataeva religiosa* is globally distributed in India, Myanmar, Sri Lanka, Malaysia, Indonesia and China. In India, it is found in Peninsular India, Western India, Gangetic Plains, and Eastern India, up to Tripura and Manipur[1]. It is mostly found along the bank of the rivers and streams and near to temple side[3,4]. The plant part used for the medicinal purpose includes Leaves, stem bark and root bark[5,6,7]. Plant is used ethnopharmacologically as diuretic, laxative, lithonotriptic, antirehumatic, antiperiodic, bitter tonic, rubifacient and counterirritant[5,6]. The bark is used in the urinary disorders including kidney and bladder stones, antiemetic, and calculous affections and as an antidote in snakebite [5]. *Crataeva religiosa* is valuable in treating *Vata* (blood flow, waste elimination and breathing), *Pitta*- (fever and metabolic disorder) and *Kapha* (joint lubrication, skin moisture, wound healing, strength and vigor, memory loss, heart and lung weakness and weak immune system[7]. The bark is contraceptive and cytotoxic and useful in kidney bladder stones, fever vomiting and gastric irritation[8]. Bark and roots are rubifacient, diuretic and vesicant[9]. Fruit juice, leaves and bark are useful to cure snakebite, infected wounds and cuts. It increases appetite and controls other skin diseases[10]. Present study deals with the pharmacognostical standardization of this rare and indigenous crude drug.

MATERIALS AND METHODS

Different bark samples (apical rind, middle bark and mature inner bark) of *Crataeva religiosa* were collected from hilly regions of Kolhapur district. Winter collection of bark was made in the last week of January and summer collection in the last week of May. The bark samples were cut into pieces, sun-dried and then oven dried at 60^0C. Dried bark samples were milled and stored in air tight plastic containers.

Qualitative Analysis

Preliminary phytochemical analysis was carried out according to the standard methods[11,12].

Quantitative Analysis

Reducing sugars

The reducing sugars were estimated according to the method introduced by Nelson[13]. The soluble sugars were extracted from 0.250mg of oven dried powdered bark tissue with 80% ethanol. The extract was filtered through Buckner's funnel using Whatman No. 1 filter paper. The filtrate obtained was condensed to 5 ml on water bath and to this 2g lead acetate and potassium oxalate (1:1)

were added for decolourization. To this 40ml distilled water was added and the solution was filtered. The volume of filtrate was measured and it served as an extract for determination of reducing sugars. To the test tube containing 0.4mL plant extract, distilled water was added and volune was adjusted to 1ml. 1ml Somogyi's alkaline copper tartarate reagent was added to each test tube and all these test tubes were transferred to boiling water bath for 10 minutes. Tubes were removed and cooled to room temperature and 1ml Arsenomolybdate reagent was added to each test tube. The contents were diluted to 10mL with distilled water and after 10 minutes, the absorbance of the reaction mixture was measured at 660nm on UV-visible double beam spectrophometer (Shimdtzu UV-190). The amount of reducing sugar was calculated with the help of standard curve obtained by using different concentration of standard glucose solution (0.1mg/ml) and was expressed in g.100g^{-1} dry tissue.

Total sugars

Phenol-sulphuric acid method described by Dey[14] was used to estimate total sugars. The plant material (0.250mg of oven dried bark powder) was suspended in 20ml of 90% ethanol in 50mL test tube. The test tubes were sealed with cork and the suspension was incubated for one hour in hot water bath maintained at 60^{0}C. The extract was filtered and the filtrate was collected in 25ml capacity volumetric flask. The residue was re-extracted with another 10ml volume of 90% ethanol. Both the fractions were collected and final volume was made 25ml with 90% ethanol. For the estimation, 0.2ml plant extract was taken in a test tube and volume was made 1ml with distilled water. 1ml (5%) phenol was carefully added and mixed thoroughly. To these test tubes, 5ml concentrated sulphuric acid (analytical grade) was added rapidly but very carefully. This was mixed thoroughly by vertical agitation with a glass rod. The mixture was cooled at room temperature in air and the absorbance was recorded at 485nm against blank containing distilled water instead plant extract. The amount of soluble sugars was estimated with the help of standard curve of glucose (0.1mg/ml) and expressed in g.100g^{-1} dry tissue.

