



*Research Paper*

**QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF  
ETHANOMEDICINAL FOLKLORE PLANT- *Clerodendrum  
colebrookianum***

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**Abstract**

*Clerodendrum colebrookianum* is popularly known as *Phuihnamb* by the Mizo community. This ethnobotanical medicinal plant of the North Eastern India is commonly used to treat and cure certain disorders such as hypertension, diabetes and gastrointestinal tract disorders. The leaves of the plant is mostly consumed by boiling. This paper attempts to compare the phytochemicals present in the raw and boiled extracts of the leaves qualitatively and quantitatively. Qualitative studies indicate the presence of carbohydrates, proteins, carotenoids, alkaloids, flavonoids, phenols, steroids, saponins, tannins, terpenoids, various glycosides such as cardiac-glycosides, coumarin glycosides and anthraquinones. Quantitative studies of total sugars, total proteins and total antioxidants showed a considerable amount present in the boiled extract having about 2.28±0.53 mg/mL glucose equivalents, 2.43±0.57 mg/mL, 0.41±0.44 mg/mL ascorbic acid equivalents respectively. Phenols and flavonoids present in the boiled extract were found to be 0.50±0.10mg/ mL Gallic acid equivalents and 0.38±0.056 mg/mL Quercetin equivalents respectively. The phytochemicals present in the boiled extract were found to be higher than the phytochemicals present in the raw extract. Thus supporting the medicinal value of the traditional method of consumption of the boiled form of the ethanomedicinal folklore plant *Cleodendrum colebrookianum* by the Mizo community in the north eastern region of India.

Key words: *colebrookianum*, *Phuihnamb*, Terpenoids, Glycosides, Antioxidants, Phytochemical Analysis.

**INTRODUCTION**

*Clerodendrum colebrookianum* is known by many vernacular names among the tribal communities in the north eastern region of India. To name a few, *Phuihnamb* by the Mizos, *Nefafuby* by the Asamesse and *Umremby* by the Nagas[1]. This plant is traditionally used for treatment and cure of many human disorders, diseases and ailments. The leaves of this plant are boiled and consumed as delicacy as well as medication. The taste of the leaves differs according to the frequency with which it is plucked. The older leaves taste bitter when compared to the young tender leaves. Traditionally it is used as a remedy for hypertension, diabetes,

gastrointestinal disorders, rheumatism, asthma and inflammatory diseases. Due to these various medicinal usage of the plant it is therefore necessary to isolate and identify its biochemically active compounds for developing semi-synthetic drugs.

The Mizo community is known to consume this plant as a boiled vegetable along with red meat or pork so as to prevent indigestion of the meat and often to reduce the fat absorption by the body. The present paper is an attempt to find out the phytochemical properties of boiled and raw leaf extract of *Clerodendrum colebrookianum*.

*Clerodendrum colebrookianum* is an evergreen wild shrub in North East India. According to Janmoni Kalita and her co-workers, "*Clerodendrum colebrookianum* is a perennial shrub which generally grows well in moist and shady habitat at higher altitudes and grows up to 4 - 8 ft. height. Stem quadrangular, branches robust and sparsely pubescent with corky internodes. Leaves often 9 inch diameter, opposite, broad-ovate, acute, entire, petiolate, small lateral veins (6-9) with few glands clustered at the petiole and scattered beneath. Inflorescence terminal, compact, corymbose cymes. Flowers white in broad terminal compact, numerous, bracteates, pedicelate (2-4 cm). Bracts lanceolate or narrowly ovate, caduceous at the time of flowering. One bract is present for each flower and glands are present on lower surface. Calyx gamosepalous, persistent, sepaloid, campanulate with several peltate glands, sepals 5 and glandular. Calyx-teeth short triangular, reddish purple. Corolla gamopetalous, petals 5, white in colour, tube nearly glabrous, stamens 4, didynamous, filiform. Gynoecium has exerted style and shorter than stamen with 4 loculi. Anthers reddish or marron, introse. Fruit is drupe, subglobose, glossy and bluish green in colour that turns on black on drying. *Clerodendrum colebrookianum* is distinguished by having broadly ovate or cordate leaf blade with large peltate glands or glands on the abaxial surface of the leaf base and corymb thyrsoid inflorescence" [1].

## MATERIALS AND METHODS

### Plant material

The 5 year old plant was transplanted from Mizoram in 2005 and was grown in United Theological College, Bangalore. The leaves were randomly selected and collected on 4<sup>th</sup> November, 2015. Two separate 10% raw and boiled aqueous extracts of the leaves were prepared and this was used to quantify the phytochemical constituents present in the leaf. To carry out qualitative analysis 10% methanolic extract of both raw and boiled leaves were prepared along with 20% raw and boiled aqueous extract.

