

QUANTIFICATION AND CORRELATION OF THE BIOACTIVE PHYTOCHEMICALS OF *CROTON BONPLANDIANUM* LEAVES OF SUB-HIMALAYAN REGION OF WEST BENGAL

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ABSTRACT

Objective: Leaves of various herbs are rich in phytochemicals which may provide protection from various diseases. Therefore, the objective of the present study was preliminary screening of the phytochemicals like tannin, phlobatannin, cholesterol, glycoside, terpenoids, phenolics, flavonoid, steroid, anthraquinone, saponin, carbohydrate, alkaloid and protein in leaf of *Croton bonplandianum* Baill. and quantify some of these phytochemical.

Method: Standardized biochemical and UV-Vis spectrophotometric methods were followed to analyze the phytochemical status of the leaves. Principal component analysis and correlation matrix on the basis of the quantity of the bioactive phytochemicals were performed in order to elucidate the interrelation between the various phytochemicals

Result: Quite a high percentage of alkaloid (59.60 ± 4.79 g/100g), saponin (17.35 ± 1.35 g/100g), phenolic content (75.39 ± 3.19 mg/g), protein (55.04 ± 2.09 mg/g), lipid (37.53 ± 2.43 mg/g), tannin (26.18 ± 2.63 mg/100g), thiamine (26.18 ± 2.36 mg/100g) and very satisfactory quantity of riboflavin or vitamin B₂ (0.55 ± 0.03 mg/100g), ascorbic acid (0.71 ± 0.05 mg/100g) has been detected in the leaves of this plant. It is fascinating to note that the phenol with lipid and the riboflavin content have displayed almost linear positive correlation with correlation coefficient of 0.999. Conclusion: It can be concluded from the present study that the leaf of *C. bonplandianum* possesses rich in various phytochemicals like alkaloid, total phenol, saponin, flavonoid, protein and tannin. These phytochemicals possess various bioactive properties and may be used as external therapeutic supplement. This study may lead to a new dimension regarding the medicinal value of *C. bonplandianum*.

Keywords: Croton, flavonoid, herbal medicine, multivariate statistics, pharmaceutical, phenol, phytochemicals, principal component analysis.

INTRODUCTION

Medicinal plants are the core of traditional medicine among the rural dwellers worldwide since the very beginning of civilization. The therapeutic use of herbs dates back to the third millennium BC during the Sumerian and the Acadian civilizations and are still utilized to treat numerous ailments in the traditional Chinese, Egyptian, Ayurvedic, Unani, Siddha and medicinal system. In western countries herbal medicine has been coined as the Complementary and Alternative Medicine (CAM). Around the globe about 3.4 billion people representing about 88% world population depends primarily on plant-based traditional medicines. The therapeutic efficiency of these herbs has mainly been attributed to the presence of various phytochemicals such as vitamins, terpenoids, phenolic acids, lignin, stilbene, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other secondary metabolites. Studies have demonstrated that many of these phytochemicals contribute as antioxidants, anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral agents [1]. Consumption of dietary supplements rich in natural phytochemicals have linear correlation with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing [2,3]. In recent years, secondary plant metabolites present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables have been extensively investigated as a source of therapeutic agents [4].

Croton bonplandianum (Euphorbiaceae) is an exotic weed commonly found in the wastelands and commonly known as 'Bantulsi' in Bengali and "Kukka tulsi" in Telugu [5]. Leaves of *C. bonplandianum* possess immense medicinal value and have demonstrated antimicrobial activity [5]. The leaves of *C. bonplandianum* are used in the treatment of skin diseases, cuts and wounds and as has been claimed to be antiseptic in nature [6]. Leaves of the many plants of this species have been found to contain diterpene resins which have been used experimentally in the studies of tumor initiation and conceivably prove to be useful in cancer therapy [7]. This species of

Croton is considered as cholagogue and purgative. The plant is reported to possess potent hepatoprotective and anti-helmenthic properties. Leaves of this exotic weed are highly medicinal and used for controlling high blood pressure [8,9,10]. Leaves infusion is used to cure fever caused due to infection of glands. Latex of the plant has wound healing activity. The fresh juice of the leaves of the plant is used against headache [11].