Starch content

For estimation of the starch, the insoluble residue along with the filter paper obtained after filtering the alcoholic extract of reducing sugar was transferred to a 100ml capacity conical flask containing 50 ml distilled water and 5ml concentrated HCL. The contents were hydrolyzed at 15lbs pressure for half an hour. The contents were cooled to room temperature and neutralized by addition of anhydrous sodium carbonate and filtered through Buckner's funnel. The volume of filtrate was measured and used for the further analysis of starch. Filtrate contains reducing sugars (glucose) formed as a result of hydrolysis of starch. These sugars represent the starch content in the residue. Other steps are essentially similar as described for estimation of reducing sugars. The amount of starch was estimated with the help of standard curve obtained by using different concentration of standard glucose solution (0.1mg/ml^{-1}) and was expressed in g.100g^{-1} dry tissue.

Amylose content

Amylose content was estimated according to the method described by Sadasivam and Manickam[15]. Powdered bark 250mg was taken in 100ml beaker. To this, 1ml distilled ethanol and 10mL 1N NaOH was added and digested on boiling water bath for 10 minutes. The reaction mixture was cooled and diluted to 100mL with distilled water. This extract was used for the estimation of amylose. In test tubes containing 2.5ml plant extract, 20mL of distilled water and few drops of phenolphthalein indicator (0.1%) were added. To each test tube 0.1N HCl was added until the pink colour disappeared. 1ml iodine reagent (1g of iodine and 10g of KI dissolved in water and diluted to 500mL) was added and volume was adjusted to 50mL. The intensity of the colour measured at 590nm on double beam UV-spectrophotometer. The amylose contents were calculated with the help of standard curve obtained by using different concentrations of standard amylose solution (1mg/mL) and expressed as g.100g^{-1} of dry tissue.

Amylopectin content

Amount of Amylopectin was calculated by subtracting the amylose content from the starch content and expressed as g.100g^{-1} of dry weight.

Crude fiber content

Crude fiber contents in the bark samples were estimated according to the method described by Maynard[16]. 2g of oven dried bark powder was transferred to 500ml conical flask and 200ml 0.255N H_2SO_4 was added to it. The contents were boiled for 30 minutes with bumping chips on hot plate. The flask was cooled and the contents filtered through muslin cloth. The residue was washed several times with hot distilled water. The residue thus obtained boiled with 200ml, 0.313N NaOH (1.25g of NaOH dissolved in 100ml distilled water). The contents were filtered through muslin cloth and the residue washed with 25ml, 1.25% H_2SO_4, three portions of water 50ml each and 25ml alcohol. The residue was removed and transferred to pre-weighed ashing dish (W_1g). The residue was dried at 130 ± 2^{0}C for 2hr. Ashing dish was cooled and weighed (W_2g). The residue was ignited at 600 ± 15^{0}C. Ashing dish was

cooled and weighed (W_3g). Crude fiber contents in the bark samples were calculated by using following formula and expressed g.100g^{-1} of dry weight.

$$\frac{\text{Loss in weight on ignition } (W_2\text{-}W_1) - (W_3\text{-}W_1) \text{ X } 100}{\text{Weight of the sample}}$$

Total polyphenols

Total polyphenol contents were estimated according to the method given by Folin and Denis[17]. Dry powdered bark (500mg) was homogenized in 15ml acetone (80%) and filtered through Buckner's funnel. The residue was washed several times with 80% acetone and final volume was adjusted to 50ml with 80% acetone. The reaction mixture in Nessler's tubes contained 1ml plant extract, 10ml 20% Na_2CO_3 and 2ml Folin-Denis reagent (prepared by mixing 100g of sodium tungstate with 20g phosphomolybdic acid in about 800ml distilled water and 200ml, 25% Phosphoric acid and the mixture was refluxed for 2-3 hours, cooled to room temperature and final volume was adjusted to 1000ml with distilled water). Final volume of the reaction mixture was adjusted to 50ml with distilled water and allowed to develop blue colour. After 20 minutes absorbance of blue colour developed was measured at 660nm on double beam UV-visible spectrophotometer. Total polyphenols were calculated with the help of standard curve of tannic acid (0.1mg/ml) and expressed as g.100g^{-1} dry weight.

