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Evaluation of Pharmacognostical, Phytochemical and In-Vitro Antioxidant Activity of Clerodendrum Colebrookianum Stem Extract

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KEYWORDS

Clerodendrum colebrookianum; phytochemicals; DPPH; Hydrogen Peroxide scavenging

ABSTRACT:

Introduction: The dependence on plant-based healthcare in developing nations arises from issues accessing allopathic medicine and concerns over its side effects. Traditional herbal remedies are assumed as safe and culturally inherited and are highly accepted as seen in Egypt's primary healthcare system. However, the preservation of this traditional knowledge is crucial due to its vulnerability over time. Globally, there's a renewed interest in herbal medicine, driven by safety concerns surrounding synthetic products. India, with its rich herbal heritage documented in texts like Ayurveda illustrates this resurgence. Recognizing and preserving traditional medicinal knowledge alongside scientific research on herbal products is essential for global healthcare. One such medicinal plant, *Clerodendrum colebrookianum*, known as "Nefafu" is prevalent in India's North-Eastern states. It holds significant traditional medicinal value, utilized for treating various ailments such as hypertension, cough, dysentery and skin diseases, emphasizing its importance in regional healthcare practices.

Objectives: This study explores the pharmacological potential and traditional significance of *Clerodendrum colebrookianum* (Nefafu) as a medicinal plant, particularly focusing on its antioxidant activity.

Methods: The research focuses on the therapeutic potential of the plant through extensive phytochemical screening and antioxidant assays such as DPPH radical scavenging and Hydrogen Peroxide activity. Additionally, the total phenolic and flavonoid content of the extract was determined.

Results: Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, phenols and fixed oils, aligning with its traditional medicinal use and suggesting new paths for pharmaceutical applications. The antioxidant assays demonstrated notable scavenging effects with an IC_{50} value of 155.27µg/ml in DPPH radical scavenging activity, indicating its efficacy in combating oxidative stress. While the test sample exhibited slightly higher percentage inhibition than ascorbic acid at lower concentrations, the latter demonstrated stronger and more consistent inhibition at higher concentrations as evidenced by an IC_{50} value of 669.7 µg/ml in Hydrogen Peroxide scavenging activity.

Conclusions: This analysis positions *Clerodendrum colebrookianum* as a promising candidate for further exploration in contemporary herbal medicine bridging traditional use with modern scientific validation.

1. Introduction

The dependence on plant-based resources for healthcare in developing countries arises from the accessibility issues and potential side effects of allopathic medicine[1]. Traditional medicine, especially herbal remedies is accepted due to its assumed safety and cultural inheritance[2,3]. This dependence is evident in Egypt where medicinal plants play a crucial role in primary healthcare[4]. However, the documentation of traditional knowledge is vital as it is vulnerable to loss over generations[5]. Globally, there is a resurgence of interest in herbal medicine driven by concerns over synthetic products' safety. India, historically rich in medicinal plants boasts a vast depot documented in ancient texts like Ayurveda[6,7,8]. These texts organize thousands of plant species for medicinal use, continuing to influence contemporary herbal practices. Therefore, the recognition and preservation of traditional

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medicinal knowledge alongside the scientific characterization of herbal products are vital for global healthcare systems[9].

Clerodendrum colebrookianum, commonly known as East Indian glory bower or locally as "NEFAFU", is a widely recognized medicinal plant found predominantly in the North-Eastern states of India[10]. This plant, flowering post-monsoon from August to December holds a significant traditional medicinal value[11]. It has been utilized for generations in treating various ailments such as cough, dysentery, stomach disorders, helminthic infections, diabetes and skin diseases. Particularly noteworthy is its role in hypertension treatment, highly regarded by different tribes in the North Eastern region[12]. The plant is distributed across tropical and subtropical regions internationally including Bangladesh, China, Indonesia, Malaysia, Nepal, Sri Lanka and Vietnam. In India, it grows mainly in states like Assam, Meghalaya, Arunachal Pradesh, Nagaland and Manipur, typically found at altitudes ranging from 1 to 4000 ft above sea level[13]. The species diversity of *Clerodendrum colebrookianum* within India, especially in Arunachal Pradesh, highlights its botanical significance within the country's flora.

Extensive literature surveys have emphasised the pharmacological and medicinal potential of Nefafu *(Clerodendrum colebrookianum)*. Various studies have focused on different aspects, ranging from its traditional use to modern scientific investigations. These include studies on rapid clonal propagation techniques[14], chemical compound isolation[15], endophytic bacterial diversity[16] and pharmacological evaluations[17]. For instance, studies have elucidated the plant's potential in antihypertensive[18,19], anthelmintic[20], antioxidant[21,22], anti-inflammatory[23] and analgesic activities[23,24]. Additionally, investigations into its molecular interactions and pharmacognostic characteristics further enhances our understanding of its therapeutic properties[25]. Such extensive research ventures not only validate its traditional use but also pave the way for potential pharmaceutical applications and efforts to protect it from disappearing. The interdisciplinary approach merging traditional knowledge with modern scientific methodologies brings out the plant's significance in both cultural heritage and contemporary healthcare practices.