Therefore, the aim of the present study was to investigate the presence of different types of phytochemicals both qualitatively and quantitatively that will lead us to a clear photochemical status of *C. bonplandianum*. Therefore, in the present study, leaf was examined for the presence of different phytochemicals such as tannin, phlobatannin, terpenoid, glycoside, phenolics, flavonoids, steroids, anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein. The quantification of tannin, alkaloid, total phenol, flavonoid, saponin, ascorbic acid, soluble sugar, thiamine, riboflavin, total protein, lipid, moisture and ash content in the leaves of the plant have also been studied.

MATERIALS AND METHODS**Sample collection**

The leaves of *C. bonplandianum*, one of the exotic weeds were collected from the campus and the adjacent areas of University of North Bengal, India. The plant was identified by Taxonomist Prof. A. P. Das of department of Botany, University of North Bengal and the voucher specimen was stored at the Botany Department Herbarium, University of North Bengal with an accession number of 09870.

Sample preparation

The leaves were washed properly first with tap water and then with double distilled water to remove dirt's. The leaves were then shade dried at room temperature for two weeks and grinded to powder. The powder was passed through a 0.5 mm metallic mesh. The

resultant crude fine powder was used for the phytochemical investigations.

Chemicals and Reagents

Chemicals were obtained from Sisco Research laboratories Pvt. Ltd. (Mumbai, India), unless otherwise indicated. Analytical grade H₂SO₄, chloroform, acetic acid, ethyl acetate, trichloroacetic acid (TCA), diethyl ether and isoamyl alcohol were purchased from Merck Specialties Pvt.Ltd. (Mumbai, India). α -naphthol, ferric chloride, sodium sulphate, bovine serum albumin (BSA), tannic acid, gallic acid, thiamine, riboflavin and were obtained from HiMedia laboratories Pvt.Ltd. (Mumbai India). HCL was supplied by Thomas Baker (Mumbai, India).

Qualitative Test of phytochemicals present in leaves of *Croton bonplandianum*

Two types of extracts from the leaves were used for the following qualitative tests those were performed according to Dey et. al. 2012 [12].

Aqueous extract of leaves

Crude dried powder (10 g) of leaves was mixed with 100 ml double distilled water taken in a 250 ml conical flask. Magnetic stirrer was used to mix the solution for 10 h. The mixture was filtered through Whatman filter paper number 1 (150 mm) and filtrate was used for the following phytochemical tests.

Methanolic extract of leaves

Crude dried powders (10 g) of leaves were taken in a 250 ml conical flask and 100 ml 70% was mixed with the leaf extract. The mixture was mixed thoroughly using magnetic stirrer at room temperature for 10 h. The mixture was then filtered with Whatman filter paper number 1. The filtrate was used for the following phytochemical tests.

Test for Carbohydrate

Molish's reagent was prepared first using 5% α -naphthol in absolute ethanol. Two milliliters of aqueous extract was then mix with 2 ml of Molish's reagent and shaken vigorously to mix properly. Two milliliters of concentrated H₂SO₄ was then added carefully with the help of pipette along the wall of the test tube. Appearance of reddish-violet ring at the junction of two liquids indicates the presence of carbohydrate.

Test for Protein

Two milliliters aqueous solution was mixed with 1 ml of 40% NaOH solution and 1-2 drops of CuSO₄ was added to it. Change in the color of the solution to violet color indicates the presence of peptide linkage in a solution which in turn is an indication of the presence of protein.

Test for tannin

Ten milliliters of aqueous extract was taken in the test tube and few drops of 0.1% FeCl₃ solution was added to it. Formation of blue black precipitation indicates the presence of tannin.

Test for phlobatannin

Ten milliliters of aqueous extract was taken in a test tube and allowed to boil. Two milliliters of concentrated HCL was added in the test tube with proper caution so that the solution dose not bumped out. The mixture was boiled for 1 minute. Deposition of red precipitate indicates the presence of phlobatannin.