Water soluble tannins

Method of Schanderl[18] was employed for determination of water soluble tannins. 500mg of powdered bark sample along with 75ml distilled water were transferred to 250ml capacity conical flask. The flask was gently heated on hot plate and material boiled for 30 minute. The contents was cooled to room temperature and centrifuged at 2000rpm for 20 minutes. The residue was discarded and volume of supernatant was adjusted to 100ml with distilled water. This extract was used for the estimation of the tannins in the bark samples. 1ml of the tannin source was transferred to 100ml capacity volumetric flask containing 75ml distilled water. To this, 5ml Folin-Denis reagent and 10ml sodium carbonate solution were and diluted to 100ml with distilled water. Contents in the flasks were thoroughly mixed and absorbance was measured after 30 minutes at 700nm on double beam UV-visible spectrophotometer (Shimadzu-190). A blank was prepared with water instead of the sample. Amount of water soluble tannins was calculated with the help of standard curve of tannic acid and expressed as g.100g^{-1} of dry weight.

Total flavonoids

Total flavonoids were estimated according to method given by Luximon-Ramma *et al*.[19]. Powdered bark (500mg) was extracted in 10ml acetone (80%) using mortar and pestle. The homogenate was filtered through Buckner's funnel using Whatman No. 1 filter paper. The volume of filtrate adjusted to 50ml with 80 % acetone. The reaction mixture contained 1.5ml the plant extract and 1.5ml, 2% methanolic Aluminum Chloride (2g Aluminium chloride dissolved in 100ml pure methanol). Blank was prepared with distilled water in place of sample. The absorbance of reaction mixture was measured at 367.5nm on a UV-visible double beam spectrophotometer (Shimadzu-190). Total flavonoid contents were calculated with help of standard curve of rutin (0.3mg/ml) and expressed as g.100g^{-1} of dry weight.

Total alkaloids

A method described by Singh *et al*.[20] was followed to determine the total alkaloid contents in the bark samples. 100mg bark powder was extracted in 10ml 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2^{0}C. The absorbance of red colour was measured at 510nm against reagent blank. Alkaloid contents were estimated and calculated with the help of standard curve of colchicines (0.1mg/ml) and expressed as g.100g^{-1}of dry weight.

Crude protein content

Crude protein contents were calculated by multiplying the total nitrogen content by factor 6.25.

Nitrate content

The nitrate contents in bark powder were determined using method given by Cataldo *et al*.[21]. In test tubes containing 100mg of dry bark powder, 10ml of de-ionized water was added. The suspension was incubated at 45^{0}C for one hour. After incubation, sample was centrifuged at 5000rpm for 15 minutes. The residue was discarded and the supernatant was taken for nitrate estimation. In 50ml test tubes, 0.2ml extract was mixed thoroughly with 0.8ml, 5% (w/v) salicylic acid (prepared in concentrated H_2SO_4). After 20 minutes at room temperature, 19ml of 2N NaOH was added slowly to raise the pH above 12. Samples were cooled to room

temperature and absorbance was measured on a double beam spectrophotometer at 410nm. The amount of nitrate (µg of $NO_3.g^{-1}$ dry tissue) was calculated with the help of a standard curve obtained by taking different concentrations of KNO_3.

Oxalic acid content

The oxalic acid contents were estimated according to the method given by Abaza *et al*.[22]. For estimation of oxalic acid, 1g powdered bark, 10ml 3N HCl and 65ml double distilled water were taken in 100ml capacity volumetric flask. The flask was kept in boiling water bath for 1hr to digest the plant material. After digestion flask was removed, cooled and diluted up to the mark of 100ml and filtered through Whatman No. 1 filter paper. Two aliquots of 50ml extract were placed in 150ml beakers and 20ml 6N HCl were added in each beaker to increase acidity and to avoid pectin retention. The mixture was evaporated to half of its original volume and filtered through Whatman No. 1 filter paper. The precipitate on the filter paper was washed several times with warm double distilled water. To this filtrate, 3-4 drops of methyl red indicator (0.01% in alcohol) were added and then concentrated ammonia solution was added until the solution turned faint yellow. The solution was heated carefully on water bath maintained at 90-100^{0}C, cooled and filtered to remove interfering ferrous ions containing precipitate. The filtrate thus obtained was heated to 90-100^{0}C on water bath and to this 10ml 5% $CaCl_2$ was immediately added along with 20-25 drops of ammonia solution to restore yellow colour. The solution was kept overnight to settle the precipitate. On next day, the solution was filtered through ashless filter paper (Whatman Filter Paper No. 44). The precipitate on the filter paper was washed several times with double distilled water to make free from Ca (to check whether the ppt is free from Ca^{++} or not, few drops of 5% sodium oxalate were added to 3ml of washing filtrate in test tube. Formation of turbidity indicated presence of Ca^{+2} and demanded further washing of ppt). Residue along with filter paper was dissolved in hot 1:5 H_2SO_4 and diluted to 125ml with double distilled water and transferred to 250ml conical flask. Contents of the conical flask wree heated to 90 – 100^{0}C and titrated against 0.05N $KMnO_4$. The percentage of oxalate was calculated by using following formula,

