Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 3 (1): 142-148
Received: 25-04-2014
Accepted: 03-05-2014

Somit Dutta
Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri–734013, West Bengal, India.
Email: somitimmuno@gmail.com
Tel: +91-7679864902

Priyankar Dey
Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri–734013, West Bengal, India.
Email: priyankardey28@gmail.com
Tel: +91-9531563048

Tapas Kumar Chaudhuri
Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri–734013, West Bengal, India.
Email: tapas.chaudhuri@gmail.com
Tel: +91-8927884784

Correspondence:
Tapas Kumar Chaudhuri
Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri–734013, West Bengal, India.
Email: tapas.chaudhuri@gmail.com
Tel: +91-8927884784, +91-9434377127

Phytochemical Investigation and Correlation Study of *Croton bonplandianus* Baill. Stem

Somit Dutta, Priyankar Dey, Tapas Kumar Chaudhuri

ABSTRACT
The present study was undertaken, to analyse the phytochemical profiles C. bonplandianus stem. Various standard biochemical and spectrophotometric methods were employed. All the analysis were performed in multiple sets. The total alkaloid, flavonoid and phenolic content were found to be 59.44±0.28 g/100 g, 3.86±0.12 mg/g and 67.37±0.46 mg/g respectively besides the presence of a certain amount of tannin (51.94±0.38 mg/100 g) and saponin (16.10±0.05 g/100 g). Among the three vitamins, total riboflavin (0.35±0.02 mg/100 g) and ascorbic acid (0.91±0.02 mg/100 g) content were found to be higher, followed by thiamine content of 0.31±0.02 mg/100 g. From the present study, it may be concluded that the stem of *C. bonplandianus* contains very high amount of various important phytochemicals. These phytochemicals are chiefly responsible for various medicinal properties of a plant. Therefore, the presence of the high quantity of phytocompounds may lead to the potent medicinal capacity of *C. bonplandianus* stem.

Keywords: Antioxidant, Croton, Herbal medicine, Phytochemical, Plant extract, Principal component analysis.

1. Introduction

Plant-based traditional medicine system continues to play a vital role in the health care system with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Modern knowledge on medicinal plant research still contains at least 25% drugs and many others, which are synthetic analogues, built on prototype compounds isolated from medicinal plants. The ongoing growing recognition of medicinal plants is due to escalating faith in herbal medicine. There are many contradictory theories on the subject of herbal medicines and their relationship regarding with human physiology and mental function [1, 2]. There is a need to develop evaluative data by using sophisticated modern techniques of standardization of Ayurvedic formulations to tackle the issues of negative criticism of Ayurvedic formulations and increased toxicity reports [3]. These kinds of phytochemical investigation both qualitatively and quantitatively will help in understanding the phytochemical composition and safety of herbal formulation.

Croton bonplandianus Baill. is a monoecious exotic weed. The plant is usually 30-40 cm in height, with whorled ranches which grows in sandy clay soil along road side, railway abandoned field in wide open ravines, and paddy or sugarcane fields [4]. It has been reported that this plant is native to Southern Bolivia, Paraguay, South Western Brazil, North Argentina, Bangladesh, South America, India and Pakistan [5, 6]. In India it is widely distributed in the Sub-Himalayan region of West Bengal. Due to the resemblance of the leaves and flower cymes to that of Tulsi, this plant is often called Ban Tulsi locally in the Sub-Himalayan region of West Bengal. *C. bonplandianus* possess immense medicinal value and its stem latex is used by different tribes of Cachar district, Assam, as a medicinal plant for the treatment of fresh cuts and wounds to stop bleeding [7]. It has got antimicrobial activity and act as good medicine for skin diseases, cut and wounds and also claimed to have the antiseptic properties [8, 9].

Hypertension, hepatoprotective, analgesic and anti-helminthic properties of *C. bonplandianus* have also been documented [10, 11, 12]. Diterpene resins present in *C. bonplandianus* stem was used experimentally for cancer therapy and conceivably result was achieved and its methanolic extract possess tremendous importance for its antitumor potentiality which was evaluated with phytotoxic analysis [13]. Local people use the root as well as stem of *C. bonplandianus* against snake bite in the remote areas of West Bengal, India.

