
**Phytochemical Analysis and Antibacterial Activity of *Croton macrostachyus* for
Management of Pneumonia**

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DECLARATION

I, Malembo Maria Gorret, declare that the research dissertation is my own original work otherwise cited, and where such has been the case, references have been stated and that the same work has not been submitted for any award of a degree in any other university or other tertiary institute of higher education.

Signature..........Date.....19...../September/2023

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APPROVAL

This research dissertation has been submitted for examination and has been approved by my supervisor.

Signature..........Date.....19/9/2023.....

DR. ORIKO RICHARD OWOR

DEDICATION

This report is dedicated to my parents and Ms. Mukoda Teopista and Mr. Nnaku Moses my siblings Kisakye Christine, Kaima Gilbert and Buseeta Francis not forgetting uncle Naika Vincent, sister Birungi Justine, and my grandmother Namukasa Theresa, all uncles and aunts who have supported me in all aspects of life in my entire stay in Busitema University. This research is also dedicated to the entire chemistry students for their special time, support and cooperation exhibited for the success of my research.

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ABSTRACT

Background. Pneumonia kills nearly one million children annually and accounts over 22 percent of all deaths in children aged 1 to 5 years. Unfortunately, there is increasing cases of pneumonia despite the presence of drugs implying that the bacteria, *Streptococcus pneumoniae* has become resistant to the approved drugs. There are medicinal plants used traditionally in Uganda for management of pneumonia and other bacterial infections which include *Croton macrostachyus*. Therefore, this study investigated the phytochemical composition, antioxidant properties antibacterial activity and herbal formulation of *C. macrostachyus* for management of pneumonia.

Objective. The main objective of this research was to determine the phytochemical composition antioxidant properties and antibacterial activities of *C. macrostachyus* and formulate herbal product for management of pneumonia.

Material and methods. *C. macrostachyus* stem bark crude was extracted using hot reflux extraction and concentration method and qualitatively analyzed for presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids and terpenoids. Phytochemical quantification was conducted using spectrophotometric method to determine the quantity of flavonoids, alkaloids, phenols and tannins in the extract. Formulation of an herbal remedy was carried out and organoleptic tests, density, pH were evaluated.

Results. The phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, phenols, saponins, steroids and terpenoids. Total flavonoid was found to be 23.28 mg/g, total alkaloid was 20.97mg/g, total tannin 4.67mg/ml and total phenol content was 6.77mg/ml. The formulated syrup had a brown colour, with moderate sweetness, pH of 6.76 and density of 0.2g/ml. The antioxidant screening with DPPH scavenging assay exhibited activity of 43.2% for the extract and 30.53% for the herbal remedy. For ferric power reducing assay yielded 22.51% of plant extract and 29.58% of the remedy. However, the antibacterial assay was not evaluated.

Conclusion. The findings of the study suggest that *C. macrostachyus* may be a potential source of antimicrobial agents for management of pneumonia due to the presence of alkaloids, tannins, phenols, saponins and flavonoids and its antioxidant activities

Recommendation. From this research, it is recommended that further research to be carried out to investigate the antibacterial activity of the plant to obtain its minimum inhibitory concentration and evaluate the efficacy in animal models of pneumonia.

LIST OF ABBREVIATION AND ACRONYMS

<i>C. macrostacyus:</i>	<i>Croton macrostachyus</i>
RSV:	Respiratory Syncytial Virus
WHO:	World Health Organization
S. pneumoniae:	Streptococcus pneumoniae
OE:	Organic Extract
µl:	microliters
ml:	milliliters
APE:	Apigenin Equivalent
PGE:	Propyl gallate Equivalent
CE:	Caffeine Equivalent
TAE:	Tannic acid Equivalent
HCl:	Hydrochloric acid
CMC:	Carboxymethyl cellulose
DPPH:	2,2-Diphenyl-1-picrylhydrazil
µg:	micrograms
UV/VIS:	Ultra Violet Visible
w/v:	weight by volume
M:	Molar

CHAPTER 1 : INTRODUCTION

1.1 Background

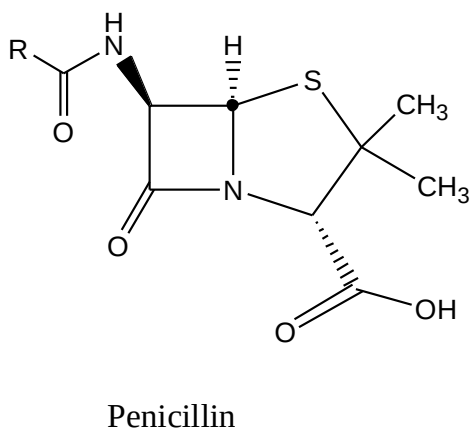
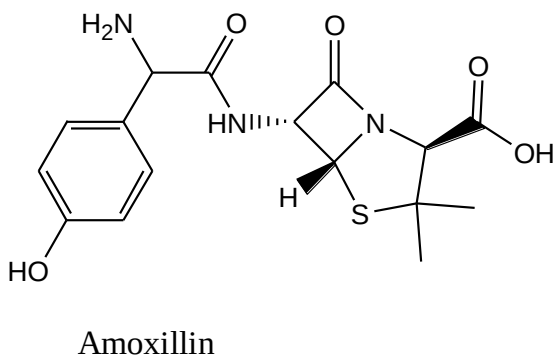
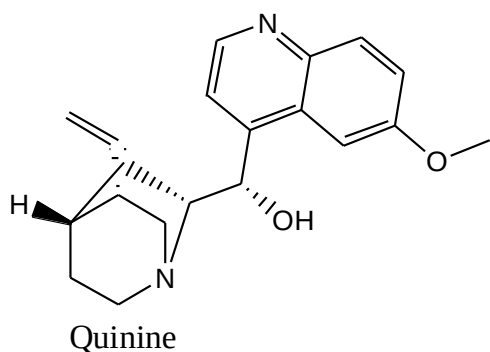
Pneumonia is a type of respiratory infection that affects the lungs (Quinton, Walkey, & Mizgerd, 2018). Symptoms of pneumonia include cough, fever, chills, shortness of breath, chest pain, and muscle aches. It can be caused by a variety of microorganisms, including bacteria, viruses, and fungi. Bacterial pneumonia is typically caused by *Streptococcus pneumoniae* (Keller, Robinson, & McDaniel, 2016), but other bacteria such as *Haemophilus influenza*, *Legionella pneumophila* and *Mycoplasma pneumoniae* also cause the disease. Viral pneumonia is usually caused by *Influenza viruses* but other viruses such as *Respiratory syncytial virus*(RSV), *Adenoviruses* and *Corona virus* can also cause pneumonia. Fungal pneumonia is often caused by the fungus *Pneumocystis jirovecii*, which is typically seen in people with weakened immune systems. Other fungi such as *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Aspergillus* species can also cause pneumonia in some cases. Other microorganisms such as *Mycobacteria*, *Chlamydia* and parasites can also cause pneumonia.