Test for Terpinoid

Five milliliters of methanolic leaves extract was mixed with 2 ml of chloroform. Three milliliters of concentrated H₂SO₄ was added to the solution slowly along the wall of the test tube. Care was taken not to stir the solution of the test tube. Reddish brown coloration formed at the junction of two liquid phases indicates the presence of terpinoid.

Test for Glycoside

Five milliliters of methanolic extract was taken in a separate test tube and the mixture of 2 ml of glacial acetic acid with 2% FeCl₃ was added to the test tube. One milliliter of concentrated H₂SO₄ was added slowly along the walls of the test tube carefully by using a pipette. Formation of brown ring at the interphase indicates the presence of glycoside.

Test for Steroid

Five milliliters of methanolic filtrate was treated with 0.5 ml of anhydrous CH₃COOH and was cooled in an ice bath for 15 min. Five hundred μ l of chloroform was added to the solution. One milliliter of concentrated H₂SO₄ was poured along the walls of the test tube very carefully. At the separation level of two liquids, a radish brown ring was formed, as an indication of the presence of steroids.

Test for Cholesterol

Two milliliters of methanolic extract was mixed with 2 ml of chloroform. Acetic acid anhydride (10-12 drops) was added to the test tube and shaken vigorously. Two drops of concentrated H₂SO₄ was added to the solution. Change of the reddish brown coloration to blue-green on addition of H₂SO₄ indicates the presence of cholesterol.

Test for Alkaloid

Two milliliters of methanolic filtrate was taken in a test tube and 2 ml of 2N HCL was added to it. The solution was shaken vigorously to mix and kept aside for 5 min. Aqueous phase was separated from the two liquid phase and a few drops of Mayer's reagent (HgCl₂ + KI in water) was added to it and shaken to observe the formation of creamy colored precipitation.

Test for Phenol

Ten milliliters of methanolic extract was mixed with 4-5 drops of 2% FeCl₃ solution and change of coloration of the solution indicates the presence of phenolics.

Test for Flavonoid

Two grams of crude powdered leaves of *C. bonplandianum* heated with 10 ml of ethyl acetate in a hot water bath for a period of 5 min. The solution was filtrated using Whatman filter paper. The methanolic filtrate (1.4 ml) was mixed with 10% dilute ammonia solution and shaken vigorously. Yellow coloration of the solution indicates the presence of flavonoid.

Test for Anthraquinone

In 100 ml conical flask 0.5 g of crude powder of leave was taken and 20 ml of benzene was added. Magnetic stirrer was used to mix the solution for about 4 h and the solution was filtered. Ten milliliters of filtrate was mixed properly with 0.5 ml of ammonia solution. At the layer phase appearance of violet color indicates the presence of anthraquinone.

Test for Saponin

Crude powder of leave (0.5 g) was boiled with 15 ml of double distilled water in a hot water bath. Intensive forth formation indicates the presence of saponin.

Quantitative Test of phytochemicals present in leaves of *C. bonplandianum*

The quantitative estimation of different phytochemicals of leaves of *C. bonplandianum* performed according to the standard protocols.

Determination of Alkaloid

The experiment was performed according to the standard method [12,13]. Five grams sample of leaves powder was taken into a 250 ml beaker and 250 ml of 20% CH₃COOH in ethanol was added to it. Magnetic stirrer was used to mix the solution for 10 h at room temperature. The solution was filtered using Whatman paper

number 1 and the resultant was placed on a hot water bath (60° C) until the extract volume turns 1/4th of its initial volume. Concentrated NH₄OH was added drop wise which form thick precipitate. NH₄OH was added till the formation of the precipitate was complete. The whole solution was allowed to settle down. The precipitate was collected by filtration, dried in an oven and weighted.