Therefore, the present paper deals with the investigation of different types of phytochemicals qualitatively and quantitatively such as tannin, phlobatannin, terpenoid, glycoside, phenolic, flavonoid, steroid, anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein for a clear understanding regarding the phytochemical status of the stem of *C. bonplandianus* which may help future investigators in their Pharmacological analysis of this species.

2. Materials and Methods

2.1 Plant material

Stem of *C. bonplandianus* was collected from the campus and the adjacent areas of University of North Bengal, India from the month of March to June 2013. The plant specimen was identified by Prof. A. P. Das, Plant Taxonomy Lab, Department of Botany, University of North Bengal, and a specimen has been deposited there in the herbarium under Voucher No. 09870.

2.2 Sample preparation

Stems were washed properly with tap water to remove soil and other dirt's and dried in shade at room temperature for two weeks and then grinded to powder. The powder of the *Croton s*tem was passed through a 0.5 mm metallic mesh to yield crude powder for the use of phytochemical investigations.

2.3 Chemicals and Reagents

Chemicals were obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), unless otherwise indicated. Analytical grade H_2SO_4, chloroform, acetic acid, ethyl acetate, trichloroacetic acid (TCA), diethyl ether and isoamyl alcohol were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). α-napthol, ferric chloride, sodium sulphate, bovine serum albumin (BSA), tannic acid, gallic acid, thiamine and riboflavin were obtained from HiMedia laboratories Pvt. Ltd. (Mumbai, India). HCL was supplied by Thomas Baker (Mumbai, India).

2.4 Qualitative profiling

Aqueous and methanolic extract of Croton stem were used for qualitative assessment for the major classes of phytochemicals namely tannin, phlobatannin, terpenoid, glycoside, phenolic, flavonoid, steroid, anthraquinone, saponin, alkaloid in addition to the major biomolecules namely cholesterol (lipid), carbohydrate and protein. The tests were performed according to various standard methods [1, 9, 10, 11]. standard methods [1, 9, 10, 11].

2.5 Quantitative profiling

The quantitative estimation of various phytochemicals present in the stem of *C. bonplandianus* was performed according to the standard protocols with slight modifications.

2.5.1 Quantification of Alkaloid content

The experiment was done according to the standard protocol with slight modifications [14, 15]. Five grams of stem powder was mixed with 250 ml of 20% CH_3COOH in ethanol in 250 ml beaker and the solution was mixed using a magnetic stirrer for 10 h at room temperature. The solution was filtered using Whatman filter paper number 1(150 mm). The resultant filtrate was placed on a hot water bath (60 °C) until the extract volume turns $^1/_4$^th^ of its initial volume. Concentrated NH_4OH was added drop wise in the final solution which formed thick precipitate. The precipitate was collected by filtration, dried in an oven and weighed to quantify the alkaloid present in the stem of *C. bonplandianus*.

2.5.2 Quantification of Flavonoid content

Total flavonoid quantification was done using standard method [16] with slight modifications. Ten grams of crude stem powder was mixed with 100 ml of 70% methanol in a 250 ml conical flask. Magnetic stirrer was used to mix the solution for 3 h and the mixture solution was filtrated using Whatman filter paper number 1(150 mm). The resultant filtrate material was re-extracted once again with 70% methanol and filtered in a similar way. Both the filtrates were transferred into a crucible and evaporated to dry in a hot water bath at 60 ^{0}C and weighed.

2.5.3 Determination of total Tannin content

Determination of tannin was performed according to the standard method [17]. One gram of crude stem powder was mixed with 50 ml of double distilled water in a 100 ml conical flask. The solution was shaked in a magnetic stirrer for 10 h at room temperature and filtered in a 50 ml volumetric flask using Whatman filter paper No. 1(150 mm). Distilled water was added up to the 50 ml of the volumetric flask. Filtered solution (5 ml) was taken in a test tube in which 0.0008M $K_4[Fe(CN)_6]$ and 0.1 M $FeCl_3$ in 0.1N HCL were added. The absorbance was measured within 10 minutes with the help of spectrophotometer at 120 nm wavelength. A blank and tannic acid as a standard were prepared and read in the same wavelength for quantification of tannin.

