# Phytochemical compounds and antimicrobial properties of Hospital too far (*Cnidoscolus Aconitifolius*).

Original Research Article

**ISSN :** 2456-1045 (Online)
**(ICV-BGS/Impact Value):** 63.78
**(GIF) Impact Factor:** 4.126
**Publishing Copyright @** International Journal Foundation
**Journal Code:** ARJMD/MDS/V-29.0/I-1/C-11/SEP-2018
**Category :** BOILOGICAL SCIENCE
**Volume :** 29.0 /**Chapter**- XI/ **Issue** -1(SEPTEMBER-2018)
**Journal Website:** www.journalresearchijf.com
**Paper Received:** 08.07.2018
**Paper Accepted:** 16.08.2018
**Date of Publication:** 10-10-2018
**Page:** 61-65

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**Citation of the Article**

**Lennox, J. A; John, G. E; Asitok, A. (2018)** Phytochemical compounds and antimicrobial properties of Hospital too far (*Cnidoscolus Aconitifolius*); *Advance Research Journal of Multidisciplinary Discoveries.29(11)pp. 61-65*

**ABSTRACT**

This study was carried out to determine the phytochemical and antimicrobial properties of *Cnidoscolus aconitifolius* (hospital too far) leaves. The plant leaves were obtained from area gardens. The useful components of the leaves were extracted with distilled water (aqueous) and methanol. The phytochemical components both qualitative and quantitative were determined and their activities tested against some pathogenic bacteria. The results of the qualitative phytochemical screening revealed the presence of saponin, alkaloids, glycosides, polyphenols flavonoids, reducing compounds and trace tannins. Phlobatanins, anthraquinones and hydroxymethyl anthraquinones were absent. Further, quantitative phytochemical analysis in (mg/100g) revealed that tannins, saponin, alkaloids, glycosides, polyphenols flavonoids and reducing compounds contain 0.21±0.02, 1.20±0.1, 2.50±0.1, 2.44±0.01, 9.18±0.1. 3.50±0.1, and 4.60±0.02 respectively. It was observed that polyphenols had the highest quantity of 9.18 and tannin the lowest quantity 0.21mg/100g. The screening of *Cnidoscolus aconitifolius* leaf extracts against some pathogenic bacteria showed some positive antibacterial activity against the selected pathogens. The antimicrobial activities against the pathogenic test organisms ranged from 12.0mm to 17.0mm zones of inhibition at 5g/ml of the aqueous extract for *S. aureus* and *E. coli*. At 10g/ml of the aqueous extract, the inhibition zones ranged from20.5mm to 26.0 for *S. typhi* and *P. aeruginosa*. For the ethanol extract at 5g/ml, the zones of inhibition were 17.0mm, 18.0mm, 20.0mm, 21.0mm, 22.0mm, and 23.0mm for *S. aureus, P. aeruginosa, K. pneumonia, S. typhi, E. coli* and *S. pyogenes* respectively. At 10g/ml ethanol extract, the zones of inhibiyion were 27.5mm, 28.0mm, 28.5mm, 29.0mm, 30.0mm and 31.0mm for *E. coli, S. aureus, K. pneumonia, P. aeruginosa, S. typhi* and *S pyogenes* respectively. All the extracts showed more effectiveness as compared to Streptomycin and Septrin and compete favorably with Ciprofloxacin and Ofloxacin. Hospital too far has the potential therefore based on the results to be used to combat most of the diseases caused by the tested pathogens.

**KEYWORDS:**

*Cnidoscolus aconitifolius*, phytochemical, antibacterial, antibiotics, Hospital too far.

## I. INTRODUCTION

Plant parts have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannins, flavonoids and phenolic compounds (Shihabudeen *et al.,* 2010). Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant (Richard *et al.,* 2000; Amish *et al*., 2016). During the last few decades, the global interest in the study of various medicinal plants has increased rapidly due to their antibacterial and antioxidant activities, low toxicity and the potential to be cheaper alternatives to costly synthetic drugs (Chew *et al.,* 2012). The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms to the currently used antimicrobials. It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs. Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties (Atikya *et al.,* 2014; Amish *et al*., 2017). It has been reported that about 70% of the human population is dependent wholly or partially on plant‐based medicine (Raven *et al*.; 2006; Sakpa and Uche‐Nwachi, 2014). These plant‐based traditional medicine systems play essential roles in health care with about 80% of the world's population relying on them due to their availability and cheap sources (Owolabi *et al*., 2007; Salahdeen and Yemitan, 2006; Anwana *et al*., 2012).