Determination of Flavonoid

A standard method [14] was followed with slight modification to quantify the total flavonoid content. Ten grams of crude leaves powder was taken in a 250 ml conical flask and 100 ml of 70% methanol was added to it. Magnetic stirrer was used to mix the solution for 3 h and filtration of the solution was done using Whatman paper number 1. The remaining powdered material was re-extracted once again with 70% methanol and filtered in a similar way. Both the filtrates were mixed and transferred into a crucible and evaporated to dryness in a hot water bath of 60°C and weighted.

Determination of Tannin

The experiment was performed according to the standard method [15]. One gram of crude leaves powder sample was taken in a 100 ml conical flask and 50 ml of double distilled water was added to it and shaken in a magnetic stirrer for 10 h at room temperature. The solution was filtered in a 50 ml volumetric flask and made up to the mark using distilled water. Five milliliters of filtered solution was taken in a test tube and 0.0008M K₄[Fe(CN)₆] and 0.1 M FeCl₃ in 0.1N HCL was added to it. The absorbance was measured in spectrophotometer at 120 nm wavelength within 10 minutes. A blank was prepared and the reading was taken in the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured.

Determination of Saponin

Estimation of saponin was done according to slightly modified standard method [16]. Ten grams of powder sample was taken in 250 ml conical flask and 100 ml of 20% ethanol was added to it. The mixture was heated in a hot water bath of 55°C for 5 h with continuous stirring. The mixture was filtrated through Whatman paper number 1 and the supernatant liquid was separated. The solid residue was mixed with 20% ethanol and heated in a similar way for about 5 h. The solution was filtered and mixed with previously filtered solution. The combined filtered solution was placed on a hot water bath of 90°C and heated still the volume was reduced to 20% of its initial volume. The concentrated sample was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added to it and shaken vigorously. The aqueous layer was separated carefully after setting down the solution. The purification process repeated again. Sixty milliliters of n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl solution. The remaining solution was heated in a water bath at 50°C until the solvent evaporates and the solution turns into semi dried form. The sample was then dried in an oven. This saponin content was calculated by 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.

Thiamine Determination

Thiamine determination was done using the standard method with slight modification [17]. Fifty grams of crude leaves powder sample was dispersed in 50 ml ethanolic 20% NaOH and stirred over a magnetic stirrer for 3 h at room temperature. The resultant was filtrated through Whatman paper number 1 in a 100 ml conical flask. Ten milliliters filtrate was mixed with equal volume of 2% potassium dichromate solution. As a result, color will develop which was read at 360 nm against a suitable blank. The blank contains all but lacks the leaves extract. The thiamine content was calculated from the thiamine standard curve.

Determination of Riboflavin

The experiment was performed according to the standard method [18] with slight modification. Ten grams crude powder of leaves was

mixed with 50% ethanol in a 250 ml conical flask. The mixture was stirred on a magnetic stirrer for about 10 h. The solution was filtrated and the filtrated solution was mixed with 25 ml of 5% KMnO₄ solution. In the solution 25 ml of 30% H₂O₂ was added with continuous stirring. The whole mixture was placed on 80° C hot water bath for about 30 minutes. Five milliliters of 40% Na₂SO₄ was added to it and the absorbance was measured at 510 nm using a spectrophotometer. The blank was prepared without leaves extract. The riboflavin content was calculated from a riboflavin standard curve.

Determination of Ascorbic Acid

Standard protocol [19] was used to estimate the ascorbic acid in leaves of *C. bonplandianum* with slight modification. First, extraction mixture was prepared using TCA and EDTA in a ratio of 2:1. Five grams powder of leaves sample was mixed with this extracted mixture and stirred on a magnetic stirrer for about 3 h at room temperature. The solution was transferred to a centrifuge tube and centrifuged at 2000 rpm for 30 min. After centrifugation the supernatant solution was filtered through Whatman number 1. Two-three drops of 1% starch solution was added to the filtrated solution and the solution was titrated against 20% CuSO₄ solution until a dark end point is reached.

Determination of Total Phenol

To quantify the total phenol [12] content the crude leaves powder sample needed to be fat free. Five grams of crude powder was mixed with 100 ml n-hexane to prepare the fat free sample using soxhlet apparatus for 2 h. The resultant was used to determination of the total phenol content.