2.5.4 Determination of total Saponin content

The experiment was done according to the standard method [18]. Ten grams of stem powder was mixed with 100 ml of 20% ethanol in 250 ml conical flask. The mixture was heated with continuous stirring on a hot water bath of 55 ^{0}C for 5 h. The mixed solution was filtered using Whatman filter paper number 1(150 mm). The solid residue was mixed again with 20% ethanol and heated in a similar way for about 5 h and filtered. The newly prepared filtered solution was added with previously filtered solution and placed on a hot water bath at 90 ^{0}C till the volume was reduced to 20% of its initial volume. Ten milliliters of diethyl ether and the concentrated sample was in a 250 ml separating funnel and shaked vigorously. After settling down the solution, the aqueous layer was separated carefully. Sixty milliliters of n-butanol extract was washed using 10 ml of 5% aqueous NaCl solution and the remaining solution was heated in a water bath at 50 ^{0}C and dried in an oven until the solvent evaporates and turns into semi dried form. Quantification of saponin was calculated with the help of the following equation:

$$\text{Percentage of Saponin} = (W_{EP} / W_S)*100$$

Where, W_{EP} = Weight of oven dried end product; W_S= Weight of powdered sample taken for test.

2.5.5 Quantification of thiamine content

Thiamine content was defected and quantitated by using the standard method with slight modifications [19]. Fifty grams of crude stem powder was dissolved in 20% NaOH prepared in ethanol. The mixture was stirred using magnetic stirrer for 3 h at room temperature. The resultant was filtrated in a 100 ml conical flask through Whatman filter paper number 1(150 mm). Ten milliliters filtrate and equal volume of 2% potassium dichromate solution were mixed well. As a result a color was developed and this colored solution was read at 360 nm against a suitable blank contains all except the stem extract. According to thiamine standard curve the total thiamine content in stem was determined.

2.5.6 Determination of total Riboflavin content

Quantification of riboflavin was done according to the standard

method with slight modification [20]. In a 250 ml conical flask 10 g crude powder of the stem was mixed with 50% ethanol. The mixture was stirred on a magnetic stirrer for about 10 h and filtrated using the above mentioned method. The filtered solution was mixed with 25 ml of 5% $KMnO_4$. Twenty five milliliters of 30% H_2O_2 was added with continuous stirring in the solution. The whole mixture solution was placed at 80 ^{0}C hot water bath for about 30 minutes for boiling. After boiling 5 ml of 40% Na_2So_4 was added to the mixture solution. The blank was prepared without stem extract and the absorbance was measured in respect to blank at 510 nm using a spectrophotometer. Quantification of riboflavin in the stem was calculated using riboflavin standard curve.

2.5.7 Determination of ascorbic acid

The experiment was done using standard protocol [21] with slight modifications. Five grams of stem powder was added in TCA and EDTA in the ratio of 2:1. The mixed solution was stirred on a magnetic stirrer for about 3 h at room temperature. The solution was then centrifuged at 2000 rpm for 30 min and the supernatant was filtered through Whatman filter number 1(150 mm). Two to three drops of 1% starch solution was added to the filtrated solution. The solution was then titrated against 20% $CuSo_4$ solution until a dark end point is reached.

2.5.8 Quantification of total Phenolic content

Determination of total phenol was done [14] from the fat free crude stem powder of *C. bonplandianus*. Five grams crude stem powder was mixed with 100 ml n-hexane using soxhlet apparatus for about 2 h for making the sample fat free and the resultant mixture was used for the following steps.

The fat free sample and 50 ml ether and was boiled for 15 min. The boiled solution was filtrated using Whatman filter paper No 1 (150 mm. Five milliliters of the filtrate solution was pipetted out in a 50 ml conical flask. Ten milliliters double distilled water, NH_4OH solution and 5 ml concentrated amyl alcohol were added to it with continuous stirring. The prepared mixture solution was incubated for 30 min at room temperature for the development of proper color. The absorbance of the developed colored solution was read against a suitable blank at 550 nm using spectrophotometer for quantification of phenol.

2.5.9 Estimation of total protein

Lowry's method [22] with slight modifications was applied to estimate the total protein. Bovine serum albumin was used for the preparation of standard curve at 750 nm.