$$\frac{\text{'ml' of } KMnO_4 \text{ used x } 0.05 \text{ x } 45.02 \text{ x } 100}{1000 \text{ x dry weight x } 50/100}$$

Total ash content

Total ash content was determined by the method described in Indian Pharmacopeias[23].1g of dry powdered bark was accurately weighed and transferred to the previously ignited and weighed silica crucible. The bark powder was spread at the bottom of the crucible and the crucible incinerated at a high temperature not exceeding 450^{0}C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight and the percent of total ash was calculated with reference to the air dried powder.

RESULTS AND DISCUSSION

While checking the potential of a crude drug with reference to its pharmacology, proper phytochemical identification is of prime important. Hence, in the present study, the bark of *Crataeva religiosa* has been evaluated biochemically. Preliminary phytochemical screening of methanolic extract of apical, middle and mature inner bark is shown in the **Table 1**. The intensity of secondary metabolites was high in the apical stem bark while lower in the mature inner bark and moderate in the middle bark. Seasonal changes in the different phytochemicals are shown in the **table 2**. The concentration of reducing sugars, total sugars, crude fibers, crude protein and total ash was higher during summer while that of amylose, Amylopectin and starch was higher during winter. During summer, apical stem bark accumulated higher amount of reducing sugars (0.34%) and present in lower concentration in mature inner bark (0.07%) during winter. Siminovitch *et al*.[24] reported 0.9% to 3.2% reducing sugars in the bark of Black locust which are higher than the reducing sugars reported in the present study. Total sugar content in the *Crataeva religiosa* bark ranged from its minimum 3.69% in mature inner bark during winter to maximum 5.82% in apical bark during summer. The sugar content in the middle bark (4.73-4.19%) was moderate. No large differences were observed in the total sugar content during both seasons. These values correlates with that of *Ficus racemosa* bark (5.70 ± 0.06%) on fresh weight basis[25] and Medicago *sativa* root bark (5.5%)[26] and are much lower than the *Eucalyptus globulus* bark (62.47%)[27]. Maximum amount of amylopectin was accounted in the apical stem bark (7.69%) during winter and minimum concentration was reported in mature inner bark (4.62%) during summer while that of amylose also was recorded maximum in apical bark (0.48%) and lowest in mature inner bark (0.17%) during winter and summer respectively.

Amylose in middle bark varied from 0.26-0.29%. Higher level of starch was reported in apical stem bark (8.17%) during winter and lower level in mature inner bark (4.78%) during summer. The levels of amylopectin, amylose and starch were differing by fractions only and not remarkable changes have been noticed during both seasons. Amount of starch reported in the present study is in the range of Black Locust (7.7%)[24] and Ash tree (6.9%)[27] and lower than the *Polyalthea longifolia* (65-70%)[28]. High amount of crude fiber content was determined in the apical stem bark (37.65%) during winter while, mature inner bark (31.05%) during summer contained lower crude fiber and in middle bark fiber content ranged from 32.03-36.35%. These values significantly correlates with *Mallotus phillippinensis* (36.26%), *Dalbergia sisso* (35.93%)[29] and Cinnamon bark (33.0%)[30]. Similar trend was observed in case of crude protein content. During summer, crude protein content was fairly high in apical stem bark (18.26%) and quite lower amount was estimated for mature inner bark (8.30%) during winter. No large differences were noticed in middle bark and mature inner bark during both the seasons. The Crude protein values in the *Crataeva religiosa* bark are in the range of *Gmelina arborea* (11.37%)[31] and higher than *Picea jezoensis* (1.6±0.14%) and *Abies bomplepis* (1.9±0.05%) as estimated by Yokoyama and Shibata[32].