Pneumonia can be particularly severe in young children, elderly and people with weakened immune systems (Abdulmir & Hafidh, 2020). The infection causes inflammation in the air sacs(alveoli) of the lungs which can fill with fluid or pus making it hard to breathe (Divyashri, Ganapathy, & Keerthisasanka, 2022). It can be a serious and sometimes life-threatening illness, particularly people who are already sick or who have other medical conditions. In Uganda, pneumonia is a common respiratory infection with estimated 4 million cases and 38,000 deaths reported each year (Connolly et al., 2004).

According to World Health Organization(WHO), pneumonia is the leading cause of death in children under five years of age in Uganda (Lindstrand et al., 2016), accounting for an estimated 15% of all child deaths.

According to ministry of health in Uganda, there were 4.2 million cases reported in 2020 an increase from the 3.8 million cases reported in 2019. The case fatality rate for pneumonia in Uganda was estimated to be 3.7% in 2020, an increase from the 3.2% reported in 2019 (Elimian et al., 2020). These statistics highlight the ongoing need for improved access to health care services and effective prevention and treatment strategies for pneumonia in Uganda.

In Uganda several drugs have been used for the management of pneumonia, the antibiotics commonly prescribed include Amoxillin, erythromycin, penicillin and azithromycin. However due to the persistence of pneumonia implying that it has become resistant to the available drugs, Ugandans in both rural and urban centres have resorted to the use of medicinal plants such as *Allium sativum*(garlic), *Combretum molle*, *zingiber officinale*(ginger) and *Croton macrostachyus*(Odongo, 2016). Therefore, this study was intended to investigate the phytochemical composition and antibacterial activities of *Croton macrostachyus* in the management of pneumonia.



1.2 Problem statement

Pneumonia is the largest infectious cause of death in children worldwide. Pneumonia killed over seven hundred forty thousand children under the age of 5 in 2019, accounting for 22% of all deaths in children aged 1 to 5 years (Karim, Afridi, Yar, Zaman, & Afridi, 2023). Pneumonia in Uganda

is a significant public health concern particularly in children under the age of five and the elderly. Besides in Uganda pneumonia drugs are inaccessible to people in rural areas. The most disturbing issue is the increase in pneumonia cases despite the presence of drugs implying that *Streptococcus pneumoniae* has become resistant to the approved drugs (Wadood et al., 2018). Thus, there is need to develop other treatment options. Fortunately, there are medicinal plants used traditionally in Uganda for management of pneumonia and other bacterial infections which include *Croton macrostachyus*. However, the major focus in this research was to investigate the phytochemistry and anti-bacterial components of *Croton macrostachyus* for management of pneumonia.

1.3 OBJECTIVES

1.3.1 General objectives

Determine the phytochemical composition of *Croton macrostachyus* for antibacterial activities

1.3.2 Specific objectives

1. Analyze the phytochemical composition of *Croton macrostachyus*.
2. Assess the efficacy of *Croton macrostachyus* against antibacterial activities.
3. Formulate herbal remedy for management of pneumonia.

1.4 Justification

Management of pneumonia used to be straight forward and penicillin generally was the antibiotic of choice(Kaplan & Mason Jr, 1998). However, rapid development and widespread of penicillin resistance and multiple-antibiotic resistance have occurred implying that *Streptococcus pneumoniae* has become resistant to the available antibiotics. Therefore, there is need to develop new antibiotics to manage the widespread of bacterial infections which is claiming the lives of over 4 million people per year in the world(Bustreo, Okwo-Bele, & Kamara, 2015). Therefore, *Croton macrostachyus* has been identified as a source of antibiotics like the way quinine was derived from *Cinchona* tree(Greenwood, 1992). Thus, this study was aimed at providing an alternative source for management of pneumonia using *Croton macrostachyus* and to justify the information about the phytochemical composition of *Croton macrostachyus*.

CHAPTER 2 : LITERATURE REVIEW

2.1 Botany of the plant

C. macrostachyus (figure 1) is a species of flowering plant in the family of Euphorbiaceae in kingdom plantae (Contini et al., 2020). It is commonly known as bastard croton or forest croton. It is native to tropical Africa, particularly in Ethiopia and Kenya and it is also widely distributed throughout East Africa. The plant is a small to medium sized tree that can grow up to 15 meters tall (Gurmessa, Soromessa, & Kelbessa, 2012). It has large, glossy, dark green leaves that are alternate and simple in shape. The inflorescence is a terminal raceme or panicle with numerous small, yellow green flowers that are unisexual. The fruit is a three lobed capsule that turns from green to brown when mature containing three seeds.



Figure 1. *Croton macrostachyus*

2.2 Traditional uses of *C. macrostachyus*

Management of malaria. The leaves and bark of *C. macrostachyus* are used to manage malaria (Tole, Diriba, & Bahiru, 2021). The plant is boiled in water and the resulting decoction is taken orally. Management of gastrointestinal disorders. The roots of *C. macrostachyus* are used to manage diarrhoea, dysentery and other gastrointestinal disorders (Rawat, Singh, & Kumar, 2017).