2.5.10 Quantification of total lipid content

Standard method [23] with slight modifications was applied to estimate the total lipid in the stem of *C. bonplandianus*. One gram of dried stem sample was macerated with 10 ml distilled water. Thirty milliliters of chloroform-methanol (2:1 v/v) was mixed with the solution and left for overnight at room temperature. Twenty milliliters of chloroform and equal volume of distilled water were added in the mixture solution and centrifuged at 1000 rpm for 10 min. Three layers were formed at the end of the centrifugation and the lower layer was collected which contained lipid dissolved in chloroform and was kept in an oven for 1 hour at 50 ^{0}C for evaporation of chloroform. Solution without the chloroform was weighed for the estimation of lipid.

2.5.11 Estimation of Total Sugar

The experiment was done using standard method [24] with slight modifications to quantify the total sugar content in the stem. Fifty grams of stem powder was macerated in a pastel and mixed with 20 ml of ethanol. This ethanolic mixture was incubated for 10 h at 30 ^{0}C and centrifuged at 1500 rpm for 20 min. The supernatant was collected separately. One milliliter of 5% phenol and 1 ml of supernatant were mixed thoroughly. Five milliliters of concentrated H_2SO_4 was added rapidly with constant stirring. The whole mixture was left at room temperature for 30 min for the development of color. Yellow orange color was developed and the optical density was measured against a blank at 490 nm. The standard curve was prepared with known concentration of glucose for the determination of total sugar content.

2.5.12 Estimation of total moisture and ash content

The specific amount of the crude stem extract was kept at 90 ^{0}C for 12 h in an oven and followed by 400 to 450 ^{0}C in a furnace for 5 min. The resultant weight of the sample was calculated for the estimation of total moisture and ash content.

3. Statistical Analysis

All the experiments for phytochemical determination, both qualitatively and quantitatively, were performed three times. KyPlot version 2.0 beta 15 (32 bit) for windows was used to analyze for descriptive statistics. Final quantifications of the phytochemicals in the stem were calculated on the basis of mean value ± SD (standard deviation) of three measurements. Principal Component Analysis (PCA) based on the correlation matrix was performed under varimax method using the SPSS statistics version 20.0 software package to find out any possible interrelation among the phytochemicals quantified in the stem of *C. bonplandianus*.

4. Result

The results of the qualitative analysis have been presented in Table 1. All the screened phytochemical parameters were present in in the stem of *C. bonplandianus* except terpenoid and anthraquinone. Based on qualitative analyses, only the phytochemicals that has been found to be present in the stem were quantified by standard protocols as mentioned above with slight modifications. The results of the quantitative analyses have been presented in Table 2.

Table 1: Display the presence/ absence of different phytochemicals in the stem of *C. bonplandianus*.

Phytochemicals	Stem
Tannin	+
Phlobatannin	+
Cholesterol	+
Terpinoid	_
Glycoside	+
Phenolic	+
Flavonoid	+
Steroid	+
Anthraquinone	-
Saponin	+
Carbohydrate	+
Protein	+
Alkaloid	+

The quantitative estimation of different phytochemicals in the stem of *C. bonplandianus* are enlisted in table 2.

Table 2: Shows the results of the descriptive statistics for the thirteen phytochemicals parameters studied, namely flavonoid, alkaloid, saponin, phenol, ascorbic acid, thiamine, riboflavin, total protein, lipid, soluble sugar, tannin, moisture and ash content. S.D. = Standard deviation; SEM=Standard error of mean; Coef. Vr=Co-efficient of Variance. All values are the mean of three replicate experiments. [d] Units are in g/100g; [e] Units are in mg/g; [f] Units are in mg/100g; [g] Units are in %.

Phytochemicals	Sum	Mean	S.D.	S.E.M.	Variance	Coef. Var.
Flavonoid[e]	11.57	3.86	0.12	0.07	0.01	0.03
Alkaloid[d]	178.33	59.44	0.28	0.16	0.08	0.00
Saponin[d]	48.29	16.10	0.05	0.03	0.00	0.00
Phenol[e]	202.12	67.37	0.46	0.27	0.21	0.01
Ascorbic acid[f]	2.74	0.91	0.02	0.01	0.00	0.02
Thiamine[f]	0.92	0.31	0.02	0.01	0.00	0.05
Riboflavin[f]	1.04	0.35	0.02	0.01	0.00	0.06
Total protein[e]	1.30	0.43	0.08	0.05	0.01	0.19
Lipid[e]	33.00	11.00	1.40	0.81	1.97	0.13
Soluble sugar[e]	3.71	1.24	0.01	0.01	0.00	0.01
Tannin[f]	155.82	51.94	0.38	0.22	0.14	0.01
Moisture[g]	151.10	50.37	0.04	0.02	0.00	0.00
Ash [g]	5.52	1.84	0.03	0.02	0.00	0.02