The fat free sample was boiled with 50 ml of ether for 15 min. The resultant mixtures were filtrated and 5 ml of the filtrate was pipetted out into 50 ml conical flask. Ten milliliters double distilled water, 2 ml of NH₄OH solution and 5 ml of concentrated amyl alcohol were added to the solution with constant stirring. The conical flask was incubated for 30 min at room temperature for color development. The absorbance of the colored solution was read using spectrophotometer at 550 nm against a suitable blank.

Total protein Estimation

Total protein was estimated according to the Lowry's method [20] with slight modification. Bovine serum albumin with known concentration was taken as standard and the OD was read at 750 nm using a suitable blank.

Estimation of Total lipid

Standard method [21] was applied to perform the experiment with slight modification. One gram of dried leaves sample was macerated with 10 ml distilled water. Thirty milliliters of chloroform-methanol (2:1 v/v) was mixed with the solution and the mixture was left overnight at room temperature. Twenty milliliters of chloroform and equal volume of distilled water was mixed with the mixture solution and the solution centrifuged at 1000 rpm for 10 min. After completion of the centrifugation three layers were formed. Out of the three layers, the lower layer was collected which contained chloroform containing lipid. The mixture was kept in an oven for 1 h at 50°C which result in the evaporation of chloroform. Weight of the remaining was taken.

Estimation of Total Sugar

Estimation of total sugar was performed according to the standard method [22] with slight modifications. Fifty grams of powder leaves sample macerated in a pastel and the mortar with 20 ml of ethanol and kept for incubation at 30°C for 10 h. The solution was centrifuged at 1500 rpm for 20 min and the supernatant was collected separately. One milliliter of 5% phenol was added With 1 ml of alcoholic extract and mixed thoroughly. Five milliliters of concentrated H₂SO₄ was added rapidly with constant stirring in the next step. This was allowed to stand at room temperature for 30 min. The color of the solution was changed to yellow orange and the OD was measured at 490 nm against a blank. Known concentration of glucose was used to prepare the standard curve of the blank

solution. The quantity of the sugar was expressed as mg/g fresh weight of the sample.

Determination of total moisture and ash content

Moisture and ash content of the leaves of *C. bonplandianum* was estimated by subjecting specific amount of the sample to 90°C for 12 h in an oven and at 400°-450°C in a furnace for 5 min respectively. The resultant weight of the sample was calculated for moisture and ash content estimation.

Statistical Analysis

All the experiments were performed three times and the data were analyzed for descriptive statistics using KyPlot version 2.0 beta 15 (32 bit) for windows. The final quantification of the phytochemicals is the value of mean \pm SD of three measurements. To find out any possible interrelation among the phytochemicals quantified, a Principal Component Analysis (PCA) based on the correlation matrix was performed using the SPSS statistics version 20.0 software package.

RESULT AND DISCUSSION

C. bonplandianum is being used as the traditional medicine not only in different parts of India but also throughout the world for the time immemorial. The result of the phytochemical screening of leaf of this plant directly correlates with the facts of using this plant as an ethnomedicine. We detected the presence of steroids, phenolics, saponin, flavonoids, terpinoids, tannins, glycoside and other phytochemicals in the leaves of the plant which are essential constituents of the herbal medicines and also determined the quantity of some of the phytochemicals and phytonutrients.

The leaf of *C. bonplandianum* showed either presence or absence of different phytochemicals. The results are listed below in the table 1 and table 2.