2. Materials and Methods

Collection of the Plant Materials: Nefafu *(Clerodendrum colebrookianum)* stems were collected from Vill-Town Bantow, Dist-Lakhimpur, Assam, India (Pin: 787031). Authentication of the collected plant samples was conducted at Assam Bio-Resource Centre, Madan Kamdev, Kamrup, Assam under Assam Science Technology and Environment Council. The samples were assigned accession number ABRC/1026/21.

Figure 1. *Clerodendrum colebrookianum*

Sample Preparation: The stems of Nefafu *(Clerodendrum colebrookianum)* were collected and thoroughly washed with tap water to eliminate all soil and dirt. Leaves underwent partial drying, exposed to both daylight and in the dark for 15 days. The dried bark was precisely fragmented and ground into powder using a mechanical grinder. The resultant powder was sieved through a number 60 mesh and carefully stored in airtight containers to shield from moisture. 60 grams of the powdered material were then subjected to extraction with 200 ml of ethanol using a Soxhlet apparatus. The resulting extract underwent concentration through evaporation, maintaining a temperature not exceeding 50ºC. Finally, the concentrated extract was dried and carefully stored in an airtight container for future utilization in subsequent experiments.

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Figure 2. Dried plant material *(Clerodendrum colebrookianum)*

Figure 3. Ethanolic extracts of *Clerodendrum colebrookianum*

Macroscopic evaluation: The macroscopic examination of a crude drug involves its visual characteristics being decoded to the naked eye as well as its sensory attributes such as odour, taste and texture. Additionally, a simple microscope with a 10x magnification may be employed to further elucidation of specific structural features[26].

Microscopic evaluation: The plant's young and dried stems were collected and rinsed with water. Thin sections were then transversely cut and stained with safranin before being examined under a Binocular camera microscope.

Phytochemical Screening[27,28]: The ethanol extract of Nefafu *(Clerodendrum colebrookianum)* was tested using specific reagents to detect the presence of bioactive compounds such as alkaloids, saponins, tannins, phenols and oils.

Alkaloid test:

1. *Mayer's Test:* Take a solution of the extract and filter it. Treat the filtrate with Mayer's reagent. The presence of alkaloids is indicated by a white or yellowish-white (creamy) precipitate with Mayer's reagent (potassium mercuric iodide).
2. *Dragendorff's Test:* Take a solution of the extract and filter it. Treat the filtrate with Dragendorff's reagent. The presence of alkaloids is indicated by orange or reddish-brown precipitates with Dragendorff's reagent (potassium bismuth iodide).
3. *Wagner's Test:* Take a solution of the extract and filter it. Treat the filtrate with Wagner's reagent. The presence of alkaloids is indicated by a reddish-brown precipitate with Wagner's reagent (iodine in potassium iodide solution).
4. *Hager's Test:* Take a solution of the extract and filter it. Treat the filtrate with Hager's reagent. The presence of alkaloids is indicated by a white precipitate with Hager's reagent (picric acid in acetic acid).

Saponin test:

A mixture consisting of 0.5 grams of the extract and 5 ml of distilled water was vigorously shaken. The formation of stable and persistent froth indicated the presence of saponins.

Tannin test:

1. *Ferric Chloride Test:* Filter a solution of the extract, then add 1 ml of the filtrate to which 2 drops of ferric chloride solution are added. The formation of a bluish-black or greenish-black precipitate indicates the presence of tannins.
2. *Lead Acetate Test*: Filter a solution of the extract, then add 1 ml of the filtrate to which 3 drops of lead acetate solution are added. The formation of a white or pale-coloured precipitate indicates the presence of tannins.

Phenol test:

1. *Ferric Chloride Test:* Similar to the test for tannins, adding ferric chloride solution to a phenol-containing solution results in the formation of a coloured complex. The colour can vary depending on the phenol present. Common colours observed include green, blue, violet, or reddish-brown, depending on the nature of the phenolic compound and the

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concentration of both the phenol and the ferric chloride solution.

2. *Millon's Test:* Adding Millon's reagent to a phenol-containing solution followed by heating results in the formation of a red precipitate or coloration.

Fats and oil test:

A quantity of 0.1 g of the extract was pressed between filter paper and the resulting paper was initially observed. Subsequently, the paper was warmed to 60°C and observed once more. The presence of translucency in the filter paper upon warming suggests the presence of fats and oils. Conversely, the disappearance of translucency upon warming typically indicates the absence of essential oils.

Total ash

The total ash technique is designed to measure the total quantity of material remaining after incineration. This includes both the "physiological ash", which is derived from the plant tissue and "non-physiological ash", which is the residue of the irrelevant matter adhere to the plant surface[29].

Method: About 3gm of crude drug powder was taken and weighed in a tared silica dish which was formerly ignited and weighed. The powdered drug was scattered on the bottom of the dish and ignited by gradually increasing the heat up to 550^0c until it is white indicating the absence of carbon. If the carbon free ash cannot be obtained, then the charred mass was exhausted with hot water. After this, the residue was collected on an ashless filter paper, the residue, filter paper and the filtrate were incinerated, evaporated to dryness and again ignited at low temperature[30]. The percentage of ash was calculated with reference to the air-dried drug using the following formula:

$$\% \text{ of total ash} = X - Y \div Z * 100$$

Where, X- weight of the dish + ash (after complete incineration)

Y- weight of the empty dish

Z- weight of the powdered drug taken

1) Acid-insoluble ash

Acid-insoluble ash is the residue obtained after boiling total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. Its measures the amount of silica present, particularly as sand and siliceous earth.