### Total Antioxidants

The total antioxidant activity of the samples was estimated by the method described by Braca [2]. A stock solution of Ascorbic acid (1000 µg/mL) was diluted suitably in order to obtain different concentrations of ascorbic acid ranging from 10µg-100µg. 0.1mL of each of the above prepared concentrations of Ascorbic acid was taken in clean dry test-tubes. The volume in each tube was made up to 3 mL with DPPH (2, 2-diphenyl-1-picrylhydrazyl). The test tubes were incubated for 10 minutes at room temperature. The contents of the tubes were mixed well and the absorbance read at 517 nm against a suitable blank. A blank containing only distilled water and control containing 3.1 mL DPPH was used. A standard curve was plotted to determine the total antioxidant content in the samples. The total antioxidant content was calculated in terms of Ascorbic acid equivalents and expressed as mg/mL of the aqueous extract.

### Total Proteins

The total protein content of the samples was determined by using Folin-Ciocalteu reagent following a slightly modified method of Lowry-*et-al* [3][4]. Bovine serum albumin (BSA) was used as a reference standard for plotting calibration curve. A volume of 0.1mL of the aqueous extract was mixed with 5mL of Alkaline Copper reagent, mixed well and allowed to stand for 10 minutes. Followed by addition of 0.6mL of the Folin-Ciocalteu reagent (diluted 1:1 with de-ionized water) and was incubated for 30 minutes for color development. The absorbance of the resulting blue color was measured at 660nm using a colorimeter. The total protein contents

were determined from 1 Optical Density (O.D.) correspondence of a standard curve prepared with BSA. The content of total protein was expressed as mg/mL of the aqueous extract.

#### **Total Phenols**

The total phenolic content of the samples was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth[5]. Gallic acid was used as a reference standard for plotting calibration curve. The reaction mixture consists of 0.1 mL of the aqueous extract which was mixed with 0.5 mL of the Folin-Ciocalteu reagent (diluted 1:1 with de-ionized water). The tubes were allowed to stand for 3 minutes and were neutralized with 2 mL of Sodium carbonate solution (20%, w/v) incubated at room temperature for 60 minutes with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 650 nm using a colorimeter. The total phenolic content were determined from 1 O.D. correspondence of a standard curve prepared with Gallic acid. The content of total phenols was expressed as mg/mL Gallic acid equivalent (GAE) of the aqueous extract.

#### **Total Sugars**

The total sugar content of the samples was determined by Anthrone method[6][7]. D-Glucose was used as a reference standard for plotting calibration curve. The reaction mixture consists of 0.1 mL of the aqueous extract which was made up to 1 mL using distilled water followed by addition of 4 mL of Anthrone reagent (10-keto- 9, 10-dihydroanthracene). The tubes were incubated in boiling water bath for 10 minutes and cooled. The absorbance of the resulting green color was measured at 630 nm using a colorimeter. The total sugar contents were determined from 1 O.D. correspondence of a standard curve prepared with glucose. The content of total sugars was expressed as mg/mL glucose equivalent of the aqueous extract.

#### **Flavonoids**

The flavonoid content of the samples was estimated using the method described by Yang[8]. (Yang et al., 2001) 0.5 to 2.0 ml aliquots of standard Quercetin solution (1 mg/1 ml) was pipetted out into different test tubes. The volume in each of the tubes was made up to 2 mL with methanol. 2 mL of methanol served as the blank. 0.1 mL of 10 % Aluminium chloride was added to each of the tubes followed by 0.1 mL of 1M Potassium acetate solution and 2.8 mL of distilled water. The test tubes were incubated for 30 min at room temperature. The contents of each tube were mixed well and the absorbance was read at 670 nm against the blank.

#### **Statistical analysis**

Samples were triplicated for the quantitative determination of each of the biochemical components. The average value for each of the triplicate samples was used for data analysis. The data were presented as Mean  $\pm$  SD for each group.

#### **Phytochemical Screening**

Chemical tests were carried out on methanolic and aqueous extracts to identify the presence of phytochemicals standard procedure to identify the constituents as described by experiments carried out previously [9][10][11][12][13][14][15][16].

**Test for Cardiac glycosides:** 5 mL of each extract was treated with 2 mL of glacial Acetic acid containing one drop of Ferric chloride solution. This was mixed with 1 mL of concentrated Sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardiac glycosides. A violet ring may appear below the ring while in the Acetic acid layer, a greenish ring may be formed.

**Test for Saponins:** 1 mL of the sample was separately boiled with 10 mL of distilled water in a water bath for 10 minutes. The mixture was filtered while hot and allowed to cool. Demonstration of frothing was carried out: 2.5 mL of filtrate was diluted to 10 mL with distilled water and shaken vigorously for 2 minutes (frothing indicated the presence of Saponins in the filtrate).