Table 1: Comparison of the presence of various phytochemicals in leaf 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. 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	Mean	S.D.	S.E.M.	Variance	Coef. Var.
Flavonoid ^e	4.36	0.48	0.27	0.23	0.11
Alkaloid ^d	59.60	4.79	2.76	23.00	0.08
Saponin ^d	17.19	1.35	0.78	1.82	0.07
Phenol ^e	75.29	3.19	1.84	10.21	0.04
Ascorbic acid ^f	0.71	0.05	0.02	0.00	0.07
Thiamine ^f	0.54	0.01	0.00	0.00	0.02
Riboflavin ^f	0.55	0.03	0.01	0.00	0.05
Total protein ^e	55.04	2.09	1.20	4.38	0.03
Lipid ^e	37.53	2.46	1.42	6.09	0.06

Soluble sugar ^e	2.53	0.40	0.23	0.16	0.15
Tannin ^f	26.18	2.63	1.52	6.95	0.10
Moisture ^g	65.20	3.19	1.84	10.18	0.04
Ash ^g	1.53	0.24	0.13	0.05	0.15

Quite a high percentage of alkaloid has been detected in the leaves of the plant (59.60 ± 4.79 g/100g). Alkaloid is a class of nitrogen containing natural compound. More than 12,000 alkaloids are known to exist in about 20% of plant species and only few have been exploited for medicinal purposes such as vinblastine and vincristine as anti-tumor agents, reserpine as anti-hypertensive and quinine as anti-malarial agent. The bioactive compounds of the plant *C. bonplandianum* can play important role in developing anti-tumor drugs in human being. The increase of the inhibition of tumor growth with the increasing concentration of plant extract is documented. It claimed that the plant conceivably prove to be useful in Cancer therapy [23]. Presence of high percentage of alkaloid in leaves of this plant, perhaps, supports these findings [18, 24].

Saponin is regarded as high molecular weight compound. A sugar molecule present in the saponin combined with triterpene or steroid glycone. It belongs to the class glycosides and has cholesterol binding property. Microbial proliferation is inhibited by saponin and used in the preparation of traditional medicines [25,26]. Therapeutically, Saponins are very important as they are shown to have hypolipidemic and anti-cancer activity. The natural anti-cancer agent saponin reacts cholesterol rich plasma membrane of various cancer cells and arrests their proliferation [27]. Very high levels of saponin (17.35 ± 1.35 g/100g) found in the leaves of *C. bonplandianum*. This high level of saponin present in the leaves directly correlates with the fact that the leaf of *C. bonplandianum* has been used traditionally as medicine for cancers [7].

Flavonoids are important group of polyphenols widely distributed among the plant flora and containing a benzopyrone which use as antioxidants or free radical scavengers [28] and also have cardioprotective roll [28]. By inhibiting the estrogen producing enzyme flavonoid suppress the progression of cancer. For example, flavonoid inhibited the estrogen synthesis which binds estrogen to its receptor. The percentage of total flavonoid in leaf was (4.36 ± 0.48 mg/g). This moderately high level of flavonoid present in the leaf could be attributed to its antioxidant capacity.

The leaf of *C. bonplandianum* has very high quantity of total phenolic compound. The host of natural antioxidant essentially represented by the phenolic compounds used as nutraceuticals, and found in apples, green-tea, and red-wine and in many medicinal plants as phytochemical or secondary metabolites. Phenolic compound has enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. They are potent vasodilator [29] and for the presence of hydroxyl group they possessing potent scavenging activity [30]. The leaf showed considerably high amount of phenolic content (75.39 ± 3.19 mg/g). The detection and quantification of total phenolic compound present in the leaf of *C. bonplandianum* may in future contribute in field of herbal remedy as potent antioxidant.

The leaf of the plant possesses very high quantity of protein (55.04 ± 2.09 mg/g). The leaf of the plant can be used as an animal feed if the non-functional factor like phytates can be removed or degraded. With rich in protein, *C. bonplandianum* contains very high quantity of lipid in leaf (37.53 ± 2.43 mg/g). For the presence of high level of lipid it can be served as an alternative source of energy in rural areas. Moderately high quantity of soluble sugar in present in the leaf (2.53 ± 0.40 mg/g). These many types of polysaccharides have turned to be bioactive and immunomodulatory agents isolated and purified from Chinese medicinal herbs [31].

