Research Article

PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND *IN-VITRO* ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *CNIDOSCOLUS ACONITIFOLIUS* LEAVES

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ABSTRACT

The phytochemical screening and *in-vitro* antimicrobial activities of methanolic leaf extract of *Cnidoscolus aconitifolius* were investigated. The proximate analysis showed that the leaves contained moisture (5.35%), ash (13.69%), crude protein (18.74%), fat (11.95%), fibre (9.81%) and carbohydrate (40.48%). The mineral analysis in mg/100g indicated that leaves contained Sodium (77.32), Potassium (58.45), Calcium (44.82), Magnesium (23.46), Zinc (0.02), Copper (0.004), Iron (0.06) while Lead was absent. The phytochemicals present in the leaves of *C. aconitifolius* were alkaloids, tannins, saponin, flavonoids and cardiac glycoside while steroids, phlobatannin and terpenoids were not detected. Antimicrobial activities were assayed using the concentrations of 500, 250, 125, 62.5 and 31.25mg/ml. The antibacterial effects of the extract showed that *Klebsiella pneumonia* had a zone of inhibition that varied from 1.0mm (125mg/ml) to 4.5mm (500mg/ml), *Psuedomonas aeruginosa* with a zone of inhibition of 1.5mm (125mg/ml) to 5.0mm (500mg/ml), similarly, *Escherichia coli* had a zone of inhibition of 1.0mm (125mg/ml) to 3.5mm (500mg/ml). *Staphylococcus aureus* had a zone of inhibition of 1.0mm (62.5mg/ml) to 6.5mm (500mg/ml). However, all test bacteria were resistant to the extract at lower concentrations of 31.25mg/ml and 62.5mg/ml except *S. aureus*. The effect of methanolic extract of *C. aconitifolius* on radial mycelial growth of the test fungi after varied hours of incubation showed that *Aspergillus tamari* had a percentage inhibition that ranged from 22% (31.25mg/ml) to 100% (500mg/ml) at 24hrs, similarly it had a percentage inhibition of 12% (31.25mg/ml) to 76% (500mg/ml) at 72hrs. *A. niger* had a percentage inhibition of 9% at 31.25mg/ml to 91% at 500mg/ml after 24hrs, but it completely inhibited the growth at 31.25mg/ml and 72% at 500mg/ml after 72hrs of incubation. This study showed that *C. aconitifolius* could be a potential antimicrobial agent for the treatment of infections and a good dietary source of nutrients.

Keywords: Phytochemical screening, Proximate analysis, Antimicrobial activities, Methanolic extract, *Cnidoscolus aconitifolius*.

INTRODUCTION

Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's developing countries.[1] These plants that possess therapeutic properties or exert beneficial pharmacological effects on animal body are generally designated as "Medicinal Plants".[2]

Cnidoscolus aconitifolius which belongs to the family Euphorbiaceae is an evergreen, drought-deciduous shrub up to 6m in height with alternate palmate lobed leaves and milky sap. The leaves are large, 32cm x 30 wide on succulent petiole.[3] It originated as a domesticated leafy green vegetable in the Maya region of Guatemela, Belize, South–East Mexico during pre-Cambrian period[3] and due to its ease of cultivation and potential productivity, the plant has spread all over the world including the tropic.[4] The leaves and shoot are taken as laxative, diuretic, circulatory stimulants, to stimulate lactation and to harden fingernails.[5,6] Like most food plants such as Lima beans, cassava and many leafy vegetables, the leaves contain hydrocyanic glycoside, a toxic compound that is easily destroyed by cooking.[5]

The medicinal plants extract exert their inhibitory activities on microorganisms in different ways which eventually cause death or static effect.[7] Some of these natural products have been approved as new antimicrobial drugs but there is an urgent need to identify novel substances/drug that are active towards pathogens with high resistance.[8]

This study was aimed to determine the nutritional value, phytochemical properties and the antimicrobial activities of *C. aconitifolius*.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of *C. aconitifolius* were collected from the University Farm, University of Ado-Ekiti, Ekiti-State. Botanical identification and authentication were done at the Herbarium section of the Department of Plant Science, University of Ado-Ekiti, Nigeria.

Preparation of Plant Extract

The leaves of the plants were air-dried at room temperature for 3 weeks. The dried leaves were ground into fine powder using laboratory mortal and pestle. The methanolic extract was prepared by soaking 1.5kg of powdered sample into 1000ml of distilled methanol for 5days. After which the supernatant was decanted and evaporated to dryness using a rotary evaporator at 40^{O}C. The dried extract was kept in the refrigerator at 4^{O}C until required for use.