Method: The total ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, ignited, cooled in a desiccator and weighed[14]. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated as follows:

$$\% \text{ of Acid-insoluble ash} = X-Y \div Z*100$$

Where, X- weight of the dish + ash (after complete incineration)

Y- weight of the empty dish

Z- total ash

2) Water soluble ash

Water soluble ash is that part of the whole ash content which is soluble in water. This is the difference in weight between the total ash and the residue after treatment of the total ash with water.

Method: The total ash is boiled with 25 ml of water for five minutes and the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15 minutes at a temperature not more than 450^0C. By subtracting the weight of insoluble part from that of the total ash, the weight of the soluble part of ash was obtained as follows:

$$\% \text{ of water soluble ash} = X-Y \div Z*100$$

Where, X- Total ash

Y- Water insoluble ash

Z- Powdered crude drug

Determination of extractive values

The extractive values of crude drugs are essential for evaluating medicinal plants, especially when the constituents cannot be quickly estimated by other methods. These values indicate the presence of polar, medium polar and non-polar components in the plant material, offering insights into its chemical nature[31]. Extractive values aid in assessing the quality of crude drugs, providing information about soluble constituents in specific solvents used for extraction, particularly when no suitable biological assay is available. The use of various solvents of increasing polarity such as petroleum ether, alcohol and water help in obtaining reliable values with each solvent extracting different phytoconstituents based on the drug's characteristics. The amount of extract obtained in a solvent serves as a measure of certain constituents present in the drug with petroleum ether extracting fixed oil, resins and volatile substances, alcohol dissolving a wide range of substances including glycosides and alkaloids and water extracting water-soluble constituents[32].

1) Alcohol soluble extractives

Method: 5gm of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in closed flask for 24 hours,

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repeatedly shook during the first 6 hours and was allowed to stand for 18 hours. Thereafter it was filtered rapidly. 25 ml filtrate was evaporated to waterlessness in a tarred flat bottom shallow dish, dried at 105° and weighed. The percentage of alcohol soluble extractive calculated by the equation as follows:

% of alcohol soluble extractive = X-Y÷Z*100

Where, X- initial weight of petri dish

Y- final weight of petri dish

Z- weight of the drug

2) Water soluble extractive

Method: 5gm of coarse powdered drug was macerated with 100 ml distilled water in closed flask for 24 hours, frequently shook during the first 6 hours and was allowed to stand for 18 hours. Thereafter it was filtered rapidly. 25 ml of the filtrate was evaporated to waterlessness in a tarred flat bottom shallow dish, dehydrated at 105˚C and weighed. The percentage of water-soluble extractive was calculated by the equation below mentioned:

% of alcohol soluble extractive = X-Y÷Z*100

Where, X- initial weight of petri dish

Y- final weight of petri dish

Z- weight of the drug

Determination of loss on drying

The percentage of active chemical constituents in crude drugs is mentioned on air dried basis. Thus, the moisture content of a drug must be determined and should also be controlled.

The moisture content of a drug should be minimized in order to avoid decomposition of crude drugs either due to chemical changes or by microbial contamination. Loss on drying is the loss in weight resulting from water and volatile matter of any kind dried under specified conditions[33].

Method: 2gm of the air-dried crude drug was accurately weighed in a dried and tarred Petridish and kept in a hot air oven, maintained at 110^0C for four hours. After cooling in desiccator, the loss in weighed was recorded. This procedure was repeat till a constant weight was obtained.

% Loss on drying = loss in weight ÷ weight of the drug in grams*100

Antioxidant activity

When testing natural antioxidants *in vitro*, it is important to consider the system composition, the type of oxidisable substrate, the methods to assess oxidation and how to quantify antioxidant activity. Antioxidant efficacy is also determined by the heterogeneity and the heterophasic nature of initiators, other components and their possible interactions. Each evaluation will be carried out under various conditions of oxidation using numerous methods to determine different products of oxidation. Due to the multifunctional nature of most of the natural antioxidants, a reliable antioxidant protocol should measure more than one property relevant to either foods or biological stems. The ambiguities in the results may be due to nonspecific one-dimensional methods to evaluate antioxidant activity. Therefore, antioxidant testing should be standardized to decrease the discrepancy in the methodologies. Particular methods should be used to obtain chemical information that can be linked directly to oxidative deterioration of food and biological system. The *in vitro* assays give an idea of the protective efficacy of the test system[34].

Following are the methods carried out to evaluate the *in-vitro* antioxidant activity of *Clerodendrum colebrookianum.*

1) Determination of DPPH radical scavenging activity

The antioxidant potential of any compound can be determined on the basis of its scavenging activity of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate). Radical scavenging activity of the methanolic extract of *Clerodendrum colebrookianum* against stable DPPH was determined spectrophotometrically using the method as described by Blois, in 1958 with trivial modifications in the method[35]. After DPPH reacts with an antioxidant, which can donate hydrogen, it becomes reduced, and the highest absorption of the stable DPPH radical in methanol occurs at 517 nm.