4.1 Multivariate statistical analysis and correlation patterns

Principal component analysis (PCA) is the simplest of the true eigenvector-based multivariate statistical analyses which allows visualizing the underlying pattern of the variables in an experiment [25]. In PCA, the variables are cumulatively arranged in the most simplified way to highlight the overall pictures of the correlation between the phytochemicals. The result of a PCA are usually discussed in terms of component scores which transformed variable values corresponding to a particular data point the weight by which each standardized original variable should be multiple to get the component score. In recent work worldwide PCA is used to analyze data sets of different branches of the biological science ranging from cytokine and chemokine study in the neuro-inflammatory pathway [26], global genetic structure analysis [27], a study of the antioxidant profile of therapeutics [25] etc. Therefore, in this investigation, PCA was performed to understand how the thirteen phytochemicals namely flavonoid, alkaloid, saponin, phenol, ascorbic acid, thiamine, riboflavin, total protein, lipid, soluble sugar, tannin, moisture and ash content contribute to the overall phytochemical profile of the *C. bonplandianus* stem extract. The loading plot (fig. 1) of first and second principal components, PC1 and PC2 accounted for 65.49% and 34.51% of the variance, respectively. Alkaloid, sugar, riboflavin, and flavonoid content were loaded high on PC1 making a separate cluster from the other variables, with a loading value of 0.985, 0.996, 0.989 and 0.954 respectively. Ascorbic acid, ash and thiamine content were highly loaded on PC2 with loading value of 0.991, 0.916 and 0.975, respectively. Besides, Protein content resided very high negative on PC1 (-0.880) and moisture content was loaded highly negative on PC2 (-0.964).

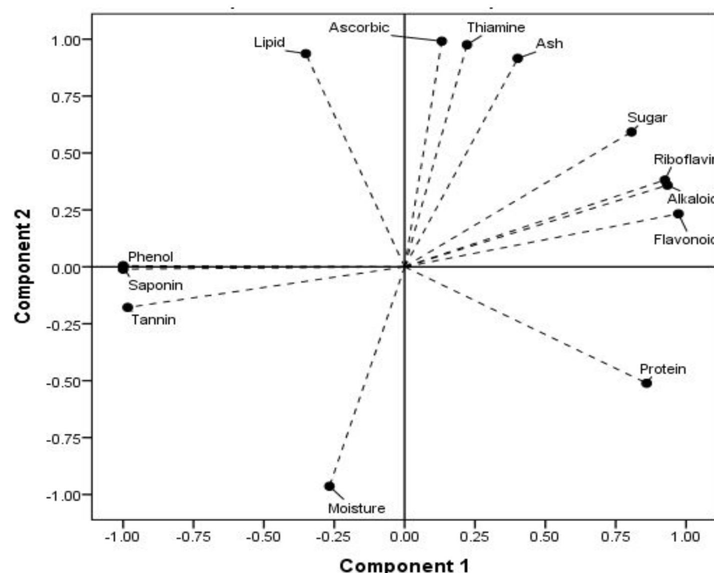

Fig 1: Represents the Principal Component Analysis (PCA) under varimax reaction for the phytochemicals of *C. bonplandianus* stem. Where, Alk = Alkaloid; Flavo = Flavonoid; Phen = Phenol; Sapo = Saponin; Lip = Lipid; Thia = Thiamine; Ribo = Riboflavin; Asco = Ascorbic Acid; Tan = Tannin; Pro = Protein; Mois = Moisture; Ash = Ash content. PCA was performed for two principal factors, the ariances of which were 65.49% and 34.51% respectively.

Table 3: Represents the correlation matrix of different phytochemicals based on the Principal Component Analysis performed by SPSS statistics version 20.0 software package. Where,*Correlation is significant at the 0.05 level (1-tailed) and **Correlation is significant at the 0.01 level (1-tailed).