Determination of Antimicrobial Activities

Source of Microorganism

The test bacteria used were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Escherichia coli* while the fungi used were *Aspergillus tamarri* and *Aspergillus niger*. They were obtained from the Research Laboratory of the Department of Obstestric and Gynaecology, College of Medicine, University of Lagos, Nigeria. The bacteria were maintained on Nutrient Agar and the fungi on Potato Dextrose Agar and stored at 4^{O}C until ready for use.

Standardizaion of inocula

The test bacteria were grown (in separate tubes) at 37°C in Mueller-Hilton (Oxoid)broth McFarland standard) at optical activity of 625 nm with Mueller- Hilton (Oxoid) broth and stored at 4°C to arrest further bacteria growth/multiplication.[9]

Antibacterial testing

Paper disc method as described by Fagbohun *et al*.,[10] was used. About 0.1ml of standardized inoculum of each of the test bacteria was aseptically transferred to each petri-dishes containing solidified nutrient agar. A sterile glass spreader was used to spread this evenly over the surface of the nutrient agar. The plates were allowed to dry for one hour of pre-diffusion. Sterile filter discs (6.00mm in diameter) were soaked in various concentrations of the methanolic extract (500, 250, 125, 62.5 and 31.25mg/ml). Enough time was allowed for the solvents to dry before transferring the discs to the surfaces of the agar plates by a pair of sterile forceps. For control experiment, the paper discs were soaked in extracting solvent. The plates were incubated at 30^{O}C for 24hours after which the zone of inhibition were measured. All plates were made in duplicate.

Antifungal testing

Radial mycelial growth assay technique of Smith[11] and Odeyemi and Fagbohun[12] were used whereby sterile plant extracts of the following concentration 500, 250, 125, 62.5 and 31.25mg/ml were introduced aseptically into sterile petri-dishes. About 18 ml of sterilized PDA was added to each of the dishes containing the various concentration of the plant extracts. The plates were swirled carefully to ensure proper mixing and allowed to set. Mycelial discs (6 mm diameter) taken from the advancing edges of 3 – 5 days old culture of each of the test fungi on PDA were placed centrally on the cooled seeded agar plates, incubated at 28°C. The radial mycelial growth was measured every 24 h for 5 days. All the plates were in duplicate and the test carried out twice. Control plates were treated as described above using the extracting solvent (methanol) only.

Phytochemical analysis of *C. aconitifolius*

Quantitative phytochemical screenings to determine the presence of alkaloids, tannins, saponins steroids, phlobatannin, terpenoids, flavonoid and cardiac glycosides using standard methods as described by Harbone[13], Trease and Evans[14], and Sofowora[15] were carried out.

Proximate analysis

The proximate analyses of the sample for moisture, ash, fibre and fat were done by the method of AOAC.[16] The nitrogen was determined by micro-Kjeldahl method as described by Pearson[17] and the percentage nitrogen was converted to crude protein by multiplying with 6.25. Carbohydrate was determined by difference. All determinations were performed in duplicates.

Mineral analysis

The mineral was analyzed by using a flame photometer (Model 405 Corning, UK), using NaCl and KCl to prepare the standards. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in duplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals was determined according to Techtron.[18] The optimum analytical range was 0.1 – 0.5 absorbance unit with a coeffient of variation of 0.87 - 2.20%. All the proximate values were reported as percentage while the minerals were reported as milligram/100 grams.

RESULTS AND DISCUSSION

The results of the proximate analysis of *C. aconitifolius* leaves are shown on Table 1. The amount of carbohydrate and fibre present were 40.48% and 9.81% respectively. The carbohydrate content is lower compared to 72.57% in *Anisopus mannii*[19], 75% in *Corchorus tridens*[20], 54.20% in *Ipomoea aquatica*[21], 51.8% in *Moringa stenopetala* leaves[22]. However, the value is higher when compared to 17.75 and 10.62% in *Cocos nucifera*[23].

Table 1: Result of Proximate analysis of *C. aconitifolius* leaves (%)

Test	Percentage of dried samples
Ash	13.69
Moisture Content	5.35
Crude Protein	18.74
Fat	11.95
Fibre	9.81
Carbohydrate	40.48