*Cnidoscolus aconitifolius* (Hospital too far) has been used nutritionally and medicinally (Diaz – Bolio, 1975). Its shoots and leaves have been said to be useful laxatives, diuretics and for good blood circulation (Rowe, 1994). *Cnidoscolus aconitifolius* is commonly called chaya or tree spinach. It is a perennial shrub of the family Euphorbiaceae commonly found in the tropics. It is one of the most productive green vegetables eaten in south western Nigeria where it is called Iyana Ipaja (Oyagbemi *et a*l., 2008). It is also eaten by the inhabitants of south eastern Nigeria where it is called "Hospital too far" (Iwalewe *et al*., 2005). The importance of this vegetable to human nutrition and wellness is still not fully harnessed and given its due place. It is still one of nature's underutilized herbs in this part of the world. However, the simple fact that many know it as "hospital too far" alone is quite gratifying and is at the same time also an indication of its healing potentials which are known to selected few in the communities.

A wide variety of claims have been made as to the medical efficacy of *Cnidoscolus aconitifolius* (Chaya; Hospital too far) as a treatment for numerous ailments, ranging from the ability to strengthen fingernails and darken graying hair, to its use as a cure for alcoholism, insomnia, venereal disease, gout, scorpion stings, and as an improvement of brain function and memory. A wild relative of Chaya, is even attributed with anti contraceptive properties (Ross-Ibara and Molina-Cruz, 2002). In South Western Nigeria, the leaves and young shoots are often squeezed with water and drank alone or with milk and tomato paste added. The local folks believe that it has a blood-boosting effect, and so is commonly taken by pregnant women and young children who are anemic (Iwalewe *et al*., 2005). Studies have also shown that *Cnidoscolus aconitifolius* has ameliorative effects on anemia and osmotic fragility induced by protein-energy malnutrition in male Wistar rats (Oyagbemi *et al*., 2008). It has been also reported that the ethanol leaf extract of *C. aconitifolius* at LC50 of 10 µg/ml showed evidence of cytotoxicity with brine shrimp larvae (Senjobi *et al*., 2011).

An unending search for new novel antimicrobials and the fight against antibiotic resistance strains has necessitated this study.



## II. MATERIALS AND MRTHODS

### Sample collection

*Cnidoscolus aconitifolius*(Hospital too far) leaves were collected from different gardens in Calabar into sterile polythene bag. The leaves were then taken to Department of Botany, faculty of Biological Sciences, University of Calabar, for identification and authentication. After, they were transported to microbiology laboratory for analysis.

### Preparation of plant extract

*Cnidoscolus aconitifolius* leaves collected were washed with clean water to remove dust and dirt. The washed leaves were then sun-dried for three (3) days. The brittle leaves were ground into fine powder using a sterilized blender and kept in a tightly covered bowl.

### Distilled water and methanol extraction

All the extractions were done by following standard methods with slight modifications as stated by Archana and Abraham, (2011). For water extraction, 20 g sample was mixed with 80 mL distilled water into a sterile bottle. This was placed in a shaker water bath at 130 r/min at 37°C overnight. The liquid sample was then filtered with Whatman No. 2 filter paper. Methanol extraction was done similarly where sample was mixed with methanol at a ratio of 2:10 and was placed in a shaker water bath following similar conditions as mentioned above. The extracted samples were stored in universal bottles and refrigerated at 4 °C prior to use

### Phytochemical Analysis of Extracts

The extract of *Cnidoscolus aconitifolius* was analyzed qualitatively and qualitatively for the presence of alkaloids, Cyanogenic glycosides, saponins, flavonoids, tannin, polyphenols, terpenoids, reducing sugar, reducing compounds, aththraqunones, and hydroxyl methyl contraquinones using the standard methods described by Eqwaikhide and Gimba (2007);

### Test microorganisms

The test bacteria pathogens used for this research study included *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyogenes*. They were previously isolated, identified and stored in the Department of Microbiology, University of Calabar, Nigeria.