	Tannin	Alka-loid	Phe-nol	Flavo-noid	Sapo-nin	Ascor-bic	Sugar	Mois-ture	Ash	Thia-mine	Ribo-flavin	Pro-tein	Lipid
Tannin	1												
Alkaloid	-0.982	1											
Phenol	0.986	-0.937	1										
Flavonoid	-0.998*	0.991*	-0.975	1									
Saponin	0.983	-0.932	1.000**	-0.971	1								
Ascorbic	-0.307	0.479	-0.942	0.359	-0.127	1							
Sugar	-0.899	0.965	-0.812	0.922	-0.803	0.693	1						
Moisture	0.434	-0.595	0.277	-0.484	0.262	-0.991*	-0.786	1					
Ash	-0.559	0.703	-0.411	0.604	-0.397	0.961	0.866	-0.990*	1				
Thiamine	-0.392	0.556	-0.231	0.442	-0.217	0.996*	0.756	-0.999*	0.982	1			
Riboflavin	-0.978	1.000**	-0.928	0.988*	-0.923	0.500	0.971	-0.614	0.721	0.577	1		
Protein	-0.755	0.619	-0.854	0.717	-0.862	-0.393	0.390	0.263	-0.123	-0.308	0.600	1	
Ipid	0.178	0.008	-0.342	-0.123	0.356	0.882	0.270	-0.809	0.716	0.835	0.033	-0.780	1

The correlation matrix (table 3) display inter-relations between the various phytochemical parameters present in the stem of *C. bonplandianus*. The correlation significant at 0.01 levels (1-tailed) found between riboflavin and alkaloid as well as saponin and phenol. On the other hand the correlation significant at 0.05 levels (1-tailed) found between flavonoid and tannin, flavonoid and alkaloid, riboflavin and flavonoid, moisture and thiamine with ascorbic acid, ash and thiamine with moisture respectively.

5. Discussion

Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals. In this study, we have identified and quantified the major phytochemicals present in the stem of *C. bonplandianus*.

Flavonoids containing a benzopyrone, one of the major classes of phytochemicals, associated with diverse pharmacological activities which include antioxidant and anti-aging properties [28]. Flavonoid also associated with anti-cancer activity by inhibiting the estrogen producing enzyme.

Flavonoid belongs to the largest phenolic group, consisting more than 600 natural compounds [29]. Generally, flavonoids with two 6-carbon rings and one 3-carbon linkage (usually forming a 3rd ring) are divided into chalcones, flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins [29, 30]. Flavonoids may bind to gamma-aminobutyric acid A receptors having sedative or anxiolytic effects on the nervous system and also can act to up-regulate the cholinergic nervous system [29, 31] and may reduce neurodegenerative processes and abnormal detoriations in cognitive performance [32]. This moderately high level of flavonoid content in the stem of *C. bonplandianus* may use in the future as the potent antioxidant and anti-cancer agent in the field of herbal remedy.

Alkaloids are nitrogen based natural compounds belong to plant secondary metabolites. The alkaloid derivative morphine from the plant *Papaver somniferum* is extensively used as a pain reliever. Furthermore, in modern medicine Artopine is used as an antidote to nerve-gas poisoning, caffeine is used as a stimulant of central nervous system, Sanguinarine is used for antibacterial, vincristine and vinblastine are treated as anti-cancer agents, reserpine and quinine used as anti-hypersensitive and anti-malarial agents respectively. Saponins are characterized by its ability of foaming in aqueous solution. Saponin has hypolipidemic and anti-cancer activity. It has cholesterol binding property and reacts with cholesterol rich plasma membrane of various cancer cells to arrest the proliferation [33]. High level of alkaloid and saponin found in the stem of *C. bonplandianus* directly correlates with the fact that it has been used traditionally as medicine for cancers [23-27].

Tannins have been considered in traditional medicine to treat various diseases. Regression of viral activities has been found with high tannin content [34]. Synergistic effects of certain tannins with various antibiotics have been proved beneficial against antibiotic resistance bacteria [35]. Moreover is tannin associated with anti-cancer, anti-mutagenic and tumor promotion inhibitory activities [35].

