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Research Article

ASSESSMENT OF BIOACTIVE PHYTOCHEMICALS PRESENT IN THE ROOT OF *CROTON BONPLANDIANUM* AVAILABLE IN THE SUB-HIMALAYAN REGION OF WEST BENGAL

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ABSTRACT

Objective: Seventy percent methanolic extract of *Croton bonplandianum* root (Family: Euphorbiaceae) was studied to preliminary screen and quantitate the presence of phytochemicals like tannin, phlobatannin, cholesterol, glycoside, terpinoids, phenolics, flavonoid, steroid, anthraquinone, saponin, carbohydrate, alkaloid and protein. Root extract of *C. bonplandianum* may provide the protection of various diseases and may also develop the resistance against some diseases because of the presence of different phytochemicals.

Method: Spectrophotometric and other standard biochemical methods provided a rapid and easy approach for the detection and quantification of the phytochemicals in roots of the plant. Principal Component Analysis (PCA) based on the correlation matrix was performed to correlate among these phytochemicals available in the root.

Result: High percentages of alkaloid (55.71 ± 0.11 g/100g), saponin (14.25 ± 0.11 g/100g), phenolic content (64.36 ± 6.82 mg/g), protein (45.71 ± 6.82 mg/g), lipid (17.05 ± 1.43 mg/g), tannin (26.68 ± .94 mg/100g), thiamine (0.37 ± 0.01 mg/100g) and also certain quantities of riboflavin or vitamin B_2 (0.25 ± 0.03 mg/100g), ascorbic acid (0.35 ± 0.03 mg/100g) have been detected in the root of this plant.

Conclusion: It may be concluded from the present study that the root of *C. bonplandianum* possesses various phytochemicals like alkaloid, total phenol, saponin, flavonoid, protein and tannin in very high quantity. These phytochemicals posses various bioactive properties which may be used as external therapeutic supplements. This study may lead to a new dimension regarding the medicinal value of *C. bonplandianum*.

Keyword: *Croton*, Flavonoid, Herbal medicine, Multivariate statistics, Pharmaceutical, Phenol, Phytochemicals, Principal component analysis, Root.

INTRODUCTION

More than three-quarters of the world population depend on traditional remedies for health care according to the World Health Organization (WHO). Ancient civilizations of Mesopotamia, Egypt, Greece, India and China used medicinal plants for inflammation, cancer and also as the preventive measure to maintenance health. The world is now looking towards India for the new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants and abundance of traditional knowledge how such as Siddha, Ayurveda etc., to cure different diseases.

Therapeutic Goods Administration (TGA) and Dietary Health and Supplement Education Act (DHSEA) of Australia regulated plant-derived medicines and classified as complementary and alternative medicines (CAM) under the Federal Drug Administration (FDA) in the USA. Phytochemicals contribute as antioxidants, anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral agents which are already studied and demonstrated [1]. Reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing have linear correlation with dietary supplements rich in natural phytochemicals [2, 3].

Croton bonplandianum Baill. (Euphorbiaceae) is an exotic weed native to the southern Bolivia, Paraguay, Southwestern Brazil, and Northern Argentina of South America and commonly known as 'Bantulsi' in Bengali and "Kukka tulsi" in Telugu [5]. These plants now grow widely along roadsides, railway abandoned field in wide open ravines, and paddy or sugarcane fields and on sandy or sandy clay soils. This plant is often called Ban Tulsi locally due to the resemblance of the leaves and flower cymes to that of Tulsi.

C. bonplandianum possess immense medicinal value. It has got antimicrobial activity [5]. It act as good medicine for skin diseases, cut and wounds and also claimed to have the antiseptic properties [6]. Hypertention, hepatoprotective and anti-helmenthic properties have also been documented [8, 9, and 10]. Diterpene resins present in *C.bonplandianum* was used experimentally for cancer therapy and conceivably result was achieved. Methanolic extract of *C.bonplandianum* possess tremendous importance as antitumor potentiality which was evaluated with phytotoxic analysis [7]. Local people using roots of *C.bonplandianum* against snake bite in the remote areas of West Bengal, India.

Therefore, the aim of the present study was to investigate the presence of different types of phytochemicals such as tannin, phlobatannin, terpenoid, glycoside, phenolics, flavonoids, steroids, anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein. The quantifications of tannin, alkaloid, total phenol, flavonoid, saponin, ascorbic acid, soluble sugar, thiamine, riboflavin, total protein, lipid, moisture and ash content were evaluated both qualitatively and quantitatively for a clean understanding regarding the photochemical status of the root of *Croton bonplandianum*.