### Antimicrobial Sensitivity Screening of Extracts

The antimicrobial activity of *Cnidoscolus aconitifolius* leaf extracts were evaluated by filter paper disc method. The test bacteria were grown in Muller Hinton broth to match the turbidity of 0.5 McFarland standards to be inoculated on Muller-Hinton agar (Kumar *et al.,* 2012). After inoculation, plates were dried for 15 min, and then the discs impregnated with the different concentration of the extracts were placed on each of the plate at some distance away from each other and not at the edge of the Petri dishes using sterile forceps. Commercially available antibiotic discs were used as positive controls in this study. The extracts were prepared in two different concentrations of 5g/ml and 10g/ml. Plates were incubated for 24 h at 37 °C to allow leaf extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zones of inhibition for different leaf extracts against different bacteria were measured in millimeter for further analysis. All antimicrobial tests were performed in triplicate and the average values were obtained.

## III. Results and Discussion

The results of the qualitative and quantitative phytochemical tests are presented in Tables 1 and 2 while the results of the sensitivity test is presented in Table 3.

**Table 1:Phytochemical analysis of *Cnidoscolus aconitifolius*(qualitative)**

| Chemical constituents | Ethanol extract | Aqueous extract |
|---|---|---|
| Alkaloids | ++ | + |
| Glycosides | ++ | + |
| Saponins | + | + |
| Tannins | - | + |
| Flavonoids | ++ | + |
| Reducing compounds | ++ | ++ |
| Polyphenols | +++ | ++ |
| Phlobatanins | - | - |
| Anthraquinones | - | - |
| Hydroxymethyl anthraquinones | - | - |

**Table 2. Phytochemical analysis of *Cnidoscolus aconitifolius*(quantitative)**

| Analysis | mg%/100g dry weight |
|---|---|
| Alcaloids | 2.50±0.1 |
| Glycosides | 2.44±0.01 |
| Saponins | 1.20±0.1 |
| Tannins | 0.21±0.02 |
| Flavonoids | 3.50±0.1 |
| Polyphenol | 9.18±0.02 |
| Reducing compounds | 4.60±0.02 |

**Each value represents the mean of 3 determinations ± SD**

**Table 3. Standard zones of inhibition of different antibiotics and leaves extracts**

| Bacteria isolate | Standard antibiotics used as control (mm) | | | | | | | | Leaves extracts | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gen 10µg | Amp 10µg | Cip 5µg | Oflx 5µg | Ery 10µg | Sxt 30µg | Met 10µg | S 10µg | W 5g/ml | W 10g/ml | Alc 5g/ml | Alc 10g/ml |
| *S. aureus* | 17 | 26 | 23 | 19 | 22 | 16 | 14 | 15 | 12±0.2 | 24±0.1 | 17±0.2 | 28±0.1 |
| *P.aeruginosa* | 18 | 24 | 24 | 20 | 21 | 17 | 16 | 15 | 16±0.1 | 26±0.2 | 18±0.1 | 29±0.2 |
| *E. coli* | 19 | 25 | 25 | 18 | 19 | 15 | 16 | 17 | 17±0.1 | 23.5±0.1 | 22±0.1 | 27.5±0.1 |
| *S. typhi* | 17 | 26 | 23 | 19 | 20 | 16 | 15 | 16 | 15±0.2 | 20.5±0.2 | 21±0.2 | 30.5±0.2 |
| *S. pyogenes* | 18 | 24 | 24 | 18 | 20 | 16 | 15 | 15 | 13±0.1 | 21±0.2 | 23±0.1 | 31±0.2 |
| *K. pneumonia* | 16 | 21 | 20 | 21 | 20 | 15 | 16 | 16 | 14±0.2 | 22±0.1 | 20±0.2 | 28.5±0.1 |

**Key:**

Oflx= Ofloxacin (sensitive=19 or more) Met= Methicillin (sensitive=14 or more)

Ery= Erythromycin (sensitive= 22 or more) Cip= Ciprofloxacin (sensitive= 23 or more)

S= Streptomycin (sensitive= 15 or more) Am= Ampicillin(sensitive= 26 or more)

Gen= Gentamycin (sensitive= 17 or more) Sxt= Septrin (sensitive= 16 or more)