Chemicals used in this assay method: 1,1-diphenyl-2-picrylhydrazyl hydrate, Ascorbic acid, Methanol.

Preparation of standard solution: Ascorbic acid was used as standard for this assay. 10 mg of ascorbic acid was dissolved in 10ml of methanol to give concentrations of 20, 40, 60, 80 and 100µg/ml.

Preparation of test sample solution: Stock solutions of samples were prepared by dissolving 10mg of extract in 10ml of methanol to give concentrations of 20, 40, 60, 80 and 100µg/ml.

Preparation of 0.3 mM DPPH solution: 11.82mg of DPPH was dissolved in 100ml of methanol and it was kept protected from light by covering the test tubes with aluminium foil.

Protocol for estimation of DPPH scavenging activity: The DPPH scavenging activity was estimated as per the method described by Blois, (1958) with trivial

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modifications in it. 1ml of 0.3mM DPPH solution was added to 2ml of each different concentration of standard solution and incubated at dark for 30 mins at room temperature after it has been shaken vigorously. This was the preparation of a standard solution of different concentrations. 1ml of 0.3mM DPPH solution was added to 2ml of each different concentration of sample solution and incubated at dark for 30 minutes at room temperature after it has been shaken vigorously. This was the preparation of a test solution of different concentrations.

1ml of 0.3 mM DPPH solution was added to 2ml of methanol and this solution was taken as control and allowed to incubate at dark for 30 mins at room temperature. After 30 min, absorbance was measured 517 nm taking methanol as blank using UV-Visible spectrophotometer. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate. Ascorbic acid was used as a reference compound. The capability to scavenge the DPPH radical was calculated as the inhibition percentage of free radical by the following equation:

$$\% \text{ Inhibition of DPPH scavenging activity} = \frac{Ao-At}{Ao} \times 100$$

Where, A_0 is the absorbance of the control and A_t is absorbance of test/standard.

The antioxidant activity of the extract was designated as IC_{50}. The IC_{50} values can be calculated by linear regression of plots where the abscissa represents the concentration of the tested plant extracts and the ordinate represent the average percent of scavenging capacity. The IC50 value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

2) Determination of Hydrogen peroxide radical scavenging activity

The ability of extracts to scavenge H_2O_2 was determined according to the method of Ruch et al. in 1989. Absorbance was measured spectrophotometrically at 230nm[36].

Chemicals used in this assay method: Hydrogen peroxide (H_2O_2), Ascorbic Acid, Phosphate buffer saline at pH 7.4.

Preparation of standard solution: 20 mg of ascorbic acid was dissolved in 20ml of distilled water and concentration was made up to 50, 100, 200, 400, 600,800 and 1000 µg/ml.

Preparation of test sample solution: 20 mg of extract was dissolved in 20ml of distilled water and final concentration was made up to 50, 100, 200, 400, 600,800 and 1000 µg/ml.

Preparation of test 0.04M H_2O_2solution: 0.068g of H_2O_2 was dissolved in 50ml of phosphate buffer at pH 7.4.

Protocol for estimation of Hydrogen peroxide (H_2O_2) radical scavenging activity: To 1ml of ascorbic acid solution of different concentrations, 0.6ml of H_2O_2 was added. Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing the phosphate buffer without H_2O_2.

To 1ml of extract solution of different concentrations, 0.6ml of H_2O_2 was added. The absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution contain the phosphate buffer without H_2O_2. 1ml of Phosphate buffer and 0.6ml of H_2O_2 was used as control. Reactions were carried out in triplicate spectrophotometrically. The percentage of H_2O_2 scavenging of both the extracts and standard compounds was calculated.

The percentage inhibition was calculated as:

$$\% \ H_2O_2 \text{ radical scavenging activity} = \frac{Acontrol-A\ test}{Acontrol} \times 100$$

Where, $A_{control}$ is the absorbance of the control and A_{test} is the absorbance in thepresence of the sample of the extracts.The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value is defined as the concentration (µg/ml) of dry extract that inhibit the formation of H_2O_2 radicals by 50%.

3) Determination of Total phenolic content: Total soluble phenolics in the extracts were determined according to the method used by Macdonald et al.,2001, with trivial modification using gallic acid as a standard phenolic compound[37]. The phenol reacts with phosphomolybdic acid in presence of alkaline medium to produce blue colour complex known as molybdenum blue complex[28].

Chemicals used in this assay method: Gallic acid, Folin-Ciocalteu reagent, Sodium carbonate

Preparation of gallic acid standard for calibration curve: 10mg of gallic acid was dissolved in 10ml of distilled water to make a solution of 1mg/ml. This solution was further diluted to get concentration of 20, 40, 60, 80 and 100 µg/ml.

Preparation of test sample solution: 10mg of extract was dissolved in 10ml of distilled water to make a solution of 1mg/ml. From this solution it was further diluted to get concentration of 20, 40, 60, 80 and 100 µg/ml.