MATERIALS AND METHODS

Sample collection

Root of *C. bonplandianum*, one of the exotic weeds, were collected from the campus and the adjacent areas of University of North Bengal, India. The scientific identification of the plant has been checked by Professor A. P. Das, Plant Taxonomy Lab., Department of Botany, University of North Bengal and the voucher specimen was stored at the Botany Department Herbarium, with an accession number of 09870.

Sample preparation

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

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).

Qualitative test of phytochemicals present in the root of *Croton bonplandianum*

Two types of root extracts were used for the following qualitative test and all the experiments were performed according to Chaudhuri et. al. 2013 [12].

Aqueous extract of *Croton* Roots

Ten grams crude dried powder of *Croton* root was mixed with 100 ml double distilled water in a 250 ml conical flask. Magnetic stirrer was used for proper mixing of the solution for 10 h. Whatman filter paper number 1 (150 mm) was used to filter the mixture and the resultant was used for the following phytochemical tests.

Methanolic extract of *Croton* root

Ten grams of crude dried powder of roots were taken in a 250 ml conical flask and mixed with100 ml of 70 percent methanol. The mixture thoroughly mixed using magnetic stirrer at room temperature for about 10 h and filtered through Whatman filter paper number 1 for the phytochemical tests qualitatively.

Tests of quantitative phytochemicals present in the root of *C. bonplandianum*

The quantitative estimation of different phytochemicals present in the root of *C. bonplandianum* performed according to the standard protocols with slight modifications.

Alkaloid Determination

The experiment was done according to the standard method [12, 13]. Five grams of root powder was mixed with 250 ml of 20% CH_3COOH in ethanol in 250 ml beaker. Magnetic stirrer was used for 10 h at room temperature to mix the solution. The solution was filtered using whatman filter paper number 1 and the resultant was placed on a hot water bath (60^0 C) until the extract volume turns $^1/_{4^{th}}$ of its initial volume. Concentrated NH_4OH was added drop wise which formed thick precipitate. The whole solution was allowed to settle down and the precipitate was collected by filtration, dried in an oven and weighted to quantify the alkaloid present in the root.

Flavonoid Determination

Standard method [14, 15] was followed with slight modification to quantify the total flavonoid. Ten grams of crude root powder was mixed with 100 ml of 70% methanol in a 250 ml conical flask and mixed using magnetic stirrer for 3 h. The mixture was filtrated using Whatman filter paper number 1. The filtrate material was re-extracted once again with 70% methanol and filtered in a similar way and both the filtrates were mixed. The filtrate solution was transferred into a crucible and evaporated to dry in a hot water bath at 60^0C and weighted.

Determination of Tannin

The experiment was performed according to the standard method [16]. One gram of crude root powder was mixed with 50 ml of double distilled water in a 100 ml conical flask and shacked in a magnetic stirrer for 10 h at room temperature. The solution was filtered in a 50 ml volumetric flask and added distilled water up to the 50 ml. Five milliliters of filtered solution 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 was added to it. The absorbance was measured within 10 minutes with the help of spectrophotometer at 120 nm wavelength. A blank was prepared and read in the same wavelength. As a standard tannic acid was prepared and measured.

Saponin Determination

Saponin estimation was done according to the standard method with slight modifications [17]. Ten grams of powder was mixed with 100 ml of 20% ethanol in 250 ml conical flask and the mixture was heated with continuous stirring in a hot water bath of 55^0C for 5 h. Mixture was filtrated and separated the supernatant through Whatman filter paper number 1. The solid residue was mixed with 20% ethanol and heated in a similar way for about 5 h. The solution was filtered and added with previously filtered solution which was placed on a hot water bath at 90^0C. Solution was heated still the volume was reduced to 20% of its initial volume. Concentrated sample was mixed with 10 ml of diethyl ether into a 250 ml separating funnel and shacked vigorously. The aqueous layer was separated carefully after settling down the solution. Sixty milliliters of n-butanol extracts was washed using 10 ml of 5% aqueous NaCl solution. Until the solvent evaporates and turns into semi dried form the remaining solution was heated in a water bath at 50^0C and dried in an oven. Saponin content was calculated with the help of 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.