A maximum level of total polyphenols was accounted for apical stem bark (1.39%) during summer and minimum amount was recorded for mature inner bark (0.33%) during winter season. Though, the level of polyphenols was higher during summer, it was more or less similar among three bark sample during summer. Kumari and Kakkar[33] estimated 19.5% phenols in the bark of *C. nurvala* which is higher than the phenol contents reported in the present study. Amount of water soluble tannins varied from its maximum value 0.26% in the apical bark to lowest 0.09% in mature inner bark during winter and tannins in the middle bark ranged from 0.11-0.20%. These values are quiet lower than *Acacia mangium* (15% to 25%)[34] and *Stryphnodendron obovatum* (20%)[35]. Flavonoids contents in the bark increased two folds during summer than winter. Maximum level of flavonoids was in apical bark (0.55%) and low concentration was noticed in mature inner bark (0.083%) during winter. Flavonoid contents in the middle bark (0.114-0.280%) were in between apical and mature inner bark. Flavonoid contents in the present study are much lower than reported for *Pinus massoniana* bark (27.1%)[36] and *Acasia nilotica* (2.14%) and *Eugenia jambolana* (1.68%) by Sultana *et al*.[36]. No large variations in the alkaloid concentrations were observed during both seasons. Maximum alkaloid amount was estimated for apical bark (0.52%) during summer, while, low level was reported for mature inner bark (0.34%) during winter and middle bark (0.37-0.40%) contained moderate alkaloid level. These figures are much lower than *Delphinium occidentale* (1.1%)[33] and *Chinchona officinalis* (9.3%)[37]. Total ash value determined was higher in the mature inner bark than apical and middle bark and increased during summer. Among all the bark samples during both the season, highest amount was noticed in mature inner bark (20.0%) and minimum ash content was estimated for apical stem bark (14.6%). These reports are higher than the ash values for *Baccaurea sumatrana* (5.34%) and *Pomelia tomentosa* (1.15%), by Whitten and Whitten[38].

Oxalate content was found increased during summer than the winter. The oxalate amount was decreased in the order of Mature inner bark > middle bark > Apical bark. Maximum oxalate was accumulated in the mature inner bark (1.25%) and lowest content was present in the apical bark (0.36%). The oxalate values determined for middle bark were between mature inner bark and apical bark i.e. 0.77-1.09%. In the present report, the total oxalate contents were lower than the *T. arjuna* (7.66 to 20.05%)[39]. Nitrate levels were found to be enhanced during summer than winter. In the apical bark, nitrate content was high (1366µg/g) and lower amount was detected in mature inner bark (546µg/g) during winter while, level was moderate for middle bark (792-1202µg/g). Similar trend of oxalate and nitrate accumulation was reported in the barks of *Anogeissus latifolia*[40] and *Pterocarpus marsupium*[41]. These nitrate values are lower than spinach petiole (6269µg/g)[42] and Parsley (1513.36µg/g)[43]. and higher than nitrate values of Tomato (11.06µg/g), Carrot (190.03µg/g), Jeeberg (623.38 µg/g) as reported by Ayaz *et al*.[43].

CONCLUSION

Phytochemical analysis of bark of *Crataeva religiosa* showed presence of secondary phytoconstituents in appreciable level. Further, their concentration is higher in the apical stem bark than the middle and mature inner bark. These secondary metabolites may acts as good sources of natural antioxidants and pharmacologically active principles during drug designing against many infectious diseases. Various biochemical parameters evaluated in the present study will helpful while ensuring the quality of the natural drug for its desire dose prescription and thereby for acute pharmacological action in the field of pharmacology.