**Test for Terpenoids:** 5 mL of each extract was mixed in 2 mL of Chloroform. 3 mL of concentrated Sulphuric acid was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of Terpenoids.

**Test for Flavonoids:** 1 mL of sample was taken and few drops of 20% Sodium hydroxide solution were added. A change to yellow colour which on addition of acid changed to colorless solution depicted the presence of flavonoids.

**Test for combined Anthraquinones:** 1mL sample of each specimen was boiled with 2 mL of 10% Hydrochloric acid for 5 minutes. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of Chloroform and the Chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % Ammonia solution was added into the Chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an Anthraquinones.

**Test for Carotenoids:** 1mL of the sample was taken to which 10 mL of Chloroform was added in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % Sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

**Test for Carbohydrates:**

- a) Molisch's test: To 1mL of the extract few drops of 1%  $\alpha$ -Naphthol was added and mixed well followed by the addition of 2mL of concentrated Sulphuric acid along the sides of the test tube. Formation of condensed purple colour shows the presence of carbohydrates.
- b) Fehling's test: To 1mL of the extract equal volumes of Fehling's A and Fehling's B solution was added and heating in boiling water bath for 5 minutes. Appearance of reddish-brown precipitate helps in the detection of reducing sugars.

**Test for Alkaloids:** 1mL of the sample extract was taken to which few drops of concentrated Hydrochloric acid was added stirred followed by the addition of Dragendroff's reagent. Appearance of orange colour indicates presence of alkaloids.

**Test for Tannins:** 1 mL of each sample was separately boiled with 20 mL distilled water for five minutes in a water bath and was filtered while hot. 1 mL of cool filtrate was distilled to 5 mL with distilled water and a few drops (2-3) of 10% Ferric chloride were observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

**Test for Phenols:** 1ml of the sample extract was taken to which 5% of ferric chloride was added. Appearance of violet colour shows the presence of phenols.

**Test for Coumarin glycosides:** 1mL of the extract was made alkaline with 10% Sodium hydroxide. Appearance of blue-green fluorescence shows the presence of Coumarin glycosides.

**Test for fixed oils and fats:** 0.5N Potassium hydroxide was added to the sample extract and few drops of Phenolphthalein was added and heated in the water bath. Appearance of pink colour indicates the presence of fats or oils.

**Test for Steroids and Phytosterols:** To 1mL of the sample glacial Acetic acid was added to which 1mL of concentrated Sulphuric acid was added carefully along the sides of the test tube. Appearance of greenish blue colour shows the presence of Steroids and Phytosterols.

**Test for Proteins:** To 1mL of the sample 5% Sodium hydroxide and 1% Copper sulphate was added. Appearance of purple colour indicates the presence of proteins and free amino acids.

## RESULT AND DISCUSSION

The various phytochemicals which were quantitatively determined is presented in table 1. The total protein content in raw extract was found to be  $1.53 \pm 0.19$  mg/mL, which was comparatively less when compared to the boiled extract which consisted of about  $2.43 \pm 0.57$  mg/mL. Similar estimation carried out by S. Majaw and J. Moirangthem[17] showed  $0.297 \pm 13.46$ mg/mL of the total protein content present in *Clerodendrum Colebrookianum* leaves. The total phenolic content present in boiled extract and raw extract was  $0.50 \pm 0.10$  mg/mL Gallic Acid Equivalents (GAE) and 0.22 mg/mL GAE respectively. The boiled extract contained twice the amount of phenol present in that of the raw extract. Hence the ratio of total phenols present in the raw extract is to boiled extract was found to be 1:2. Similarly S. Majaw and J. Moirangthem[17] showed  $2.52 \pm 0.041$  g/L (GAE) of phenolics was found to be present in the leaves of *Clerodendrum Colebrookianum*. The total sugars present in the raw extract was  $1.53 \pm$

0.32 mg/mL glucose equivalents and  $2.28 \pm 0.53$  mg/mL glucose equivalents was found to be present in the boiled extract. Hence the total sugars present in the boiled extract was found to be higher than the raw extract. This may suggest that many of the polysaccharides and unavailable carbohydrates are broken down during the process of hydrolysis due to boiling. The total antioxidants present in the boiled extract was found to be higher than in raw extract having about  $0.41 \pm 0.44$  mg/mL and  $0.015 \pm 0.007$  mg/mL Ascorbic acid equivalents respectively. Studies carried out by the Botanical Survey of India showed that *Clerodendrum Colebrookianum* has an  $IC_{50}$  value of  $3333.99 \pm 516.22$   $\mu$ g/L DPPH radical scavenging activity. Total flavonoids present in the raw extract was found to be  $0.23 \pm 0.025$  and about  $0.38 \pm 0.056$  of total flavonoids was found to be present in boiled extract. Similarly S. Majaw and J. Moirangthem showed that about  $32.83 \pm 0.49$  mg/g of flavonoids present in the leaves of *Clerodendrum Colebrookianum*. [17]

The phytochemicals were found to be present in higher amounts in the boiled extract when compared to the raw extract, this infers that many of the phytochemicals present in the bound form are released and made available to react when boiled. Hence the boiled extract proves to be a good source of natural medicine. This could be related to the traditional medicinal use of the leaves of *Clerodendrum colebrookianum* by the Mizo tribe as a source of vegetable in its boiled form.