The roots are pounded and boiled in water and the resulting decoction is taken orally. Management of wounds. The leaves of *C. macrostachyus* are used to treat wounds. The leaves are crushed and applied topically to the affected area. Management of respiratory disorders. The roots and bark of *C. macrostachyus* are used to treat coughs and other respiratory disorders (Haile, Tsegay, Seid, Adnew, & Moges, 2022). The roots and bark are boiled in water and the resulting decoction is taken orally. Management of skin infections. The leaves of *C. macrostachyus* are used to treat skin infections such as ring worm. The leaves are crushed and topically applied to the affected area (Wanga & Nyamboki, 2023). The plant is also used for timber, fuelwood and shade in agroforestry systems.

2.3 Phytochemistry of *C. macrostachyus*

C. macrostachyus has been subject to several phytochemical screenings, with some studies reporting the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids and saponins (Agidew, 2022). Alkaloids are nitrogen containing compounds that have diverse pharmacological properties. *C. macrostachyus* has been found to contain alkaloids such as *crotonoside*, *isocrotonoside* and *macrostachyine*. Flavonoids are a group of compounds that have antioxidant and anti-inflammatory properties. *C. macrostachyus* contains flavonoids such as *quercetin*, *kaempferol* and *rutin* (Al-Timimi, Shafeeq, & Hasan, 2022). It contains terpenoids such as *carvacrol*, *thymol* and *caryophyllene*. Phenolic compounds include *gallic acid*, *protocatechuic acid*, and *ellagic acid* (Rotich, 2022). *C. macrostachyus* also contains tannins such as catechins and epicatechins.

Several compounds have been isolated from *C. macrostachyus* including diterpenoids, flavonoids, phenolics and triterpenoids (Terefe, Okalebo, Derese, Muriuki, & Batiha, 2021). Diterpenoids such as microstachyusin A and B, crotonoside and croton microlactone have been isolated from *C. macrostachyus*. Flavonoids such as quercetin, kaempferol and apigenin have been isolated from this plant (Metsämuuronen & Sirén, 2019). Phenolic compounds such as caffeic acid, chlorogenic acid and gallic acid have been identified and triterpenoids such as lupeol and β -amyrin have been isolated from this plant.

2.4 Diseases that have been tested using *C. macrostachyus*

Several infections have been tested using *C. macrostachyus* including diabetes, malaria and inflammation (Meresa, 2019). Extracts from this plant have shown to possess anti-diabetic, anti-malarial and anti-inflammatory activities in various studies. However, *C. macrostachyus* is used to manage respiratory infections but no specific research has been performed on its anti-bacterial activities on pneumonia therefore this means there exists that gap and therefore my research focuses on the use of this plant to manage pneumonia.

2.5 Pharmacology of the plant

C. macrostachyus is a plant species that has been used for medicinal purposes in Africa. The plant contains several phytochemicals that have been identified as having potential pharmacological activities. One of the main active components of *C. macrostachyus* is 1,8-cineole also known as eucalyptol. This compound has been shown to have anti-microbial anti-inflammatory and analgesic properties. It has also been found to have broncho dilatory effects making it useful in the management of asthma and other respiratory conditions (Omara, 2021).

Another active compound in *C. macrostachyus* is carvacrol which has been shown to have antimicrobial, anti-inflammatory and antioxidant properties (Laksemi et al., 2022). Carvacrol has been found to have potential as an anti-cancer agent. *C. macrostachyus* also contains flavonoids such as quercetin and kaempferol which have antioxidant and anti-inflammatory properties. These compounds may have potential in the treatment of inflammatory conditions such as arthritis.

2.6 Traditional treatment of pneumonia

Traditional treatments of pneumonia vary depending on cultural and historical context. In many cultures, medicinal plants have been used to treat respiratory infections including pneumonia. For example, in traditional Chinese medicine, a combination of herbs known as ma xing shi gan tang has been used to treat pneumonia (Guo et al., 2021). This formula includes herbs such as ephedra, apricot seed licorice root and gypsum which are believed to have anti-inflammatory, antitussive and expectorant properties. In Ayurvedic medicine which originated from India, herbs such as Indian gooseberry, licorice root and turmeric have been used to treat pneumonia and these herbs are believed to have anti-bacterial, anti-inflammatory and antioxidant properties (Verma, Arya, & Aman, 2022). In many cultures, home remedies such as steam inhalation, hot compresses and herbal teas are also used to alleviate the symptoms of pneumonia (Mars & Fiedler, 2014). For

example, in some parts of Africa, steam inhalation with herbs such as eucalyptus or peppermint is used to relieve respiratory symptoms associated with pneumonia.

2.7 *Streptococcus pneumoniae*

Streptococcus pneumoniae also known as pneumococcus is a Gram-positive bacterium that commonly colonizes the human upper respiratory tract (Iovino, Nannapaneni, Henriques-Normark, & Normark, 2020). It is a major cause of bacterial pneumonia, meningitis and other invasive infections particularly in children, the elderly and immunocompromised individuals (Cillóniz, Garcia-Vidal, Ceccato, & Torres, 2018). *Streptococcus pneumoniae* is a spherical or ovoid shaped bacterium that is typically arranged in pairs or chains. It is a gram-positive bacterium meaning that it has a thick peptidoglycan layer in its cell wall that stains purple with gram staining (Perez et al., 2021). It produces a range of virulence factors that enable it to colonise and infect the host. These include capsule which is a polysaccharide layer that protects the bacterium from phagocytosis by the host immune system, pneumolysin which is a cytotoxic protein that can cause tissue damage and immune evasion. *Streptococcus pneumoniae* is a common cause of bacterial pneumonia particularly in young children and the elderly (Feldman & Anderson, 2014). It is also the leading cause of meningitis, sepsis and otitis media. The bacterium is highly transmissible and can spread through respiratory droplets or close contact with infected individuals (Marks, Reddinger, & Hakansson, 2014).