The leaves of *C. bonplandianum* possess very satisfactory quantity of riboflavin or vitamin B₂ (0.55 ± 0.03 mg/100g). In the combination with ultraviolet ray, riboflavin has been proved to kill harmful pathogen found in blood which causes disease. Besides, this riboflavin has anti-jaundice, anti-migraine and pain relieving effects. The fresh juice of the leaves of the plant is used against headache [10]. So the presence of very high quantity of riboflavin supports this

finding. There is also very high quantity of thiamine present in the leaf (26.18 ± 2.36 mg/100g). Good amount of ascorbic acid found in the leaf as well (0.71 ± 0.05 mg/100g). The potent antioxidant, ascorbic acid terminate the chain radical reaction by electron transfer and scavenges free radicals.

Tannin is phenolic compounds of high molecular weight used as antiseptic and this activity is due to presence of the phenolic group. This is also associated with antiviral activity. In presence of very high quantity of tannin, there are many viruses like polio virus, herpes simplex viruses have been found to get inactivated [32]. The leaf of *C. bonplandianum* contains very high level of tannin (26.18 ± 2.63 mg/100g). The presence of very high quantity of tannin supports the leaf of this plant has antimicrobial activity.

The leaf of the plant have been found to contain (1.53 ± 0.24 %) ash, the end product when the organic matter is burnt at very high temperature (>550) in presence of an oxidizing agent, usually oxygen. The leaf has been found to contain (65.20 ± 3.19 %) of moisture.

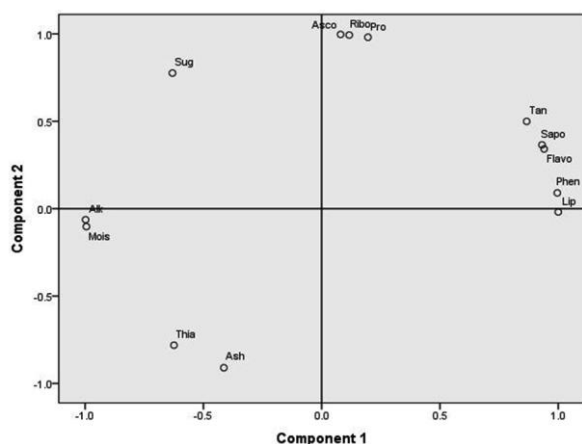


Figure 1: Represents the principal component analysis (PCA) under varimax rotation for the phytochemicals of *C.*

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 ^{NS}Correlation 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).

Correlation	Tannin	Alkaloid	Phenol	Flavonoid	Saponin	Ascorbic Acid	Sugar	Moisture	Ash	Thiamine	Riboflavin	Protein	Lipid
Tannin	1.000												
Alkaloid	-0.896 ^{NS}	1.000											
Phenol	.908 ^{NS}	-1.000 ^{**}	1.000										
Flavonoid	.985 ^{NS}	-0.960 ^{NS}	0.967 ^{NS}	1.000									
Saponin	.989 [*]	-0.952 ^{NS}	0.960 ^{NS}	1.000 ^{**}	1.000								
Ascorbic Acid	.567 ^{NS}	-0.143 ^{NS}	0.169 ^{NS}	0.415 ^{NS}	0.438 ^{NS}	1.000							
Sugar	-0.159 ^{NS}	0.581 ^{NS}	-0.559 ^{NS}	-0.328 ^{NS}	-0.304 ^{NS}	0.723 ^{NS}	1.000						
Moisture	-0.913 ^{NS}	0.999 [*]	-	-0.970 ^{NS}	-0.964 ^{NS}	-0.181 ^{NS}	0.548 ^{NS}	1.000					
Ash	-0.813 ^{NS}	0.470 ^{NS}	-	-0.700 ^{NS}	-0.717 ^{NS}	-0.941 ^{NS}	-	0.505 ^{NS}	1.000				
Thiamine	-0.931 ^{NS}	0.672 ^{NS}	-	-0.853 ^{NS}	-0.866 ^{NS}	-0.828 ^{NS}	-	0.701 ^{NS}	0.969 ^{NS}	1.000			
Riboflavin	0.597 ^{NS}	-0.179 ^{NS}	0.205 ^{NS}	0.443 ^{NS}	0.471 ^{NS}	0.999 [*]	0.697 ^{NS}	-0.217 ^{NS}	-	-0.849 ^{NS}	1.000		
Protein	0.695 ^{NS}	-0.257 ^{NS}	0.283 ^{NS}	0.519 ^{NS}	0.540 ^{NS}	0.993 [*]	0.638 ^{NS}	-0.295 ^{NS}	-	0.888 ^{NS}	0.997 [*]	1.000	
Lipid	0.857 ^{NS}	-0.997 [*]	0.994 [*]	0.993 ^{NS}	0.924 ^{NS}	0.061 ^{NS}	-	-0.993 [*]	-	0.974 ^{NS}	0.097 ^{NS}	0.177 ^{NS}	1.000
							0.646 ^{NS}		0.396 ^{NS}				