Preparation of 40ml of 0.2N Folin-Ciocalteu reagent: 4ml of Folin-Ciocalteu was dissolved in 36ml of distilled water.

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Preparation of 100ml of 75g/ml of Sodium carbonate: 7.5g of sodium carbonate was dissolved in 100ml of distilled water.

Protocol for estimation of total phenolic content: 1ml of each concentration of gallic acid was mix with 5ml of Folin-Ciocalteu reagent (diluted 10-fold) and 4ml of sodium carbonate. Absorbance was measured spectrophotometrically in triplicates at 765 nm and calibration curve was plotted.

Similarly, 1ml of each concentration of extract solution was mix with 5ml of Folin-Ciocalteu reagent (diluted 10-fold) and 4ml of sodium carbonate. Absorbance was calculated spectrophotometrically in triplicates at 765 nm. 1ml of distilled water was mixed with 5ml of Folin-Ciocalteu reagent and 4ml of sodium carbonate. This was taken as control and absorbance was measured spectrophotometrically at 765mm.

The total phenol content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

$$T = C \times \frac{V}{M}$$

Where, T- Total phenol contents, mg g^{-1} plant extract in Gallic Acid Equivalent (GAE)

C-Concentration (mg ml) of Gallic acid obtained from calibration curve

V- Volume of extract (ml)

M- Weight (mg) of plant extract

4) Determination of Total flavonoid content: Total soluble flavonoid content of the fractions was determined with aluminium chloride using quercetin as the standard according to the method of Ghasemi et al., in 2009 with trivial modifications in the method[38].

Chemicals used in the assay: Quercetin, Aluminium chloride, Potassium acetate.

Preparation of standard solution: 10mg of quercetin was dissolved in 10ml of methanol to prepare concentration of 1mg/ml or 1000 µg/ml and finally diluted to 50, 100, 150, 200, 250 µg/ml.

Preparation of test sample solution: 10mg of extract was dissolved in 10ml of methanol to prepare concentration of 1mg/ml or 1000µg/ml and finally diluted to 50, 100, 150, 200, 250 µg/ml.

Preparation of 10% Aluminium chloride and 1M Potassium acetate: 2g of Aluminium chloride was dissolved in 20ml of distilled water to prepare 10% Aluminium chloride.

0.98g of Potassium acetate was dissolved in 10ml of distilled water to prepare 1M of potassium acetate.

Protocol for estimation of total flavonoid content: To 1ml of each different concentration of quercetin, 2ml of methanol was added to each concentration. Then it was mixed with 0.2ml of aluminium chloride and 0.2ml of potassium acetate and finally 5.6ml of distilled water was added to each concentration. To 1ml of extract, 2ml of methanol was added. Then it was mixed with 0.2ml of aluminium chloride and 0.2ml of potassium acetate and finally 5.6ml of distilled water was added to each concentration. All the samples were incubated for 30mins at room temperature and absorbance was measured at 415 nm against control. The total flavonoid content in the fraction was determined as µg quercetin equal by using the standard quercetin graph and using the following formula:

$$T = C \times \frac{V}{M}$$

Where, T- Total flavonoid content (mg g^{-1} plant extract) in Quercetin Equivalent (QE)

C- Concentration (mg ml^{-1}) of Quercetin obtain from calibration curve

V-Volume of extract (ml)

M- Weight (mg) of plant extract

3. Results and Discussion

Table 1. Macroscopic characteristics of the stems of *Clerodendrum colebrookianum*

SL. No.	*Characteristics under investigation*	*Stem characteristics*
1.	Colour	Light grey
2.	Odour	Characteristic
3.	Taste	Bitter
4.	Shape	Quadrangular
5.	Texture	Rough

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Transverse section:

Figure 4. T.S of stem of *Clerodendrum colebrookianum*

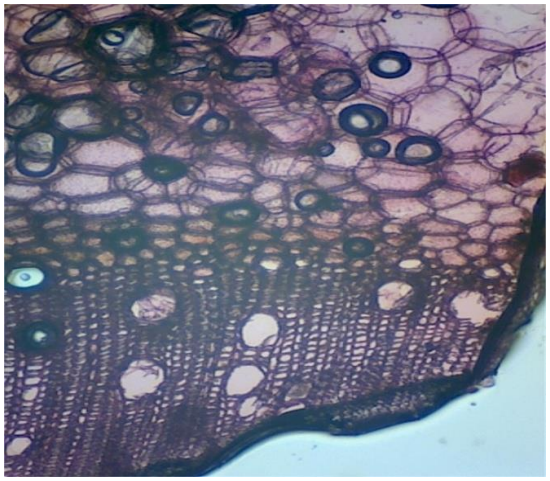

Table 2. Phytochemical screening of ethanolic extract of *Clerodendrum colebrookianum* stem