2.8 Treatment and failure of treatment

Pneumonia can be treated with antibiotics. The antibiotics of choice for first line treatment are amoxicillin dispersible tablets and penicillin (Graham et al., 2016). Most cases of pneumonia require oral antibiotics (Lodha, Randev, & Kabra, 2016), which are often prescribed at a health Centre. These cases can also be diagnosed and treated with low-cost oral antibiotics at the community level by trained community health workers. Hospitalization is recommended only for severe cases of pneumonia. However, emergence of antibiotic resistance among *S. pneumoniae* (Cillóniz et al., 2018) has called for change to this approach and also the rapid development and spread of resistance available antibiotics requires that further research is made to ensure that more effective remedies are developed to manage such infections.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Plant material

C. macrostachyus was collected from its natural habitat in Kapchorwa district. It was identified and verified at Makerere University Herbarium by Ms. Caro Kawuma, a lecturer in Biology department at Busitema University Nagongera Campus. The stem bark of *C. macrostachyus* was dried in the shade. The dried plant samples were grounded into powder using an electric mortar. After grinding, the sample was stored in an air tight container for further analysis

3.2 Extraction

The shade dried and ground powder of *C. macrostachyus* stem bark extract (300g) was extracted using hot reflux extraction and concentration method (NSG-50L, China) with ethanol/water (7:3, v/v) as solvent and temperature set at 50°C. The extract was concentrated under reduced pressure to obtain crude.

3.3 Preliminary phytochemical analysis

The crude extract of the stem bark of *C. macrostachyus* was dissolved in corresponding solvents to obtain their solutions unless stated in the procedure. Their solutions were subjected to different chemical tests separately for the identification of various active constituents which were as follows;

3.4 Test for flavonoids

3.4.1 Ferric chloride test

Few drops of neutral ferric chloride solution were added to 1 mL of an alcoholic solution of a crude extract using a test tube. Formation of blackish red colour indicated the presence of flavonoids.

3.4.2 Lead-acetate test

Few drops of aqueous basic lead acetate solution were added to 1 mL of alcoholic extract using a test tube. The appearance of a reddish-brown bulky precipitate indicated the presence of flavonoids.



Figure 2. Lead acetate test for flavonoids.

3.5 Detection of Steroids

3.5.1 Salkowski test

1 mL of concentrated Sulphuric acid was added to 5 mL of a chloroform solution of crude extract (a little part of crude extract was dissolved in chloroform and filtered) using a test tube. After shaking, the test tube was allowed to stand for 5 minutes. Lower layer turning into red in colour indicated the presence of steroids.



Figure 3. Salkowski test for steroids.

3.5.2 Liebermann-Burchard test

Add few drops of acetic anhydride to 5 mL of a chloroform solution of crude extract followed by adding 1 mL of concentrated Sulphuric acid through the wall of the test tube and allow to stand for 5 minutes. Formation of a reddish-brown ring at the junction of the two layers and a green colour in upper layer indicated the presence of steroids.



Figure 4. Liebermann Burchard test for steroids

3.6 Test for terpenoids

3.6.1 Salkowski test

2 ml of the extract were mixed with 1 ml of chloroform and 1 ml of concentrated Sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the interface was formed which indicated the presence of terpenoids.



Figure 5. Salkowski test for terpenoids

3.7 Test for tannins

3.7.1 Ferric chloride test

Few drops of ferric chloride solution were added to 1 mL of aqueous solution of crude extract (a little part of the crude extract was dissolved in 1 mL of distilled water and filtered) using a test tube. A blackish precipitate indicated the presence of tannins



Figure 6. Ferric chloride test for tannins

3.7.2 Lead acetate test

Few drops of aqueous basic lead acetate solution were added to 1 mL of aqueous solution of crude extract using a test tube. Reddish-brown bulky precipitate indicated the presence of tannins.



Figure 7. Lead acetate test for tannins

3.8 Test for saponins

A little part of the crude extract was mixed with 20 mL of distilled water and then agitated in a graduated cylinder (test tube also could be used) for 10 minutes. Foam formation indicated the presence of saponins.

3.9 Test for Alkaloids

3.9.1 Mayer's test

1 mL of Mayer's reagent (potassium mercuric iodide solution) was added to 1 mL of an acidic solution of crude extract. Cream colour precipitate indicated the presence of alkaloids.

3.9.2 Wagner's test

1 mL of Wagner's reagent (iodine in potassium iodide) was added to the 1 mL of an acidic solution of crude extract. The formation of reddish-brown precipitate indicated the presence of alkaloids.



Figure 8. Wagner's test for alkaloids

3.10 Detection of Phenols

3.10.1 Ferric chloride test

1 mL of ferric chloride solution was added to 5 mL of an aqueous/ alcoholic solution of crude extract (little part of crude extract was dissolved in 5 mL of water or ethyl alcohol and filtered) using a test tube. Formation of an intense colour indicated the presence of phenols.



Figure 9. Ferric chloride test for phenols

3.10.2 Ellagic acid test

Few drops of 5% glacial acetic acid and 5% sodium nitrite solution were added to 2 mL of an aqueous/ alcoholic solution of crude extract using a test tube. Formation of muddy or brown precipitate indicated the presence of phenols.



Figure 10. Ellagic acid test for phenols

3.11 Estimation of Phytochemical contents

3.11.1 Total flavonoid content

The total flavonoids content in the *C. macrostachyus* crude extract was determined by aluminium chloride spectrophotometric method (Ayele, Akele, & Melese, 2022). Apigenin was used as a standard and flavonoid content determined as Apigenin equivalent. From the standard Apigenin solution, the concentrations (450, 225, 112.5, and 56.25 $\mu\text{g/ml}$) were prepared in methanol.

100 μl of each of the apigenin dilution was mixed with 1500 μl of distilled water followed by 100 μl of 5% sodium nitrate solution and allowed to stand for 6 min. Then 150 μl of 10% aluminium chloride solution was added then allowed to stand for 5 minutes after which 200 μl solution of 1 M sodium hydroxide solution was added. The absorbance of this reaction mixture was measured at 416 nm using single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for methanolic extract solution of *C. macrostachyus*. All measurements were

performed in triplicate for each analysis. The total flavonoids content was determined from the linear equation of a standard curve prepared with Apigenin and expressed as mg/g Apigenin equivalent (APE) of dry extract.