Determination of thiamine

Determination of thiamine was done using the standard method with slight modifications [18]. Fifty grams of crude root powder was disolved in some of 20% NaOH prepared in ethanol. The mixture was stirred using magnetic stirrer for 3 h at room temperature and resultant was filtrated in a 100 ml conical flask through Whatman filter paper number 1. Ten milliliters filtrate and equal volume of 2% potassium dichromate solution was mixed well and as a result a color was developed. The colored solution was read at 360 nm against a suitable blank contains all but not the root extract. The total thiamine content in root was determined using thiamine standard curve.

Determination of Riboflavin

The experiment was done according to the standard method [19]. In a 250 ml conical flask 10 g crude powder of root was mixed with 50% ethanol and the mixture was stirred on a magnetic stirrer for about 10 h. The filtrated solution was mixed with 25 ml of 5% $KMnO_4$ and in the solution 25 ml of 30% H_2O_2 was added with continuous stirring. The whole mixture solution was placed in a 80^0 C hot water bath for about 30 minutes and after boiling 5 ml of 40% Na_2So_4 was added to the mixture solution. The blank was prepared without root extract. The absorbance was measured with respect to blank at 510 nm using spectrophotometer. Riboflavin content in the root was calculated using riboflavin standard curve.

Determination of ascorbic Acid

Ascorbic acid in the root of *C.bonplandianum* was estimated using Standard protocol [20] with slight modifications. Extraction mixture was prepared using TCA and EDTA in the ratio of 2:1. This extracted mixture was mixed with 5 grams powder of root and stirred on a magnetic stirrer for about 3 h at room temperature. The solution was then centrifuged at 2000 rpm for 30 min. Supernatant was filtered through whatman filter number 1. Two to three drops of 1% starch solution was added to the filtrated solution and titrated against 20% $CuSo_4$ solution until a dark end point is reached.

Determination of total Phenol

Determination of total phenol was done [13] from the fat free crude powder of root. Five grams crude root powder was mixed with 100 ml n-hexane using soxhlet apparatus for about 2 h for making the sample fat free. The resultant mixture was used for the following steps.

The fat free sample and 50 ml of ether was boiled for 15 min and the boiled solution were filtrated using Whatman filter paper No 1. In 50 ml conical flask 5 ml of the filtrate was pipetted out. Ten milliliters double distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were added to the solution and stirred continuously. The conical flask was incubated for 30 min at room temperature for the development of proper color. The absorbance of the colored solution was read against a suitable blank at 550 nm using spectrophotometer.

Estimation of total protein

Lowry's method [21] with slight modifications was applied to estimate the total protein present in the root of *C.bonplandianum*.

Bovine serum albumin with known concentration was taken and the OD was read using a suitable blank at 750 nm for preparing the standard curve.

Estimation of total lipid

Standard method [22] was applied to estimate the total lipid with slight modifications. One gram of dried root sample was macerated with 10 ml distilled water and after proper macerated; 30 ml 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 was added in the mixture solution and centrifuged at 1000 rpm for 10 min. Three layers were formed and the lower layer was collected which contained lipid dissolved in chloroform. The mixture was kept in an oven for 1 hour at 50^0C to evaporation of chloroform and weighted for the estimation of lipid in root.

Estimation of Total Sugar

Standard method [23] with slight modifications was performed to estimate total sugar. Fifty grams of root powder was macerated in a pastel and mixed with 20 ml of ethanol and incubated for 10 h at 30^0C. The solution was centrifuged at 1500 rpm for 20 min. The supernatant was collected separately after centrifugation. One milliliter of 5% phenol was added with 1 ml of supernatant and mixed thoroughly. Five milliliters of concentrated H_2SO_4 was added rapidly with constant stirring and left at room temperature for 30 min as the next step. Yellow orange color was appeared and OD was measured of the colored solution against a blank at 490 nm. The standard curve was prepared with known concentration of glucose.

Estimation of total moisture and ash content

Specific amount of the sample was kept at 90^0C for 12 h in an oven followed by 400^0-450^0C in a furnace for 5 min. For the estimation of total moisture and ash content the resultant weight of the sample was calculated.

Statistical Analysis

All the experiments were performed three times and KyPlot version 2.0 beta 15 (32 bit) for windows were used to analyze for descriptive statistics. Final quantifications of the phytochemicals in the root were calculated on the mean value ± SD 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 root of *C.bonplandianum*.