Phytochemical tests	***Result***
Alkaloids	
Mayer's test	Positive
Dragendorff's test	Positive
Wagner's test	Positive
Carbohydrates	
Molisch's test	Negative
Fehling's test	Negative
Benedict's test	Negative
Proteins and Amino Acids	
Millon's test	Negative
Biuret test	Negative
Xanthoprotein test	Negative
Glycoside	
Keller Killiani test	Negative
Bontrager's test	Negative
Saponins	
Froth formation test	Positive
Tannins and phenols	
Ferric Chloride test	Positive
Lead acetate test	Positive
Detection of fixed oils	
Spot test	Positive

The phytochemical tests revealed that the crude drug tested positive for alkaloids, saponins, tannins, phenols and fixed oils indicating potential pharmacological properties. On the other hand, the crude drug tested negative for carbohydrates, proteins, amino acids and glycosides. Alkaloids, saponins, tannins, phenols, and fixed oils are present. These findings offer insights into the chemical composition of the crude drug aiding its characterization and potential therapeutic uses.

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Table 3. Ash values, extractive values and loss on drying values

SL. No.	*Parameters*	*Ash value and extractive value (% w/w)*
1.	Total Ash	0.94 ±0.04
2.	Acid insoluble ash	0.40 ±0.07
3.	Water soluble ash	0.92 ±0.09
4.	Water soluble extractive	1.42 ±0.25
5.	Alcohol soluble extractive	21.2 ±1.23
6.	Loss on drying	64.5 ±2.80

The data provided includes the total ash content, acid insoluble ash content, water soluble ash content, water soluble extractive value, alcohol soluble extractive value and loss on drying of the sample. These parameters offer insights into the purity, inorganic residue, water and alcohol soluble components and moisture content of the sample. Such information is vital for assessing the quality, purity and potential therapeutic properties of the sample helping in its characterization and standardization for medicinal or pharmaceutical purposes.

Table 4. DPPH free radical scavenging activity of test sample and standard

SL. No.	*Concentration(µg/ml)*	*Percentage inhibition (Mean ± S.E.M)*	
		Test sample	*Ascorbic acid*
1.	20	54.8±9.82	16.76±9.08
2.	40	60.60±6.95	21.28±6.42
3.	60	67.28±5.67	25.98±5.24
4.	80	73.53±4.91	33.09±4.54
5.	100	79.41±4.39	39.44±4.06

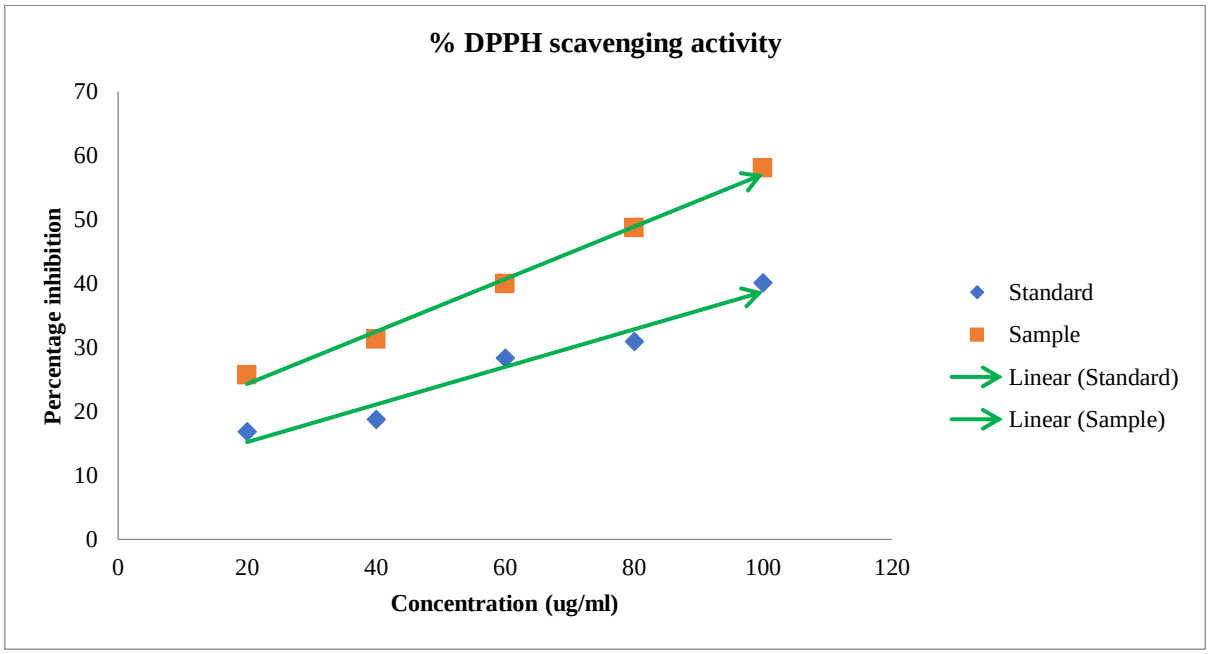

Figure 5. DPPH scavenging activity

The above results indicates that the test sample consistently displayed higher percentage inhibition of the target compared to the standard, ascorbic acid across all tested concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml).