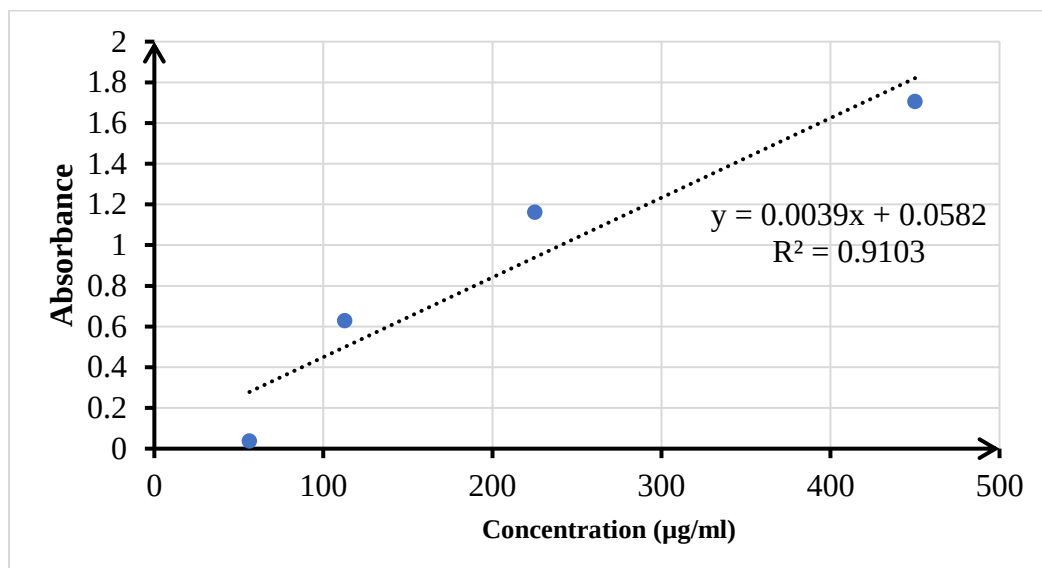


Figure 11. A calibration curve for the determination of total flavonoids using standard Apigenin solution

Regression equation $y = 0.0039x + 0.0582$

At $\lambda_{\max}$ 416 nm, the average absorbance of the sample prepared = 0.149

Thus
$$C = \frac{(0.149 - 0.0582)}{0.0039} = 23.28205128 \mu\text{g/ml}$$

$$\text{APE} = \text{Conc. of apigenin in the sample} \times \text{Volume of sample} \times \text{Dilution factor (D.F)}$$

$$\text{APE} = 23.28205128 \times 10 \times 1 = 232.8205128 \mu\text{g/ml}$$

$$\begin{aligned} \text{Total flavonoid content} &= \frac{\text{APE} \times \text{Vol. of sample (ml)} \times \text{D.F}}{\text{Weight of the sample (g)}} = \frac{232.8205128 \times 10 \times 1 \times 100}{10} \\ &= 23282.05128 \mu\text{gAPE/g} \end{aligned}$$

$$\text{Total flavonoid content} = 23.28205128 \text{mgAPE/g}$$

3.11.2 Total phenolic content

Folin-Ciocalteu method was used to estimate the total phenol content in a plant extract (Lamuela-Raventós, 2018). In this method, a methanolic solution of the extract (1 mg/ml) was added to 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml 7.5% Na_2CO_3 or NaHCO_3 . A Blank was similarly prepared containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 or NaHCO_3 . The samples were incubated in a thermostat at 45 °C for 45 min and the absorbance was determined using a spectrophotometer at λ_{max} 765 nm using single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for the standard solution of propyl gallate. The calibration curve was constructed using standard propylgallate solution prepared at concentrations of 0.5, 0.25, 0.125, 0.0625 mg/ml. The concentration of phenolics is read (mg/ml) from the calibration line. Thereafter, the total phenol content in a plant extract was expressed in terms of propyl gallate equivalent (mg of PGE/g of extract)

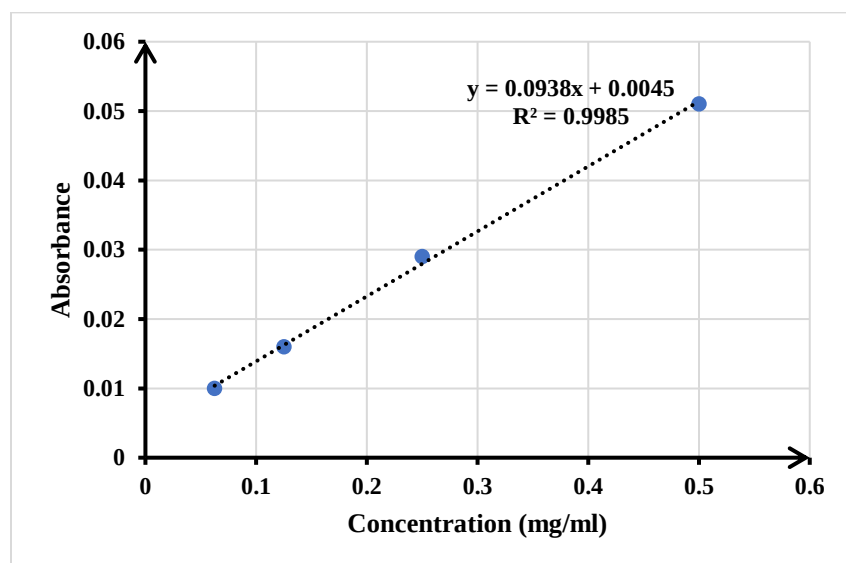


Figure 12. A calibration curve for the determination of total phenol content using standard propyl gallate solution