RESULT AND DISCUSSION

The results of the phytochemicals screening both qualitatively and quantitatively in the root of *C.bonplandianum* directly correlate with the facts of using this plant as an ethnomedicine. We have detected the presence of steroids, phenolics, saponin, flavonoids, terpinoids, tannins, glycoside and other phytochemicals in the root of the plant which are essential constituents of the herbal medicines.

The roots of *C. bonplandianum* showed the presence or absence of different phytochemicals in the table1 and table 2 bellow.

Table 1: Qualitative analysis of various phytochemicals in roots of *C. bonplandianum.*

Chemicals	Leaves
Tannin	+
Phlobatannin	+
Cholesterol	–
Terpinoid	+
Glycoside	+
Phenolics	+
Flavonoid	+
Steroid	+
Anthraquinone	-
Saponin	+
Carbohydrate	+
Protein	+
Alkaloid	+

Table 2: Describes 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.

Phytochemicals	Mean	S.D.	S.E.M.	Variance	Coef. Var.
Flavonoid[e]	2.71	0.31	0.18	0.10	0.11
Alkaloid[d]	55.71	0.11	0.07	0.01	0.01
Saponin[d]	14.25	0.11	0.07	0.01	0.01
Phenol[e]	64.36	6.82	3.94	46.56	0.11
Ascorbic acid[f]	0.35	0.03	0.02	0.00	0.07
Thiamine[f]	0.37	0.01	0.01	0.00	0.03
Riboflavin[f]	0.25	0.03	0.02	0.00	0.12
Total protein[e]	45.71	6.82	6.30	118.98	0.24
Lipid[e]	17.05	1.43	0.83	2.06	0.08
Soluble sugar[e]	2.45	0.02	0.01	0.00	0.01
Tannin[f]	26.68	0.94	0.54	0.88	0.04
Moisture[g]	52.30	0.30	0.17	0.09	0.01
Ash [g]	1.72	0.01	0.00	3.33	0.00

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 %.

High percentage of alkaloid (55.71 ± 0.11 g/100g) and saponin (14.25 ± 0.11 g/100g) have been detected in the roots of *C.bonplandianum*. Alkaloid is a class of nitrogen containing natural compound are known to exist in about 20% of plant species. Only few natural compounds present in alkaloid have been exploited for medicinal purposes. To mention few of them are vinblastine and vincristine as anti-tumor agents, reserpine as anti-hypertensive and quinine as anti-malarial agent. The inhibition of tumor growth increases with the increasing concentration of plant extract and it is claimed that the plant conceivably prove to be very useful in Cancer

treatment [24]. Saponin inhibits microbition and used in the preparation of traditional medicines [25, 26]. It also has hypolipidemic and anti-cancer activity. Saponin has cholesterol binding property and reacts with cholesterol rich plasma membrane of various cancer cells to arrest the proliferation [27]. Very high level of alkaloid and saponin found in the root of *C. bonplandianum*. High level of alkaloid and saponin present in the root directly correlates with the fact that the root of *C. bonplandianum* has been used traditionally as medicine for cancers [7, 18-24].

Flavonoids containing a benzopyrone which is used as antioxidants or free radical scavengers [28] and also have cardioprotective roll [28]. Flavonoid suppresses the progression of cancer by inhibiting the estrogen producing enzyme. 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 and has enormous ability to combat cancer. Heart ailments to an appreciable degree are prevented by phenolic compound. Phenolic compound sometimes serves as anti-inflammatory agent, potent vasodilator [29] and also potent scavenging activity due to the presence of hydroxyl group [30]. The percentage of total flavonoid and phenolic content were (2.79 ± 0.31 mg/g) and (64.36 ± 6.82) mg/g. This moderately high level of flavonoid and very high level of phenolic content may in future contribute in the field of herbal remedy as potent antioxidant and anti-cancer agent.

Root of *C.bonplandianum* possesses very high quantity of protein (45.71 ± 6.82 mg/g), lipid (17.05 ± 1.43 mg/g) and moderately high quantity of soluble sugar (2.45 ± 0.02 mg/g). If the non-functional factor like phytates can be removed or degraded, can be used as an animal feed. It can serve as an alternative source of energy in rural areas for the presence of high level of lipid. Various polysaccharides isolated and purified from different Chinese medicinal herbs are found to possess potent immunomodulatory activity [31].

