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The Evaluation of Phytochemical and Antimicrobial Properties of *Cnidoscolus aconitifolius* (Hospital Too Far) Leaf Extracts on Selected Organisms

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ABSTRACT

Objectives: The aim of this investigation was to determine the phytochemicals and antibacterial properties of *Cnidoscolus aconitifolius* leaves. **Methods**: The herbs were obtained from a farmer in the village of Eziobodo. Distilled water and methanol were utilised to extract the beneficial constituents of the leaves. The qualitative and quantitative phytochemical components were identified, and their activity were evaluated against several pathogenic bacteria. In the qualitative phytochemical screening results, saponins, flavonoids, tannins, alkaloids, anthraquinones, terpenoids, glycosides, and steroids were identified, along with their respective potencies. The characterization and identification of the isolates were conducted using gramme staining, motility testing, catalase testing, coagulase testing, oxidase testing, among other methods. **Results**: The evaluation of *Cnidoscolus aconitifolius* leaf extracts shown substantial antibacterial efficacy against the chosen pathogenic microorganisms. The antibacterial efficacy against the pathogenic test organisms exhibited inhibition zones ranging from 12.0 mm to 17.0 mm at a concentration of 5 g/ml of the aqueous extract for *Streptococcus aureus* and *Escherichia coli*. The ethanol extract at 5g/ml exhibited zones of inhibition of 17.0mm for *Streptococcus aureus* and 22.0mm for *Escherichia coli*. All the extracts shown more efficacy than Septrin and can compete effectively with Ciprofloxacin. **Conclusion**: Consequently, the results indicate that *Cnidoscolus aconitifolius* (commonly known as chaya leaf) possesses nutritional potential to treat various diseases induced by the examined pathogens.

***Keywords**: Phytochemical, Antimicrobial, Cnidoscolus aconitifolius, Chaya leaf, Microorganisms, Hospital too far*.

INTRODUCTION

Since ancient times, plant components have been utilised as herbal medicine due to their therapeutic characteristics[1]. This plant contains bioactive chemicals that confer its therapeutic significance. According to Shihabudeen et al.[2], the most significant bioactive chemicals found in plants include phenolic compounds, alkaloids, tannins, and flavonoids. Different plants may have different therapeutic properties due to differences in concentration [3, 4]. Antibacterial, antioxidant, and potentially less toxic than produced medications, several medicinal plants have attracted a great deal of attention from researchers around the world in recent decades [5]. In light of the growing worldwide concern over the increasing resistance of germs to current antimicrobials, it is of utmost importance to evaluate the antibacterial activities of different medicinal plants [6-9]. The widespread usage of commercial antimicrobials has led many to believe that harmful germs are becoming more resistant to these drugs [10]. The prevention and treatment of an increasing number of illnesses caused by bacteria, parasites, viruses, and fungi are being threatened by the rise of antimicrobial resistance. Therefore, it is crucial to find compounds that can be used to make new drugs that are more effective against bacteria [11, 12]. A substantial portion, if not all, of the world's population uses medicinal plants [13, 14]. Because of their widespread availability and low cost, traditional medicine systems based on plants play an essential role in healthcare for around 80% of the world's population [15, 16].

Nutritionally and medicinally, *Cnisdoscolus aconitifolius* (Hospital too far) has been recommended for a number of ailments, such as obesity, cholelithiasis, hypercholesterolaemia, ophthalmic disorders, nephrolithiasis, haemorrhoids, and digestive problems. Tree spinach, or *Cnidoscolus aconitifolius*, goes by a few different names. It is a tropical perennial plant that belongs to the family Euphorbiaceae. Oyagbemi et al.[15] listed it as one of the most prolific green vegetables eaten in southwest Nigeria. Inhabitants of southeastern Nigeria also consume it, referring to it as “Hospital too far” [17]. It is utilised locally for the treatment of malaria, liver and renal disorders, infertility, and enhancement of haematologic parameters. It is purported to possess antidiabetic, hepatoprotective, hypoglycaemic, and antioxidant effects, and to promote bone marrow function [18-20]. The significance of this vegetable in the context of human nutrition and wellness has been neglected and unappreciated. It is still regarded as one of the most neglected herbs in nature in this region. The term "hospital too far" is flattering and indicative of its efficacy, despite the fact that only a small percentage of community members have recognised its healing potential.