The qualitative analysis carried out showed the following results as tabulated in table 2. Alkaloids were found to be present both in aqueous and organic extract of the leaves, whereas Saponins were found to be extracted only in the aqueous solvent and tannins were found to be extracted only in the organic solvent. The phytochemicals such as alkaloids, saponins and tannins are known to have antibiotic properties [18] [19]. Experiments previously carried out also showed that leaves which taste bitter contains an alkaloid which is capable of reducing headaches associated with hypertension [20]. Studies carried out on tannins and saponins reported that they constitute the main components in traditional herbal medicine preparation [21] [22] [23]. Many studies have reported the antibacterial properties of tannins [24]. Flavonoids was found to be present in both aqueous and organic extract. Experiments on various flavonoid compounds and tannins have indicated that tannins and flavonoids are responsible for anti-diarrheal properties. The mode of action of these phytochemicals were found to increase colonic water and electrolyte reabsorption, while some components were shown to inhibit enteropathogens [25]. Phytosterols and steroids were found to be present in both aqueous and organic extract. Steroids are found to be anti-inflammatory and analgesic agents [26]. Phenolic acids, tannins, flavonoids are known for their potent antioxidant properties. They help in reducing oxidative stress, tumor suppression, anti-diabetic agent and many other human disorders and diseases [27]. The presence of carbohydrates, carotenoids proteins and fixed oils accounts for the nutritional value of the leaves of *Clerodendrum Colebrookianum* [28] [29]. Dietary carotenoids are believed to reduce the risk of certain cancers and eye disease [30] [31]. Anthraquinones were found to be extracted in the boiled aqueous extract. Anthraquinones are known for their stimulating laxative effect, which act directly on the intestinal mucosa by increasing peristalsis and reducing transit time. They influence several pharmacological targets. They help in reabsorption of water from the colon thus making the stool more liquid and easing bowel movements hence often used to relief constipation [32]. Cardiac-glycosides were also found to be present in both the extracts. Cardiac glycosides, are the earliest therapies discovered for congestive heart failure and arrhythmia [33]. Coumarin glycosides are also found to be present in both aqueous and organic extracts. In general coumarins have antifungal, hypotensive, anticoagulant and antimicrobial properties [34]. Terpenoids were found to be present both in aqueous and organic extracts. Terpenoids among the secondary metabolites form a group of defense system for the plant. Terpenoids are used for developing sustainable pest control and abiotic stress protection [35].

Table 1: Quantitative analysis of phytochemicals

PARAMETERS	RAW EXTRACT	BOILED EXTRACT
Total Proteins ( mg/mL)	1.53±0.19	2.43±0.57
Total Phenols ( mg/mL)	0.22±0.04	0.50±0.10
Total Sugars ( mg/mL)	1.53±0.32	2.28±0.53
Total Antioxidants ( mg/mL)	0.015±0.007	0.41±0.44

Table 2: Qualitative analysis of phytochemicals

PARAMETERS	AQUEOUS EXTRACT		ORGANIC EXTRACT	
	RAW	BOILED	RAW	BOILED
<b>Alkaloids</b>	+	+	+	+
<b>Anthroquinones</b>	-	+	-	-
<b>Carbohydrates</b>	+	+	+	+
<b>Cardiac-glycosides</b>	+	+	+	+
<b>Carotenoids</b>	-	-	+	+
<b>Coumarin-glycosides</b>	+	+	+	+
<b>Fixed oils</b>	+	+	+	+
<b>Flavonoids</b>	+	+	+	+
<b>Phenols</b>	+	+	+	+
<b>Phytosteroids</b>	+	+	+	+
<b>Proteins</b>	+	+	+	+
<b>Saponins</b>	+	+	-	-
<b>Steroids</b>	+	+	+	+
<b>Tannins</b>	-	-	+	+
<b>Terpenoids</b>	+	+	+	+

## CONCLUSION

The leaves of *Clerodendrum Colebrookianum* improves to be a potential herbal medicine. The various plant compounds present in the aqueous extract of *Cleodendrum colebrookianum* could act as an antidote for the many of the lifestyle related disorders such as stress, diabetes and hypertension. We are currently working on how these phytochemical compounds would combat stress and its related disorders by exvivo experiments. We would like to conclude that this plant extract would prove to be a potential drug to curb stress and stress-related disorders in the near future.

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