Regression equation $y = 0.0938x + 0.0045$

At λ_{max} 765 nm, the average absorbance of the sample prepared = 0.068

Thus
$$C = \frac{(0.068 - 0.0045)}{0.0938} = 0.67697228145 \text{ mg/ml}$$

PGE = Conc. of propyl gallate in the sample $\times$ Volume of sample $\times$ Dilution factor (D.F)

$$\text{PGE} = 0.67697228145 \times 10 \times 1 = 6.7697228145 \text{ mg/ml}$$

$$\begin{aligned} \text{Total phenol content} &= \frac{\text{PG} \times \text{Vol. of sample (ml)} \times \text{D.F}}{\text{Weight of the sample (g)}} = \frac{6.7697228145 \times 10 \times 1}{10} \\ &= 6.7697228145 \text{ mgPGE/g} \end{aligned}$$

$$\text{Total phenol content} = 6.7697228145 \text{ mgPGE/g}$$

3.11.3 Total Alkaloid content

A solution of 1 mg/mL of plant extract is prepared using Methanol. 1 mL of 2 M HCl is added to 1 mL of Methanol dissolved extracts and the resulting mixture is filtered using filter paper. The filtrate is transferred to a 250 mL separating funnel and to this solution, 5 mL of 0.1% Bromocresol green (dissolved in methanol) is added followed by 5 mL of phosphate buffer (pH 6.6). Chloroform (1 mL) is added into the separating funnel and the mixture was vigorously shaken, after which the funnel is allowed to stand to allow the mixture to separate into different layers. The lower layer is collected in a 10 mL volumetric flask. The process was repeated with 2, 3, and 4 mL of chloroform. Caffeine was used to construct a standard curve using a concentration range of 1.0–0.0625 mg/mL. The absorbance of the sample and standard solutions is recorded at a wavelength of 470 nm against a reagent blank using single beam UV-VIS spectrophotometer (6705 JENWAY). The total alkaloid content was expressed as milligram caffeine equivalent/ gram of extract (mg CE/g). The calibration curve was constructed using a standard tannic acid solution prepared at concentrations of 1.0, 0.8, 0.6, and 0.4 mg/ml.

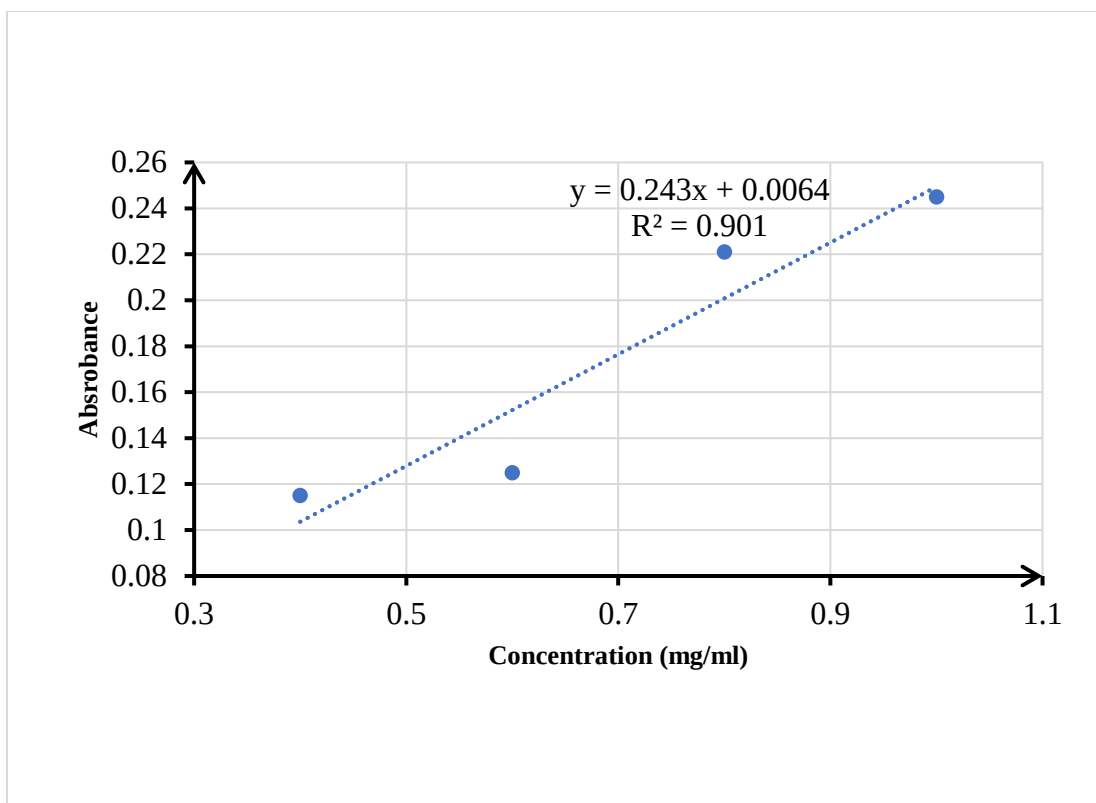


Figure 13. A calibration curve for the determination of total Alkaloid content using standard caffeine solution

Regression equation; $y = 0.243x + 0.0064$

At $\lambda_{\max}$ 470 nm, the average absorbance of the sample prepared = 0.516

Thus
$$C = \frac{(0.516 - 0.0064)}{0.243} = 2.09711934156 \text{ mg/ml}$$

CE = Conc. of caffeine in the sample $\times$ Volume of sample $\times$ Dilution factor (D.F)

$$CE = 2.09711934156 \times 10 \times 1 = 20.9711934156 \text{ mg/ml}$$

$$\begin{aligned} \text{Total Alkaloid content} &= \frac{CE \times \text{Vol. of sample (ml)} \times \text{D.F}}{\text{Weight of the sample (g)}} = \frac{20.9711934156 \times 10 \times 1}{10} \\ &= 20.9711934156 \text{ mg CE/g} \end{aligned}$$

$$\text{Total Alkaloid content} = 20.9711934156 \text{ mg CE/g}$$

3.11.4 Total Tannin content

100 μL of 10 mg/mL extracts were added to a clean test tube containing 7.5 mL of distilled water. The Folin-Ciocalteu reagent (0.5 mL) was added to the mixture and vortexed thoroughly. 10 mL of a 35% solution of sodium carbonate (Na_2CO_3) was added to the mixture. The mixture in the tube was transferred to a 10 mL volumetric flask and the volume of the mixture made up to 10 mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 minutes in the dark. Tannic acid was used as a standard and reference standard solutions (1.0–0.425 mg/mL) were prepared. The absorbance for the solutions was measured at 725 nm against a blank reagent blank using single beam UV-VIS spectrophotometer (6705 JENWAY). The calibration curve was constructed using a standard tannic acid solution prepared at concentrations of 1.0, 0.8, 0.6, and 0.4 mg/ml.