Many different claims have been made about the medicinal benefits of *Cnidoscolus aconitifolius* (Chaya; Hospital too far). Some of these claims include that it can strengthen fingernails, darken grey hair, treat alcoholism, insomnia, venereal diseases, gout, scorpion stings, and improve memory and cognitive function. Anticontraceptive effects have been attributed to a wild relative of Chaya as well [21]. The young shoots and leaves are often washed and eaten in southwestern Nigeria, either on their own or mixed with tomato paste and milk. Pregnant women and children with anaemia often consume it because, according to the local population, it increases blood production [22]. Anaemia and osmotic fragility brought on by protein energy deficit in male Wistar rats can be alleviated by *Cnidoscolus aconitifolius*, according to research by Oyagbemi et al. [15]. According to Senjobi et al. [23], brine shrimp larvae were shown to be cytotoxic by the ethanol leaf extract of *C. aconitifolius*, with an LC50 value of 10 µg/ml. The challenge of antibiotic resistance and the continuous search for new antimicrobials necessitate this inquiry. Examining the phytochemical and antimicrobial properties of hospital too far has been the primary goal of this research.

MATERIAL AND METHODS

A. *Sample Collection and Preparation*

The farm in Eziobodo hamlet was surveyed for the collection of *Cnidoscolus aconitifolius* (Hospital too far) leaves. To protect my skin from any potential irritation caused by the leaf juice, I made sure to wear gloves. To prevent the sap from being spread, each stem that was removed was carefully wrapped in a clean polyethylene bag. After being exposed to sunshine for three days, the leaves began to dry out. After being finely ground into a powder using an electric blender, the delicate leaves were placed in a tightly sealed container for storage.

B. *Extraction of Plant Materials*

Using ethanol and water as solvents, the plant materials' active components were isolated. A digital balance was used to measure twenty (20) grammes of pulverised plant materials. Then, 100 ml of sterile distilled water was added to each of the 250 ml conical flasks in a 2:5 (w/v) ratio. For ethanol extraction, the procedure was restated. Before letting it rest for 18–24 hours, the conical flask was mechanically shaken vigorously for 30–60 minutes. A portion of the filtrate was used for qualitative phytochemical analysis after the extract was filtered using Ashless No. 42 filter paper. The rest was dried in the air, and the resulting powder was used for testing bacteria.

C. Phytochemical Screening

Enenebeaku et al. [24] outlined the procedures that were followed for the phytochemical screening. The phytochemical components analyzed includes, alkaloids, flavonoids, glycosides, tanins, terpenoids, anthraquinone, steroids and saponin.

D. Microscopic Identification of Bacteria Isolates

Isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from food samples. Analysis was carried out in Anthony van-Leeuwenhoek Research center, Imo State, Nigeria. The characterization and identification of the isolates was done following the procedures below.

i. Gram Staining

This was executed as outlined by Cheesbrough [25]. A smear of the isolates was prepared on clean slides and fixed with crystal violet stain for 30-60 seconds, then rinsed with clean water and treated with 1% iodine for 50 seconds. After rinsing with water, the iodine was decolorised with acetone alcohol for a short period of time. After that, the smear was let to sit in neutral red stain for two minutes before being rinsed with water. After a thorough cleaning, the back of the slide was set on a draining rack to let the smear dry naturally. To begin the microscopical analysis of the smear, the staining and material distribution were examined through the 40X objective. Subsequently, the bacteria and cells were identified using the oil immersion objective.

E. Biochemical Tests

i. Motility Test

A solitary colony of each organism was injected into designated test tubes containing 5 ml of peptone water, and the tubes were cultured at 37°C overnight. A droplet of the incubated organism culture was positioned on a coverslip, with the periphery encircled by immersion oil. A microscope slide was positioned over the coverslip, ensuring that the slide did not contact the drop suspended by the oil immersion on the coverslip. The slide was subsequently inverted swiftly yet delicately [25]. The preparation was subsequently examined under a microscope (100 X objective) to assess bacterial motility.

ii. Catalase Test

A drop of 3% hydrogen peroxide was applied to a glass slide. A specimen of each isolate was obtained from the media with a wire loop and emulsified in the droplet. Bubbling signified a positive test result.