W = Water Alc = Alcohol

Plants contain various bioactive compounds which impart different characteristics to the plant. The results of phytochemical analysis of *Cnidoscolus aconitifolius* show 7 different phytochemical compounds as can be seen in Table 1. These include alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols and reducing compounds. This is in line with the results reported by Igbinaduwa *et al*., (2012) except anthraquinone which was not detected in our sample. Again, the results of the phytochemical analysis are in accordance with previous reports by (Awoyinka *et al.,* 2007; Yakubu *et al.,* 2008). ). The absence of phlobatannins is in complete contrast to recent reports by Peixoto Sobrinho *et al*., (2012). Yuan *et al*. (2007) reported isolation of fifteen flavonoids from *Cnidoscolus texanus*. Phlobatannin and saponin were found in appreciable amounts in the aqueous extract than the ethanolic extract according to Mordi and Akanji (2012) which is in contrast to our findings.

The quantitative analysis of the extract of Cnidoscolus aconitifolius as displayed in Table 2 shows the mean amounts of each phytochemical component present in the leaves. It can be seen that polyphenols had 9.18mg/100g which is the highest and tannins 0.21mg/100g turned out to be the lowest concentration. Only tannins and flavonoids concentrations observed in this research work compared favorably with the findings of Akachukwu *et al*., (2014).

The extracts of the *Cnidoscolus aconitifolius* were tested against the test organisms, *E. coli, Klebsiella species, Staph. aureus, P. aeruginosa, S. pyogenes* and *S. typhi*. The extracts showed antimicrobial activities against the test organisms with the strongest antimicrobial activity found to be 26mm at a concentration of 10g/ml against *Pseudomonas aeruginosa.* The least activity was observed against *Staphylococcus aureus* with a zone inhibition of 12mm at a concentration of 5g/ml.

The antimicrobial showed a broad spectrum of activity. All the extracts showed appreciable activity against *E. coli, Klebsiella pneumoniae, Staphylococcus aureus, P. aeruginosa, S. pyogenes* and *S. typhi* and this agrees with the work of Abu-Shanab *et al*., (2006). In an earlier work, Awoyinka *et al.,* (2007) reported the antimicrobial activities of the various extracts of *C. aconitifolius* against *S. typhi* and *S. aureus* but at very low zones of inhibition compared to what we obtained in this study. This disparity could be attributed to the different species of *C. aconitifolius* and the geographysical location of the plant. Alcoholic extract of the plant exhibited higher antimicrobial activity on all the test organisms at all concentrations as compared with the aqueous extract. This could be as a result of the antimicrobial properties of alcohol and importantly the ability to extract the active ingredient more than water. The strongest antimicrobial activity was found to be 31mm at a concentration of 10g/ml against *Streptococcus pyogenes*. The least activity was observed against *E. coli* with a zone of inhibition of 27mm at a concentration of 10g/ml for alcoholic extract. For the aqueous extract, the highest activity was seen against *Pseudomonas aeruginosa* with an inhibition zone of 26mm at a concentration of 10g/ml while the lowest was 20.5mm against *S. typhi* at 10g/ml.

All the extracts showed more effectiveness than some of the commercial antibiotics (Streptomycin and Septrin) and compete favorably with others (Ciprofloxacin and Ofloxacin) held in high esteem as antimicrobial agents (Table 3). A proper exploration of the antimicrobial potentials of this plant may result in emergence of lead antibiotic with very broad spectrum of activity. The antimicrobial activity from this plant could be as a result of some antimicrobial components contained in the leaves such as polyphenols, alkaloids, flavonoids and tannins (Papuc *et al*., 2017).

## IV. CONCLUSION

Based on this work, it was concluded that the ability of C. aconitifolius to show sensitivity to two different strains of bacteria (gram positive and negative) points to its possible use as a broad spectrum antimicrobial agent and paving way for further investigation to identify the active compounds responsible for the plant biological activity with the required MIC for use in drug development for safe health care delivery. Further studies of this plant extract could be exploited as future alternatives to control contamination in foods and diseases associated with common pathogenic bacteria. Thus, the need to identify proximate composition and antioxidant activities of the plant species may lead to the development of new excellent alternative natural antioxidant.



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