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Additionally, both the test sample and ascorbic acid exhibited a dose-dependent increase in percentage inhibition with increasing concentration. The extract showed antioxidant activity with IC_{50} value of 155.27 µg/ ml. However, the known antioxidant ascorbic acid exhibited IC_{50} value of 170.64µg/ml on DPPH radical. Hence, *Clerodendrum colebrookianum* had significant scavenging effects with increasing concentration when compared with that of ascorbic acid and was found to be comparatively higher.

Table 5. Hydrogen peroxide radical scavenging activity of test sample and standard

SL. No.	*Concentration(µg/ml)*	*Percentage inhibition (Mean ± S.E.M)*	
		Test sample	*Ascorbic acid*
1.	100	5.65±19.96	1.03±27.25
2.	200	17.68±14.11	14.68±19.27
3.	400	30.03±11.52	36.39±15.73
4.	600	43.05±9.98	56.32±13.62
5.	800	56.09±8.92	66.823±12.18

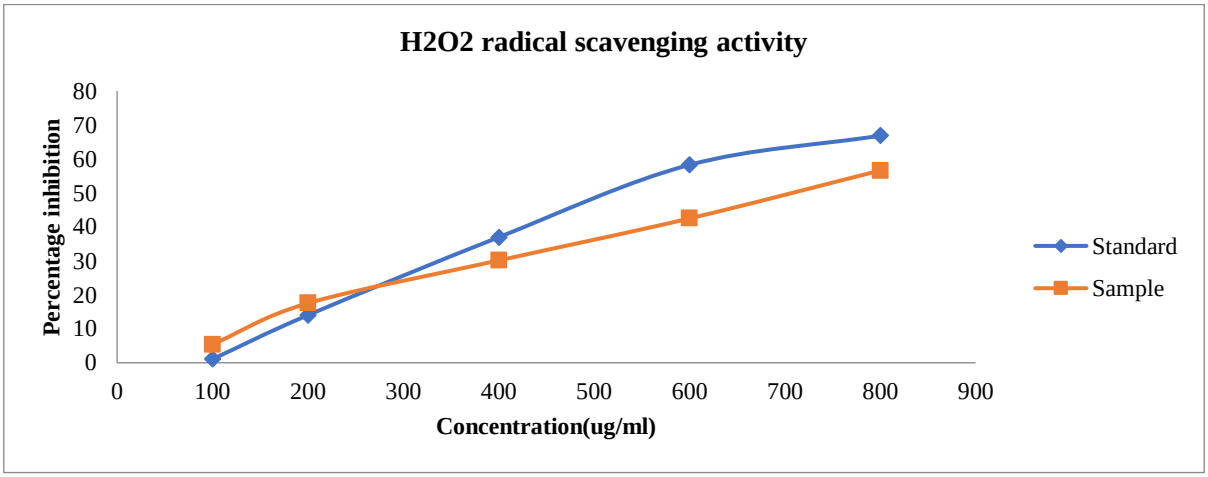

Figure 6. Hydrogen peroxide radical scavenging activity

The above data indicates that at the lowest concentration (100 µg/ml), the test sample showed slightly higher percentage inhibition than ascorbic acid but with high variability in results. However, as concentration increased (200 µg/ml, 400 µg/ml, 600 µg/ml, and 800 µg/ml), the test sample consistently exhibited lower percentage inhibition compared to ascorbic acid. Notably, at higher concentrations (600 µg/ml and 800 µg/ml), the difference in percentage inhibition between the test sample and ascorbic acid became clearer with ascorbic acid consistently demonstrating higher inhibition rates. The IC_{50} values of extract and ascorbic acid were found to be 669.7 µg/ml and 575.02µg/ml respectively. Hence, while the test sample displayed some antioxidant activity, ascorbic acid exhibited stronger and more consistent inhibition across all concentrations tested indicating its superiority in terms of antioxidant activity.

Table 6. Calibration curve in Total Phenolic Estimation

SL. No.		*Concentration (mg ml^{-1})*	*Absorbance (Mean ± SEM)*
1.	**STANDARD**	20	0.267±0.29
2.		40	0.421±0.20
3.		60	0.96±0.16
4.		80	0.747±0.14
5.		100	0.83±0.12
6.	**SAMPLE**	100	0.046±0.006