iii. Coagulase Test

This study employed the slide method test. Two distinct locations on a grease-free slide received a drop of saline, followed by the addition of a speck of the test organism culture, which was emulsified at both sites. A drop of plasma was added to one location, while a drop of saline was added to the other. A positive test demonstrated coagulation in the emulsion at the location where plasma was introduced.

iv. Indole Test

The test organism was cultivated in peptone water and incubated at 37°C for 24 hours to achieve optimal indole accumulation. A positive test result was indicated by the observation of red colouration in the uppermost layer of the tube following the addition of 0.5 ml of Korac's reagent to 5 ml of peptone water culture.

v. Citrate Utilization Test

For each isolate, 10 ml of citrate medium was allocated into five test tubes and sterilised using autoclaving at 121°C for 15 minutes. The test organism was subsequently inoculated into citrate medium and cultured at 37°C for 48 hours. A royal blue hue signified a favourable outcome. A test tube containing solely the citrate medium functioned as a control.

vi. Oxidase Test

A section of filter paper was saturated with several drops of oxidase reagent. A colony of the test organism was subsequently applied to the filter paper. An organism that produces oxidase oxidises phenylenediamine in the reagent, resulting in a deep purple hue.

vii. Urease Test

Inoculate the test organism abundantly in a bigou bottle containing 3 ml of sterile Christensen's modified urea broth. Incubate at 35-37°C for 3-12 hours in a water bath. The presence of pink colouration in the medium signified a positive urease test.

F. Preparation of Test Organism

Test organisms for standardisation were subcultured onto nutrient agar at 37°C for 24 hours. Organisms were then cultivated on a slant for preservation [26]. Test isolates were standardised using the McFarland method. The McFarland solution comprises Barium Chloride and Sulphuric Acid.

i. Susceptibility Test

In order to determine how sensitive the test isolates were to the extract, a well diffusion technique was employed. On Mueller Hinton Agar that had been injected with 24-hour-old standardised cultures of Staphylococcus aureus and Escherichia coli, four 6.25 mm deep wells were bored using a sterile cork borer. Different concentrations of ethanol and water extract were added to the wells, with concentrations ranging from 500 mg/ml to 62.5 mg/ml. The plates were kept at 37°C for 24 hours for incubation. The well inhibition zones were measured and recorded in millimetres (mm) following a 24-hour interval.

G. Minimum Inhibitory Concentration (MIC) Assay

The extract's sensitivity to the test isolates was established using a well diffusion approach. After

injecting Mueller Hinton Agar with standardised cultures of *Staphylococcus aureus* and *Escherichia coli* that were 24-hours old, a sterile cork borer was used to bore four wells that were 6.25 mm deep. The wells were replenished with ethanol and water extract at varying concentrations, from 500 mg/ml to 62.5 mg/ml. The incubation process required 24 hours at 37°C for the plates. Measurements and records of the well inhibition zones were made in millimetres (mm) at 24-hour intervals.

H. *Minimum Bactericidal Concentration (MBC) Assay*

After 24 hours of incubation on nutritional agar, the Minimum Bactericidal Concentration is the lowest bacterial count that was recorded on the plate. A surface of freshly prepared, dried nutrient agar was streaked with a loop containing different proportions (post-spectrophotometric analysis), and the mixture was left to incubate overnight. To determine the MBC, the concentrations of growth after incubation were used.

I. Preliminary Phytochemical Screening

Following standard protocols, the medicinal plant extract will be tested for the presence of alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, coumarins, carbohydrates, and flavonoids [27].