Other variables too have displayed very close correlations, like tannin has almost linear positive correlation with flavonoid and saponin with correlation coefficient of 0.985 and 0.989, respectively. The phenol content possesses close positive correlation with flavonoid (0.967) and saponin (0.960). Saponin with lipid (0.924),

bonplandianum leaf. 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 variance of which were 67.80% and 32.19%, respectively.

In this present investigation, 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* leaf extract. The loading plot (figure 1) was used to draw an overview of the significance among the quantification of the phytochemicals and the correlation matrix (table 3) 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, phenol, flavonoid, saponin, ascorbic acid, riboflavin, protein and lipid content were heavily loaded positively on the PC1 with squared cosine value of 0.999, 0.890, 0.977, 0.982, .600, 0.629, .689 and 0.835, respectively. Whereas only the soluble sugar content displayed high quantum of positive loading on PC2 with squared cosine value of 0.776.

The antioxidant capacity of the plant extracts are mainly attributed to the phenolic and flavonoid species of compounds [33] and it is very interesting to note that these compounds have resided in the same cluster with very high PC1 positive value. From the results of PCA, it can be said that the phytochemicals of *C. bonplandianum* leaf resides in three major clusters. The largest cluster bearing the tannin, saponin, flavonoid, phenol and lipid content may contribute highest to the bioactive profile of the plant, followed by the second major cluster riboflavin and bearing ascorbic acid, protein. Table 3 represents the correlation matrix of the various phytochemicals quantified. Though interrelation between the variables were non-significant ($p > 0.05$) for the most cases but it is very interesting to note that two important variables namely, flavonoid and saponin are completely correlated (coefficient of correlation 1.00) with each other.

ascorbic acid with protein (0.993), riboflavin with protein (0.997) and lipid with flavonoid (0.993) content have displayed very close positive correlation between them. It is fascinating to note that the phenol with lipid and the riboflavin content have displayed almost linear positive correlation with correlation coefficient of 0.999. Form

the correlation matrix it can be concluded that out of all the parameters studied, the variable which contributed in the correlation pattern of PCA is the protein content, followed by riboflavin content with a cumulative correlation coefficient of 3.390 and 3.314, respectively.

CONCLUSION

It can be concluded from the present study that the leaf of *C. bonplandianum* possesses various phytochemicals like alkaloid, total phenol, saponin, flavonoid, protein and tannin in very high quantity. These phytochemicals possess various bioactive properties and may be used as external therapeutic supplement. Medicinal value of *C. bonplandianum* is well recognized throughout the world in different ethnopharmacological practices and the presence of high quantities of these bioactive phytochemicals may attribute to its medicinal value. Present study is the first of its kind that provides not only the overall phytochemical screening of the leaves of this plant but also elucidates the correlation in quantities among the different phytochemicals present in it. PCA is one of the rational approaches that can be taken to correlate the quantities of different phytochemicals present in the leaf sample. We are now trying to identify and isolate the different phytochemicals from the leaf of *C. bonplandianum* and to test these bioactive compounds for their antioxidant, immunomodulatory, hepatoprotective and anti-cancer activity.

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