Very satisfactory quantity of riboflavin or vitamin B_2 (0.25 ± 0.03 mg/100g), satisfactory quantity of thiamine (0.37 ± 0.01 mg/100g) and good amount of ascorbic acid (0.35 ± 0.03 mg/100g) were also found in the root of *C.bonplandianum*. Riboflavin has been proved to kill harmful pathogens found in blood which cause disease in the combination with ultraviolet ray and it has anti-jaundice, anti-migraine and pain relieving effects. Fresh juice of the the plant is very useful against headache [10]. Ascorbic acid terminates the chain radical reaction by scavenging free radicals. So the presence of satisfactory quantity of riboflavin, thiamine and ascorbic acid present in the root supports this finding.

Phenolic group tannin is used as antiseptic and antiviral activity which is also present in the root of *C.bonplandianum*. There are many viruses like polio virus, herpes simplex viruses have been found to get inactivated due to the presence of very high quantity of tannin [32]. The presence of very high quantity of tannin (26.68 ± 0.94 mg/100g) in *C. bonplandianum* show antimicrobial activity as well [6]. Root of the plant has contains (1.72 ± 0.01 %) ash and (52.30 ± 0.30%) moisture.

In this present study, Principle component analysis (PCA) was performed to understand how the thirteen parameters 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. bonplandianum* root extract. To draw an overview of the significance among the quantification of the phytochemicals and the correlation matrix (table 3), the loading plot (figure 1) was used which describes how intricately the correlation between the phytochemical constituents exists. The loading of first and second principal components, PC1 and PC2 accounted for 67.80% and 32.19% of the variance respectively (figure 1). The loading plot demonstrated that tannin, ash, thiamine and ascorbic acid were heavily loaded positively on the PC1 with squared cosine value of 0.820, 0.908, 0.971 and 0.989 respectively. Whereas alkaloid, riboflavin and sugar displayed high quantum of positive loading on PC2 with squared cosine value of 0.925, 0.817 and 0.932.

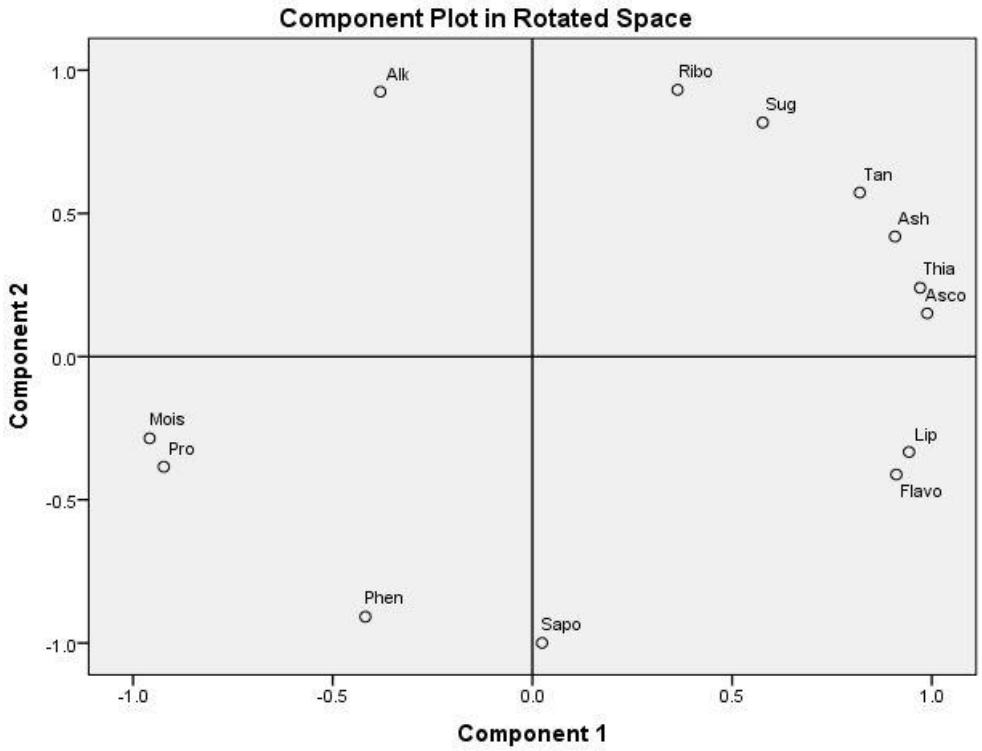

Fig. 1: Represents the principal component analysis (PCA) under varimax rotation for the phytochemicals of *C. bonplandianum* roots.

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 variances of which were 67.80% and 32.19%, respectively.