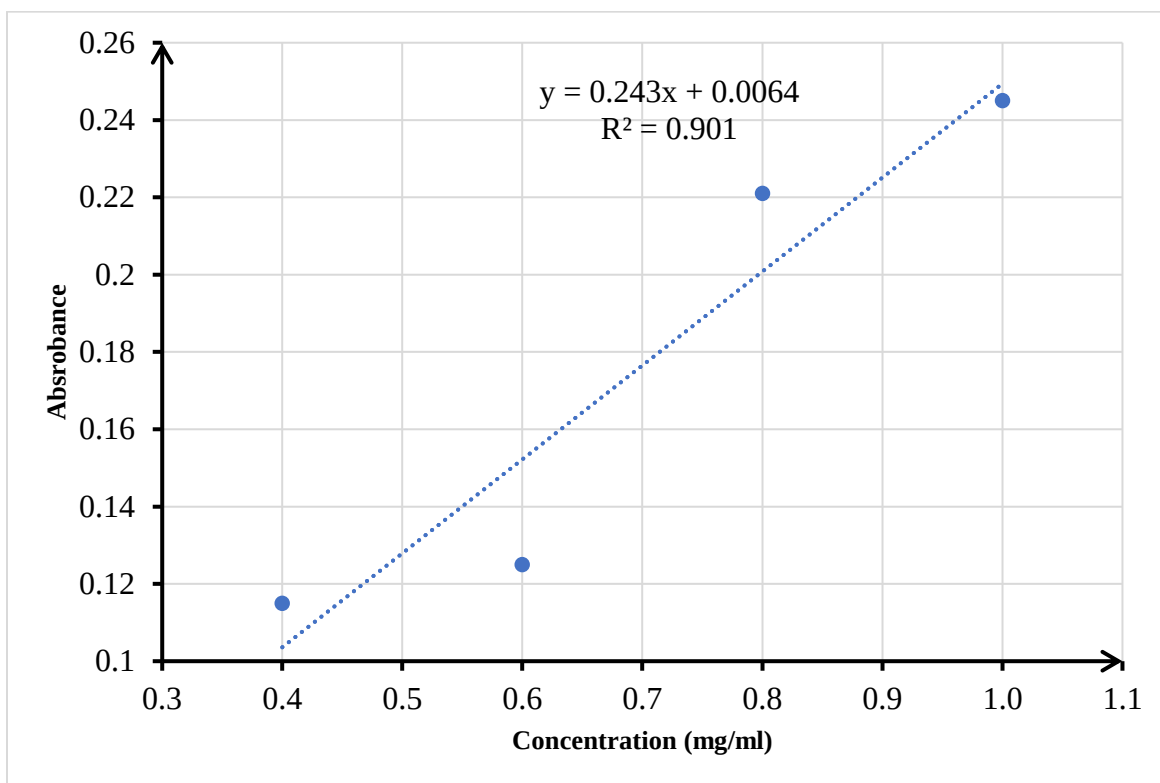


Figure 14. A calibration curve for the determination of total Tannin content using standard tannic acid solution

Regression equation; $y = 1.4885x + 0.1073$

At λ_{max} 725 nm, the average absorbance of the sample prepared = 0.803

Thus

$$C = \frac{0.803 - 0.1073}{1.4885} = 0.46738327175 \text{ mg/ml}$$

TAE = Conc. of tannic acid in the sample $\times$ Volume of sample $\times$ Dilution factor (D.F)

$$\text{TAE} = 0.46738327175 \times 10 \times 1 = 4.6738327175 \text{ mg/ml}$$

$$\begin{aligned} \text{Total tannin content} &= \frac{\text{TE} \times \text{Vol. of sample (ml)} \times \text{D.F}}{\text{Weight of the sample (g)}} = \frac{4.6738327175 \times 10 \times 1}{10} \\ &= 4.6738327175 \text{ mgTE/g} \end{aligned}$$

$$\text{*Total tannin content} = 4.6738327175 \text{ mgTE/g}$$

3.12 Formulation of the Herbal Remedy

Table 1. The ingredients that were mixed to formulate the syrup

Ingredient	Quantity	Use
Potassium sorbate	100mg	Preservative
Honey	7.5ml	Sweetener
Carboxymethyl cellulose (CMC)	120mg	To increase the viscosity
Water	42.5ml	Solvent
<i>Croton macrostachyus</i>	200mg	Active ingredient

3.13 Antioxidant screening

3.13.1 Free radical (DPPH) scavenging assay:

Free radical scavenging activity of the plant extracts was quantified using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method. Different concentrations of the extract (250-15.63 $\mu\text{g/mL}$) are prepared to a volume of 1 mL of the solution. L-ascorbic acid was used as a standard by preparing the same concentration range as the extracts. To this 1 mL solution, 2 mL of 0.2 mmol/L DPPH solution was added and vortexed thoroughly. All the prepared mixtures were left to stand in the dark for 30 min. The control solution was prepared by adding 2 mL of 0.2 mmol/L DPPH to 1 mL of distilled water. After the elapsed time, the solutions are analyzed with a UV/VIS spectrophotometer. The absorbance of the solutions was read at 575 nm.

$$\% \text{ scavenging} = \left(\frac{Ac - As}{Ac} \right) \times 100$$

Where *Ac* is the absorbance of the control solution, *As* is the absorbance of the extracts

$$\text{For plant extract, } \% \text{ scavenging} = \left(\frac{0.285 - 0.152}{0.285} \right) \times 100 = 43.16\%$$

$$\text{For the remedy, } \% \text{ scavenging} = \left(\frac{0.285 - 0.198}{0.285} \right) \times 100 = 30.53\%$$