Phenolic compounds are used as nutraceuticals, and found in apples, green-tea, and red-wine and also in many medicinal plants as phytochemical or secondary metabolites. Antioxidant and reactive oxygen species (ROS) scavenging capacity of the plant is primarily attributed to the presence of phenolic compounds that hold the ability to inhibit the formation of ROS by inhibiting the activation of redox sensitive transcription factor like nuclear-factor κβ [36]. Various phenolic compounds derived from medicinal plant such as curcumin, genistein, resveratrol and catechins act as potent inhibitors of growth factor and signaling pathways associated with cancer [36]. Resveratrol the phenolic compound has the ability to halt cell-cycle at various stages in different cancer models [36]. Phenolic compounds have also demonstrated immunomodulatory

activity by modulating cytokines and chemokine's [37, 38].
Vitamins not only act as micronutrients but also as one of the essential components for the prevention of diseases. Riboflavin proved to kill harmful pathogens found in blood, which cause disease in the combination with ultraviolet ray. It has also anti-jaundice, anti-migraine and pain relieving activity. The fresh juice of this plant is very useful against headache [39]. Ascorbic acid terminates the chain radical reaction by scavenging free radicals is essential for growth and repair of tissue. On the other hand thiamine plays a major role in the electrolyte balance of muscle and nerve cells. So the presence of satisfactory quantity of riboflavin, thiamine and ascorbic acid present in the stem supports this finding.
Therefore, phytochemicals play a major role in the prevention of diseases. Moreover, out of 250,000 plant species, more than 70,000 species are utilized as therapeutic of different diseases in different ethno medicinal practices [40]. The medicinal property [7-13] of *C. bonplandianus* may be due to the presence of the phytochemicals.

6. Conclusion

The present study may conclude that the stem of *C. bonplandianus* possess various phytochemicals like alkaloid, total phenol, saponin, flavonoid, protein and tannin in a high quantity and possess various bioactive properties. Throughout the world *C. bonplandianus* is well recognized in different ethnopharmacological practices and the presence of high quantities of these bioactive phytochemicals may attribute to its medicinal value. The Bio-induction study will be helpful for improving the yield of these metabolites. Detailed phytochemical analysis will be performed using HPLC and GC-MS in the future. All these information leads us to conclude that stem of *C. bonplandianus* Baill. harbours immense qualities and can further prove a pivotal role in the field of phytochemical research.

7. Acknowledgements

The authors would like to thank Mr. Bijoy Mahanta for his contribution towards collection of plant material and would like to sincere thanks to Professor A. P. Das, Department of Botany, NBU, for identification of the plant.