It is very interesting to note that the antioxidant capacity of the root extract is mainly attributed to the phenolic and flavonoid species of compounds [33] resided in the same cluster with very high PC1 positive value. It can be said that the phytochemicals of *C. bonplandianum* root resides in the three major clusters as documentedin the result of PCA. The largest cluster bearing the tannin, ash, thiamine and ascorbic acid may contribute highest to the bioactive profile of the plant, followed by the second major cluster alkaloid, riboflavin and sugar. Table 3 represents the correlation matrix of the various phytochemicals quantified from the root of *C.bonplandianum*.

Table 3: Represents the correlation matrix of different phytochemicals based on the Principal Component Analysis performed by SPSS statistics version 20.0 software package.

Correlation	Tannin	Alkaloid	Phenol	Flavonoid	Saponin	Ascorbic Acid	Sugar	Moisture	Ash	Thiamine	Riboflavin	Protein	Lipid
Tannin	1.000												
Alkloid	.217^{NS}	1.000											
Phenol	-.863^{NS}	-.681^{NS}	1.000										
Flavonoid	.511^{NS}	-.728^{NS}	-.007^{NS}	1.000									
Saponin	-.553^{NS}	-.933^{NS}	.898^{NS}	.434^{NS}	1.000								
Ascorbic Acid	.897^{NS}	-.237^{NS}	-.550^{NS}	.839^{NS}	-.127^{NS}	1.000							
Sugar	.941^{NS}	.536^{NS}	-.983*	.189^{NS}	-.803^{NS}	-.127^{NS}	1.000						
Moisture	-.949^{NS}	.101^{NS}	.660^{NS}	-.756^{NS}	.262^{NS}	-.803^{NS}	-.127^{NS}	1.000					
Ash	.984*	.042^{NS}	-.760^{NS}	.655^{NS}	-.397^{NS}	.262^{NS}	-.803^{NS}	-.127^{NS}	1.000				
Thiamine	.933^{NS}	-.148^{NS}	-.624^{NS}	.786^{NS}	-.217^{NS}	-.397^{NS}	.262^{NS}	-.803^{NS}	-.127^{NS}	1.000			
Riboflavin	.831^{NS}	.723^{NS}	-.998*	-.052^{NS}	-.923^{NS}	-.217^{NS}	-.397^{NS}	.262^{NS}	-.803^{NS}	.577^{NS}	1.000		
Protein	-.977^{NS}	-.004^{NS}	.735^{NS}	-.683^{NS}	.362^{NS}	-.923^{NS}	-.217^{NS}	-.397^{NS}	.262^{NS}	-.988*	-.694^{NS}	1.000	
Lipid	.582^{NS}	-.667^{NS}	-.092^{NS}	.996*	.356^{NS}	.362^{NS}	-.923^{NS}	-.217^{NS}	-.397^{NS}	.835^{NS}	.033^{NS}	-.742^{NS}	1.000

Where NSCorrelation is not-significant (1-tailed); *Correlation is significant at the 0.05 level (1-tailed) and **Correlation is significant at the 0.01 level (1-tailed).

Though the interrelationship between the variables were non-significant ($p>0.05$) for the most of the cases, but other variables too have displayed very close correlations, like tannin has almost linear positive correlation with ascorbic acid, sugar, ash content and thiamine with correlation coefficient of 0.897, 0.941, 0.984 and 0.933 respectively. The phenol content possesses close positive correlation with saponin which is 0.898 and displayed very close positive correlation of flavonoid with Saponin which is 0.996.

CONCLUSION

From the present study it may be concluded that the root of *C. bonplandianum* possess various phytochemicals like alkaloid, total phenol, saponin, flavonoid, protein and tannin in a very high quantity and possess various bioactive properties which may be used as external therapeutic supplement. Throughout the world medicinal value of *C. bonplandianum* is well recognized in different ethnopharmacological practices and the presence of high quantities of these bioactive phytochemicals may attribute to its medicinal value. This is the first report based on the work on root of *C.bonplandianum* in which not only the overall phytochemical screening was done but also elucidates the correlation in quantities among the different phytochemicals present in it. To correlate the quantities of different phytochemicals present in the root, PCA is one of the rational approaches that can be taken. We are now trying to identify and isolate the different bioactive compounds from the root of *C. bonplandianum* and to test these bioactive compounds for their antioxidant, immunomodulatory, hepatoprotective and anti-cancer activity.

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