J. Preparation of Plant Extracts

Petroleum ether, chloroform and methanolic extract. Alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, coumarins, carbohydrates, and flavonoids will be quantified in the medicinal plant extract according to established techniques [27].

i. Aqueous Extract

Similarly, 400 millilitres of distilled water will be dissolved in 50 grammes of powdered leaves. On a hot plate, this will be heated until it boils. After one hour of stirring the combinations every three to five minutes, they will be filtered using No. 1 Whatman filter paper. The filtrate will undergo a five-hour concentration process in a water bath heated to 80°C. Chilling the filtered extract is the next step [28].

ii. Screening for Alkaloids

The alkaloids in the plant extract will be identified using Wagner's assay. After adding a few drops of 1% HCl to three millilitres of extracts in a test tube, the mixture will be heated for fifteen minutes. The combination will be tested for the presence of alkaloids by looking for a reddish-brown precipitate after cooling, which can be achieved by adding two drops of Wagner's reagent [27].

iii. Screening for Saponins

To determine whether saponins are present, the foaming test will be performed. Before letting the mixture sit for 10 minutes, add 9 millilitres of distilled water to 1 millilitre of extract in a measuring jar, shake vigorously for 15 seconds, and then drain. According to Thilagavathi et al. [29], saponins are present when stable foam (1 cm) forms.

iv. Screening for tannins

According to Edori et al. [27], the presence of tannins can be determined by adding 10% freshly prepared potassium hydroxide (KOH) to 3 ml of the extract in $Mn(OH)_2$. A dirty white precipitate will then be observed, confirming the presence of tannins.

v. Screening for steroids

The presence of steroids can be determined by adding three drops of concentrated H_2SO_4 to three millilitres of the extract; the resultant red colouration indicates the presence of steroids [27].

vi. Screening for flavonoids

The powdered leaves, weighed at 2 g, were completely deproteinised with acetone. Warm water will be used to remove the residue once the acetone has evaporated in a water bath. While the mixture is still hot, it will be filtered. Allow the filtrate to cool before using it. Five millilitres of 20% sodium hydroxide will be mixed with the same volume of the detanned water extract for the sodium hydroxide test. The presence of flavonoids is indicated by a yellow solution [30].

vii. Screening for Anthraquinones

In order to execute this, we will implement Bontrager's testing methodology. The mixture will be vortexed for 5 minutes prior to the addition of 5 ml of chloroform to a dried test tube containing approximately 0.5 g of the extract. The extracts will be combined with a 100% ammonia solution of the same volume after they have been filtered. Maria et al.[31] discovered that free anthraquinones are present when the ammoniacal layer (the lower layer) exhibits a pink, violet, or scarlet hue.

viii. Screening for cardiac glycoside

A ten millilitre mixture of concentrated H_2SO_4 and three millilitres of the extract will be cooked for fifteen minutes in a water bath set to a high temperature. The next step is to add 10 cm^3 of Fehling's solution while the mixture is still boiling. Look for a brick-red colour, which means glycosides are present [27].

RESULTS AND DISCUSSION

Plants possess diverse bioactive chemicals that influence numerous properties of the plant. The phytochemical investigation of *Cnidoscolus aconitifolius* reveals eight distinct phytochemical components, as presented in Table 3. Comprising saponins, flavonoids, tannins, alkaloids, anthraquinones, terpenoids, glycosides, and steroids. These results are in agreement with those of Awoyinka et al. [32] and Igbinaduwa et al. [33], which concerned the phytochemical screening and in vitro bioactivity of *Cnisdoscolus aconitifolius* and the methanolic extract of *Jatropha tanjorensis* leaf, respectively. Table 3 displays the results

Table 1. Colonial and Microscopic Characteristics of Test Cultures

Colonial Characteristics	Spore Formation	Motility	Gram Reaction	Identity of Isolates
Small circular moist and shiny golden yellow colonies on nutrient agar (NA) and light yellow colonies on mannitol salt agar (MSA)	-	-	+S	*Staphylococcus aureus*
Small circular moist and shiny purple metallic sheen on eosin methylene blue agar (EMBA) and pink colonies on MacConkey agar (MCA)	-	+	-R	*Escherichia coli*

Key: R, rod shaped; S, spherical/cocci; E, ellipsoidal

Table 2. Microscopic and Biochemical Characteristics of Test Cultures

NO_3 reductio n	H_2S production	Ure	Oxi	Cat	Coag	In	MR	VP	S	L	G	M	Identity of isolates
+	-	-	-	+	+	-	+	-	+	+	+	+	*Staphylococcus aureus*
+	-	-	-	+	-	-	-	+	-	-	-	-	*Escherichia coli*

Key: NO_3, nitrate reduction test; H_2S, hydrogen sulphide production test; Ure, Urease; Oxi, oxidase test; Cat, Catalase test; Coag, coagulase test; In, indole test; MR, Methyl Red test; VP, Voges Proskaeur test; S, sucrose; L, lactose; G, glucose; M, maltose; nd, not done.