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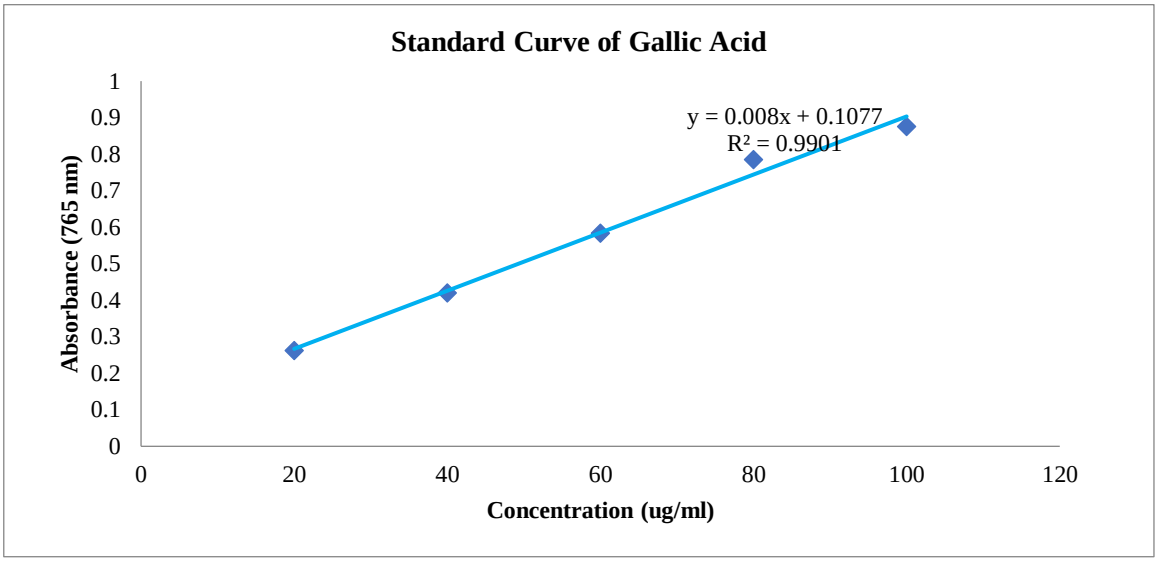

Figure 7. Standard curve of Gallic Acid

Table 7. Calibration curve in Total Flavonoid Estimation

SL. No.		*Concentration (mg ml^{-1})*	*Absorbance (Mean ± SEM)*
1.	**STANDARD**	50	0.238±0.31
2.		100	0.490±0.22
3.		150	0.862±0.18
4.		200	0.891±0.15
5.		250	0.987±0.14
6.	**SAMPLE**	250	0.821±0.11

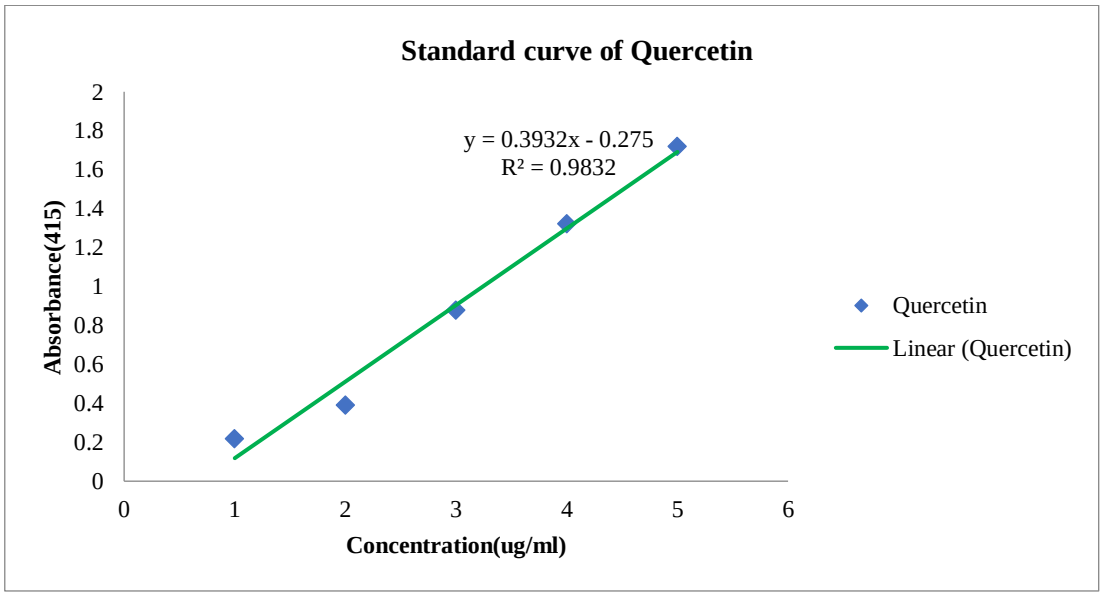

Figure 8. Standard curve of Quercetin

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4. Conclusion

The comprehensive study highlights the pharmacological potential and traditional significance of *Clerodendrum colebrookianum* (Nefafu) as a medicinal plant. Through extensive phytochemical screening and evaluation of antioxidant activity, the study underscores its therapeutic promise. The plant is rich in alkaloid, saponin, tannin, phenol and fixed oil content as revealed by the phytochemical tests highlighting its traditional medicinal use and suggest paths for pharmaceutical applications. The antioxidant assays demonstrate the plant's notable scavenging effects, particularly evidenced by its IC_{50} value of 155.27 µg/ml in DPPH radical scavenging activity indicating its efficacy in combating oxidative stress. While the test sample exhibits slightly higher percentage inhibition than ascorbic acid at lower concentrations, the latter demonstrates stronger and more consistent inhibition at higher concentrations, as reflected in the IC_{50} value obtained in Hydrogen Peroxide scavenging activity with an IC_{50} value of 669.7 µg/ml. This comparative analysis positions *Clerodendrum colebrookianum* as a promising candidate for further exploration in contemporary herbal medicine, bridging traditional use with modern scientific validation.

5. Conflict of Interest

The authors declare no conflict of interest with respect to research, authorship and/or publication of this research article.

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