8. References

1. Sriwastava NK, Shreedhara CS, Aswatha RHN. Standardization of Ajmodadichurna, a polyherbal formulation. Pharmacognosy Res 2010; 2:98-101.
2. Qureshi S, Diab AA, Al-Anazi FA, Al-Hassan MI, Qureshi MF, Qureshi VF *et al*. Negative aspects of the beneficial herbs: An over view. J Herb Med Toxicol 2012; 6:1-14.
3. Saper BR, Kales NS, Paquin J, Burns JM, Eisenberg MD, Davis BR *et al*. Heavy metal content of Ayurvedic herbal medicine products. Jama 2004; 292(23):2868-2873.
4. Nassir E, Ali SI. Flora of Pakistan No. 172. Shamim Printing Press, Karachi, 1986, 43.
5. Pande CS, Tewari JD. Journal of the Indian Chemical Society 1962; 39:545-552.
6. Satish S, Bhakuni DS. Constituents of Indian and Other Plants. Phytochem 1972; 11(9):2888-2890.
7. Kumar Das A, Dutta BK, Sharma GD. Medicinal plant used by different tribes of Cachar district, Assam. Ind j trad med 2008; 7 (3):446-454.
8. Vadlapudi V. *In vitro* antimicrobial activity of methanolic extract of selected Indian medicinal plants. Pharmacophore 2010; 1(3):214-219.
9. Nishanta R, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharm Biol 2002; 40(3):235–244.
10. Chaudhuri AB. Endangered medicinal plants. Daya publishing House, Delhi, 2007, 226.
11. Bhakat RK, Sen UK. Ethno medicinal plant conservation through sacred groves. Tribes and Tribals 2008; 2:55-58.
12. Maria CMT, Joao CA, Gilvendete MPS, Manoel AN, Edilberto RS, Leticia VCL *et al*. Larvicidal and nematicidal Activities of the leaf essential oil of *Croton regelianus.* J Chem Biodiv 2008; 5(12):2724-2728.
13. Islam MS, Rahman MM, Rahman MA, Qayum MA, Alam MF. *In vitro* evaluation of *Croton bonplandianum* Baill. as potential antitumor properties using *Agrobacterium tumefaciens*. J Agr Technol 2010; 6:79-86.
14. Dutta S, Dey P, Chaudhuri TK. Quantification and correlation of the bioactive phytochemicals of *Croton bonplandianum* leaves of Sub-Himalayan region of West Bengal. Asi J Pharceu cli Res 2013; 3(6):579-587.
15. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci 2001; 8:203-208.
16. Harborne JB. Phytochemical methods. Chapman and Hall, London, 1983.
17. Boham AB, Kocipai DC. Flavonoid and condensed tannins from leaves of *Hawaiian vaccinumvaticulum* and *vicalycimum*. Pracific Sci 1994; 48:458-463.
18. Van-Burden TP, Robinton WC. Formation of complexes between protein and tannin acid. J Agric Food Chem 1981; 1:77-82.
19. Nahapetian A, Bassiri A. Changes in concentration and interrelationship of phytate, P, mg, Cu, Zn, in wheat during maturation. J Agric Food Chem 1974; 32:1179-1182.
20. Poornima GN, Ravishankar RV. Evaluation of phytonutrients and vitamin contents in a wild yam, *Dioscorea belophylla* (Prain) Haines. Afr J Biotechnol 2009; 8(6):971-973.
21. Abe F, Yamauchi K. Cardenolidetriosides of oleander leaves. Phytochem 1992; 31(7):2459-2463.
22. Barakat MZ, Shahab SK, Darwin N, Zahemy EI. Determination of ascorbic acid from plants. Anal Biochem 1993; 53:225-245.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol Reagent. J Biol Chem 1951; 193:265–275.
24. Jayaraman J. Laboratory manual in biochemistry. Wiley Eastern Limited, New Delhi, 1981.
25. Dey P, Chaudhuri TK. Antioxidant capacity of *N. indicum*: a correlation study using principal component analysis and multivariate statistical approach. Int J Pharma and Pharmaceu Sci 2013; 5(3):931-937.
26. Helmy A, Antoniades CA, Guilfoyle MR, Carpenter KLH, Hutchinson PJ. Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. Plos one 2012; 7(6):e39677.
27. Athanasiadis G, Moral P. Spatial principal component analysis points at global genetic structure in the Western Mediterranean. J Hum Genetics 2013; 58(11):762-5.
28. Sharma DK. Pharmacological properties of flavonoids from plants. J Sci and Indus Res 2006; 65:477-484.
29. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Advnc in Nutrition 2011; 2: 32–50.
30. Bowsher CS, Tobin M. Plant biochemistry A. New York:

Garland Science, 2008.
31. Kim DH, Jeon SJ, Son KH, Jung JW, Lee S, Yoon BH *et al*. The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. Neuro biol Learning and Memory 2007; 87:536–46.
32. Spencer J. The impact of fruit flavonoids on memory and cognition. Brtsh J Nutrition 2010; 104:40–7.
33. Zulak K, Liscombe D, Ashihara H, Facchini P. Alkaloids, Plant secondary metabolism in diet and human health. Blackwell Publishing, Oxford, 2006, 102–36.
34. Bajaj YPS. Medicinal and aromatic plants (Biotechnology in agriculture and forestry). Springer-Verlag, Berlin, 1988.
35. Okuda T, Ito H. Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. Molecules 2011; 16:2191-2217.
36. Wahle KWJ, Brown I, Rotondo D, Heys SD. Plant phenolics in the prevention and treatment of cancer. Advnc in Exp Med and Biol 2010; 698:36-51.
37. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 2006; 71:1397-1421.
38. Shen F, Chen SJ, Dong XJ, Zhong H, Li YT, Cheng GF. Suppression of IL- 8 gene transcription by resveratrol in phorbol ester treated human monocyte cells. J Asn Nat Pro Res 2003; 5:151-157.
39. Maria CMT, Joao CA, Gilvendete MPS, Manoel AN, Edilberto RS, Leticia VCL *et al*. Larvicidal and nematicidal Activities of the leaf essential oil of *Croton regelianus.* J Chem Biodiv 2008; 5(12):2724-2728.
40. Dey P, Saha MR, Sen A. Hepatotoxicity and the present herbal hepatoprotective scenario. Int J Green Pharmacy 2013; 7:265-73.