Table 3. Phytochemical Analysis of plant Extracts

Phytochemical parameters	Leaf extract
Saponin	++
Flavonoid	++
Tannin	+
Alkaloid	+++
Anthraquinone	++
Terpenoid	+++
Glycosides	+
Steroids	+++

Key: The +++ indicates that Steroids, Terpenoids and Alkaloids are highly present, ++ indicates that Saponin, Flavonoid and Anthraquinone are moderately present while + indicates that Tannin and Glycosides are lowly present.

Table 4. Sensitivity of Plant Extract to Test Organisms in Diameter (mm)

Test isolates	CEF	500 mg/ml	250 mg/l	125 mg/l	62.5 mg/l	Ethanol Extract
Staphylococcus aureus	40	15.0	10.0	20.0	0	
Escherichia coli	30	20.0	10.0	15.0	0	
						Aqueous Extract
Staphylococcus aureus	40	0	0	10.0	10.0	
Escherichia coli	30	0	0	0	0	

Table 5. Minimum Inhibitory Concentration (MIC) of Plant Extracts on Test isolates (ƛ=340 nm)

Ethanolic Plant Extract	Test Organisms	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
	Escherichia coli	0.136	0.172	0.136	0.215
	Staphylococcus aureus	0.174	0.261	0.199	0.187
Aqueous Plant Extract					
	Escherichia coli	0.175	0.270	0.279	0.297
	Staphylococcus aureus	0.291	0.175	0.265	0.235

Table 6. Minimum Bactericidal Concentration (MBC) of Plants Extract on Test Organisms

Test isolates	MBC of Aqueous Extract	MBC of Ethanol Extract
Staphylococcus aureus	500 mg/l	500 mg/ml
Escherichia coli	500 mg/ml	500 mg/ml

of the quantitative analysis of the *Cnidoscolus aconitifolius* extract, which shows the main quantities of each phytochemical constituent extracted from the leaves. Alkaloids, terpenoids, and steroids exhibit the highest level of positive activity due to their significant prevalence. Flavonoid chemicals exhibit antioxidant, anti-inflammatory, and antiseptic properties. Saponins in Chaya can markedly reduce blood cholesterol levels and act as an inhibitor of tumour cell proliferation. Tannins facilitate the precipitation of proteins and function as metal chelators. Alkaloids are potent analgesics with antibacterial, antidiabetic, and antimalarial properties. Anthraquinones, glycosides, and terpenoids exhibit antifungal and antiviral properties that help mitigate illnesses [34].

This study tested the *Cnidoscolus aconitifolius* extracts against *Staphylococcus aureus* and *Escherichia coli*. The extracts showed antimicrobial action against the organism that was tested, with the most potent showing very high activity. Table 5 displays the MIC values of plant extracts in relation to test isolates using both water and ethanolic extracts. The extracts have all demonstrated superior efficacy compared to certain commercial antibiotics, such as streptomycin and septrin, and they may hold their own against other, more esteemed antibacterial medications, such ciprofloxacin and ofloxacin. An innovative, broad-spectrum antibiotic could be discovered by conducting in-depth research into the antibacterial characteristics of this plant. Alkaloids, terpenoids, steroids, and tannins are some of the antimicrobial substances found in this plant's leaves, which are responsible for its antibacterial activity [11, 35-37].

CONCLUSION

This study came to the conclusion that *Cnidoscolus aconitifolius* is sensitive to two different bacterial strains, namely gram-positive and gram-negative strains. This finding suggests that the plant has the potential to act as a broad-spectrum antimicrobial agent. However, additional research is required to determine the active compounds that are responsible for the plant's biological activity, as well as the minimum inhibitory concentration (MIC) that is required for drug development in the healthcare industry. It is possible that additional research on this plant extract could be employed as potential therapeutic solutions for the management of food contamination and diseases that are associated with prevalent dangerous microorganisms. Consequently, with the identification of the proximate components and antioxidant activity of the plant species, it may be possible to accelerate the development of superior alternative natural antioxidants.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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