REFERENCES

1. Williamson M. Major herbs of Ayurveda. Churchill Livingston publication, Elsevier Sci., Ltd., 2002; Pp111-116.
2. Pullaiah T. Encyclopedia of world medicinal plants. Regency Publication, 1Edn, 2006; Vol 1:256.
3. Panda H. Handbook of medicinal plants. Asia Pacific Publication Delhi 2004; Pp 112.
4. Warrier PK. Indian medicinal plants: A compendium of 500 species. Arya V. S. (Ed), Orient Publication, France 1995; Vol.3:280.
5. Bhatachargee SK. Handbook of Medicinal Planate. Aaviscar Publication and Distributors, Jaispur 2001; Pp.117.
6. Nadkarni AKC. Indian Materia Medica. Popular publication Pvt. Ltd., Mumbai 1979; Pp.387.
7. Anonymous. *Crataeva*: wealth of India. CSIR Publication, New Delhi 1987; 2:366.
8. Gagandeep M, Khadilkar SB. Chemical constituents of *Crataeva nurvala* (Buch-ham) leaves. Int. J. Pharm. Sci. 2006; 68:804-806.
9. Krishnaraju AV, Rao TVN, Sundarraju D, Vanaree M, Tsay H, Subbaraju, GV. Assessement of Bioactivity of Indian Medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. Int. J. of Appl. Sci. and Engg. 2005; 3(2):125-134.
10. Sapkota J. Biologically active pentacyclic triterpenes lupeol and their current medicine signification. J. of Appl. Biomed. 2003; 31:7-12.
11. Brindha P, Sasikala P, Purushothaman KK. Pharmocognostic studies on Merugan kizhangu. Bull. Med. Eth. Bot. res. 1981; 3:84-96.
12. Lala PK. Lab manuals of Pharmacognosy, CSI Publishers and Distributors, Calcutta. 1993; Pp105.
13. Nelson N. A photometric adaptation of the Somogyi's method for determination of glucose. J. Biol. Chem. 1944; 153:375-380.
14. Dey PM. Methods in plant biochemistry, Vol.-II. Carbohydrates, (Publ.) Acad. Press London, 1990; Pp224.
15. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences, 1992; Pp12-13.
16. Maynard AJ. Methods in food analysis, Academic Press New York, London, 1970; Pp176.
17. Folin O, Denis W. A colorimetric estimation of phenol and phenol and derivatives in urine. J. Biol. Chem., 1915; 22:305-308.
18. Schanderl SH. In: Methods in Food Analysis. Academic Press, New York, London, 1970; Pp709.
19. Luximon-Ramma A, Bahorum T, Soobratee MA, Aruoma OI. Antioxidamt activities of fiavonoid componoids in extracts of *Cassia fistula*. Journal of Agriculture and food chemistry 2002; 50:5042-5047.
20. Singh DK, Srivastva B, Sahu A. Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals. Analytical Sci 2004; 20:571-573.
21. Cataldo DA, Harcon M, Schrader LE, Youngs VL. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. and Plant analyst 1975; 6:71-80.
22. Abaza RH, Blake TT, Fisher EI. Oxalate determination, analytical problem encountered with certain plant species. *I. O., A. O. A. C.,* 51:5. Original KMnO4 method of dye W. B. Weeds., 1968; 4:55-56.
23. Indian Pharmacopeia. Government of India, ministry of health and family welfare, the controller of Publications, Delhi, 1996; Vol. I, Pp423.
24. Siminovitch D, Briggs D R.). Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. Vii. A possible direct effect of starch on the susceptibility of plants to freezing injury. Plant Physiol. 1954; 29(4): 331–337.
25. Ahmed F, Asha MR, Asna U, Bhat KK. *Ficus racemosa* bark: Nutrient composition, physiochemical properties and its utilization as nutra tea. Inter. J. of Nutrition and Metabolism 20102(2): 33-39.
26. Habben JE, Volenec JJ. Starch grain distribution in taproots of defoliated *Medicago sativa* L. Plant Physiol. 1990; 94:1056-106.
27. Essiamllh S, Eschrich W. Changes of starch content in the storage tissues of deciduous Trees during winter and spring. IAWA Bulletin 1985; 6(2):97-106.
28. Khatoon S, Singh, N, Kumar S, *et al*. Authentication and quality evaluation of an important ayurvedic drug Ashoka bark. J. of Sci. and Indus. Res. 2009; 68: 393-400.
29. Prajapati A. Nutrient analysis of important food tree species of Asian elephant (*Elaphas maximus*) in hot-dry season in bardia national park, Nepal. A M. Sc. dissertation submitted to Kathmandu University, Dhulikhel, Nepal, 2008.
30. Gul S, Safdar M. Proximate composition of mineral analysis of Cinnamon. Pak. J. of Nutrition 2009; 8(9):1456-1460.
31. Santra AK, Pan S, Samanta AK, Das S, Halder S. Nutritional status of forage plants and their use by wild elephants in South West Bengal. India. Tropical Ecology 2008; 49:251-257.
32. Yokoyama S, Shibata E. Characteristics of Sasa nipponica grassland as a summer forage resource for Sika deer on Mt Ohdaighara, central Japan. Ecolo. Res. 1998; 13:193-198.
33. Kumari A, Kakkar P. Screening of antioxidant potential of selected barks of Indian medicinal plants by multiple *in vitro* assays. Biomedical and environmental sciences 2008; 21:24-29.
34. Hoong YB, Paridah MT, Luqman CA, Koh MP, Loh YE. Fortification of sulfeted tannin from the bark of *Acacia mangium* with phenol formaldehyde for use as plywood adhesive. Industrial crops and products 2009; 30(3):416-421.
35. Sanches ACC, Lopes GC, Nakamura CV, Filho BPD, de Mello JCP. Antioxidant and antifungal activities of extracts and condensed tannins from *Stryphnodendron obovatum*. Benth. Brazilian Journal of Pharmacological Sciences 2005; 41(1):101-107.
36. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica , Terminalia arjuna , Acacia nilotica* and *Eugenia jambolana* trees. Food Chemistry 2007; 104:1106–1114.
37. Martinez F, Mercadt A, Vagas LM. Estimation of the tannin content in the bark of 13 species of the *Pinus* growing on the tops of de Collantes. Wood and Fiber Science 1995; 27(4):379.
38. Whitten JEJ, Whitten AJ. Analysis of bark eating in a tropical squirrel. Biotropica, 1987; 19(2):107-115.
39. Pandey AK, Kori DC. Variation in tannin content and oxalic acid content of *Terminalia arjuna* (Arjuna) bark. Pharmacognosy magazine 2009; 5(18):159-164.
40. Patil UH, Gaikwad DK. Seasonal dynamics in the nutritional and antinutritional status of stem bark of *Anogeissus latifolia*. International journal of applied Biology and Pharmaceutical Technology 2011a; 2(1):20.
41. Patil UH, Gaikwad DK. Biochemical Standardization of Stem Bark of *Pterocarpus marsupium*. Pharmacophore 2011b. In Press