3.13.2 Ferric reducing power assay

The ferric-reducing power of extracts was determined by measuring the formation of Pearl's Prussian blue at 700 nm and monitor the Fe^{2+} concentration. Extracts (2.5 mL) prepared to a concentration between 625-39 $\mu\text{g/ml}$ were mixed with 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% aqueous potassium ferricyanide in test tubes respectively. The mixtures were vortexed after the addition of solutions. The mixtures are incubated at 50°C for 20 minutes. 2 mL of 10% w/v aqueous trichloroacetic acid were added to the test tubes after incubation. The mixtures were centrifuged at 3000 rpm for 10 min and 5 mL of the resulting supernatant was transferred to a clean test tube. To this solution, 5 mL of distilled water and 1 mL of 0.1% aqueous ferric chloride was added consecutively with thorough mixing after each addition. A UV/VIS spectrophotometer was used to read the absorbance of solutions at 700 nm wavelength. The blank for this procedure was prepared in the same manner, however, the extract was replaced by an equal amount of distilled water. L-ascorbic acid is used as a positive control.

$$\text{For the plant extract} = \frac{0.382 - 0.96}{0.382} \times 100 = 22.51\%$$

$$\text{For the remedy} = \frac{0.382 - 0.269}{0.382} \times 100 = 29.58\%$$

CHAPTER 4 : RESULTS AND DISCUSSION

4.1 Phytochemical analysis

The phytochemical screening of *C. macrostachyus* stem bark extract indicated the presence of alkaloids, flavonoids, phenols, saponins, tannins, steroids and terpenoids as shown in the table 2 and 3.

Table 2. Phytochemical analysis of *C. macrostachyus* extracts

Phytochemical	Test	Present(+), Absent(-)
Flavonoids	Ferric chloride test	+
	Zinc-HCl reduction test	++
	Lead acetate test	+++
Tannins	Ferric chloride test	++
	Lead acetate test	+++
Saponins	Foam formation	++
Phenols	Ferric chloride test	++
	Ellagic acid test	++
Steroids	Salkowski's test	++
	Liebermann-Burchard test	++
Alkaloids	Wagner's test	+++
	Dragendorff's test	++
Terpenoids	Salkowski's test	+++

Key +: Weakly present, ++: Moderately present, +++: Strongly present, -: Absent

Table 3. Showing the total quantity of each phytochemical expressed as the corresponding standard solution equivalence

Phytochemical	Quantity (mg/g)
Phenolics	6.77
Flavonoids	23.28
Tannins	4.67
Alkaloid	20.97

The antibacterial activity of *C. macrostachyus* is attributed to the presence of alkaloids, flavonoids, phenols, saponins, tannins, steroids and terpenoids and which may act synergistically to hinder the

growth of the bacteria. Alkaloids contain nitrogen and have pharmacological properties (Bribi, 2018). Flavonoids have polyphenolic compounds are known to contain antioxidant, anticancer and anti-inflammatory properties (Tungmunthum, Thongboonyou, Pholboon, & Yangsabai, 2018). Tannins are polyphenolics compounds that have caustic properties. Phenols are aromatic compounds that have antioxidant properties (Skotti, Anastasaki, Kanellou, Polissiou, & Tarantilis, 2014). However, the antibacterial evaluation was not done but the present literature reveals that *C. macrostachyus* is a good remedy for antibacterial activity.

4.2 The physiochemical evaluation of the formulated herbal remedy

Table 4. Showing the properties of the formulated syrup

Features	Evaluation
Colour	Brown
Odor	Pleasant
Taste	Sweet
pH	6.76
Density	0.2g/ml

C. macrostachyus extract was found to possess phytochemical constituents with therapeutic values and therefore it was used to formulate herbal remedy. The product is an herbal syrup containing *C. macrostachyus* extract as the active ingredient which has reported to contain unique phytochemicals and shown to possess antibacterial activities

4.3 Antioxidant screening

Table 5. Showing results for antioxidant screening

Free radical (DPPH) scavenging assay	Plant extract	43.16%
	Remedy	30.53%
Ferric reducing power assay	Plant extract	22.51%
	Remedy	29. 58%

The extract and the syrup have the ability to neutralize 43.16% and 30.53% of DPPH free radicals respectively indicating their potential as antioxidant agents. The effectiveness of the plant extract may vary depending on factors such as extraction method, plant species and sample preparation (Muyumba, Mutombo, Sheridan, Nachtergaeel, & Duez, 2021).

The values of the ferric reducing power assay indicate that the sample exhibits ferric reducing ability. This means both the extract and the syrup contain a significant amount of reducing agents that are capable of reducing ferric ions to ferrous ions.

The antioxidant activity of a sample enables the inhibition of bacterial enzyme activity (Yao, Xiao, & Zhou, 2021). Plant antioxidants enhance the immune response by promoting production of immune cells that help eliminate bacterial pathogens.

CHAPTER 5 : CONCLUSION AND RECOMMENDATION

5.1 Conclusion.

The results from this study showed that the extract from the stem bark of *C. macrostachyus* contains alkaloids, flavonoids, phenols, saponins, tannins, steroids and terpenoids. This study therefore, contributes to justifying the traditional use of the *C. macrostachyus* for the management of respiratory tract infections with formulation of an herbal syrup. Therefore, new research outcomes could top to greater protection and assistance to people who use *C. macrostachyus* to manage pneumonia that is contributing to an improved access to health care and thus an excellent lifespan. The findings of the study suggest that *C. macrostachyus* may be a potential source of antimicrobial agents for management of pneumonia due to the presence of alkaloids, tannins, phenols, saponins and flavonoids and its antioxidant activities

5.2 Recommendation.

From this research, it is recommended that further research to be carried out to investigate the antibacterial activity of the plant to obtain its minimum inhibitory concentration and evaluate the efficacy in animal models of pneumonia.

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