42. Anjana J, Shahid U, Muhanmad I, Abrol YP. Are nitrate concentrations in leafy vegetables within safe limits? Current Science 2006; 92(3): 355-360.

43. Ayaz A, Topcu A, Yurtagul M. Survey of nitrate and nitrite levels of fresh vegetables in Turkey. J. of Food Tehcnol 2007; 5(2): 177-179.

Table 1: Qualitative analysis of stem bark samples of *Crataeva religiosa*.

Sr. No.	Parameter	Samples		
		Apical Bark	Middle Bark	Mature inner Bark
1	Polyphenols	+++	++	+
2	Flavonoids	+++	++	+
3	Tannins	+++	++	+
4	Alkaloids	+++	++	+
5	Flavones	+++	++	+
6	Terpenoids	+++	++	+
7	Saponins	+++	++	+
8	Cardiac glycosides	+++	++	+
9	Sterols	+++	++	+

'+++'- High concentration; '++'- Moderate concentration; '+'- low concentration and '-' absent

Table 2: **Qualitative analysis of stem bark samples of *Crataeva religiosa***

Sr. No.	Parameter	Samples					
		Apical Bark		Middle Bark		Mature inner Bark	
		Summer	Winter	Summer	Winter	Summer	Winter
1	Reducing Sugars	0.34	0.16	0.22	0.10	0.13	0.07
2	Total Sugars	5.82	5.13	4.73	4.19	3.92	3.69
3	Amylopectin	7.46	7.69	4.91	5.57	4.62	4.80
4	Amylose	0.43	0.48	0.26	0.29	0.17	0.21
5	Starch	7.89	8.17	5.17	5.86	4.78	5.01
6	Crude fiber	35.1	37.65	32.3	36.35	31.05	35.8
7	Crude Protein	18.26	11.31	14.75	9.54	14.54	8.30
8	Total ash	17.2	14.6	18.3	15.2	20.00	16.2
9	Total Polyphenols	1.38	0.99	1.29	0.63	1.17	0.33
10	Tannin	0.26	0.16	0.20	0.11	0.14	0.09
11	Flavonoids	0.55	0.181	0.280	0.114	0.116	0.083
12	Total Alkaloid	0.52	0.43	0.40	0.37	0.36	0.34
13	Total Oxalate	0.48	0.36	1.09	0.77	1.25	1.09
14	Nitrate (µg of $NO_3.g^{-1}$ dry tissue)	1366	1311	1202	792	847	546

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