The plant is a good source of carbohydrate when consumed because it meets the Recommended Dietary Allowance (RDA) values e.g. children (40%), adults (40%), pregnant women (30%) and lactating mothers (25%).[24] The fibre content of the leaves is higher compared to 3.7% in *Ipomoea batatas* leaves.[20] But lower when compared to 17.67% in *Ipomoea aquatica*[21], 45.70-46.05% (dry weight) reported as dietary fibre in Japanese *Ipomoea batatas*[25] and 89.64% reported in *Anisopus mannii*[19]. High fibre content in food causes intestinal irritation and lower nutrient bioavailability.[26] Apart from

negative effect, intake of fibre can stimulate weakening hunger, stimulating peristaltic movement and lower the serum cholesterol level.[27,21] The composition of ash and moisture content in the leaves of *C. aconitifolius* are 13.69% and 5.35%. The amounts of moisture content compared favourably with 8.41% in *Anisopus mannii*.[19] However, it is lower when compared to 50.19, 63.51 and 29.19% in the leaf, stem and root of *Nypa fructican* respectively.[28] High moisture content enhances microbial growth and enzyme activity.[29] This suggests that dried leaves of *C. aconitifolius* will not promote microbial growth and enzyme activity since its water content is low. Also, the ash content (13.69%) compared favorably with 10.36% reported in *Anisopus mannii*[19] and 14.44% in leaves of *Ipomoea aquatica* grown in Vietnam.[30] It is lower compared to 17.87% found in leaves of *Ipomoea* sp grown in Swaziland.[31] But it is higher compared to 1.19 and 1.66% in healthy and infected *Cocos nucifera*.[23] The ash content of these leaves is an indication that the leaves contain nutritionally important mineral elements.[21]

The leaves also contained 18.74% of crude protein. It is high when compared to 8.40% in *Anisopus mannii*[19], 6.30% in *Ipomoea aquatica* leaves.[21] However, it is lower compared to 24.37-29.46% reported in *Ipomoea batatas*.[32] It compared favourably with 17.50% in *Cinetum africana*[33] and 19.79% in *Urena lobata*[10]. This showed that *C. aconitifolius* leaves have potential benefit as proteins are essential for the synthesis of body tissues and regulatory substance such as enzyme and hormones.[34] The plant is considered a good source of protein because it provides more than 12% of caloric value of protein.[17]

The value for fat in the leaves was 11.95%. This value is lower when compared to 20.27% and 40.75% in healthy and infected *Cocos nucifera*[23] but compared moderately with 8.71% in *Baseila alba*[35] and 10.21% in *Urena lobata* L.[10] Dietary fat increases the palatability of food by absorbing and retaining flavours.[36]

Table 2: Result of Mineral analysis of *C. aconitifolius* leaves (mg/100g)

Tests	Results (mg/100g)
Sodium	77.32
Potassium	58.45
Calcium	44.82
Magnesium	23.46
Zinc	0.02
Copper	0.004
Iron	0.06
Lead	Absent

The mineral compositions of *C. aconitifolius* leaves in mg/100g were shown in Table 2. It contained Sodium (77.32) and Potassium (58.45). The value of Potassium in *C. aconitifolius* leaves is lower when compared to that reported for *Anisopus manni* (1700)[19] and higher when compared to 0.91mg/100g in leaves of *Boerhavia diffusa* and 0.78mg/100g in *Commelina nudiflora*.[37] Sodium is associated with Potassium in the body in maintaining acid-base balance and nerve transmissions.[38] High concentration of Sodium is disadvantageous because Potassium depresses blood pressure while Sodium raises blood pressure, thus the level of Sodium in these leaves may cause hypertension and atherosclerosis when consumed.[21]

The value of iron present in the leaves was 0.06mg/100g. The concentration of iron in *C. aconitifolius* leaves is lower compared to 156mg/100g in *Anisorus mannii*[19], 75.9mg/100g and 102.40mg/100g in whole seeds and seed nut of *Tamarindus indica* respectively.[39] However, it compared favourably with 0.91mg/100g reported for *Asparagus officinalis*.[40] This low amount of iron in leaves of *C. aconifolius* is an indication that it could not serve as a good source of Iron, since daily requirement is 1.00mg/100g.[41,25]

The values of Manganese and Copper in the leaves were 23.46mg/100g and 0.004mg/100g respectively. This suggests that the plant could be an important modulator of cells functions, play a vital role in the control of diabetes[34] and cannot be used for substitute as blood forming leafy vegetables[41] respectively.

The calcium content was 44.82mg/100g. This value is lower compared to 101mg/100g in Vietnamese *Ipomoea aquatica* leaves[30] and 100mg/100g in Indian *Solanum tubirosam*[42]. But higher when compared to 10mg/100g in whole seed of *Tamarindus indica* and compared favourably with 31mg/100g in seed nuts of *T. indica*[39]. Therefore, it is possible for *C. aconitifolius* to serve as a rich source of minerals involved in bone formation[43].

Zinc value was 0.02mg/100g. This implies that the leave is not a good source of Zinc and therefore may not be involved in normal functioning of immune system.

In this study, Lead was not detected in the leaves. This is in great contrast to the findings of Proph *et. al.*,[44] who reported Lead to have a concentration of 2.71mg/100g in *Caesalpina pulcherrima*. Sometimes, anti-nutrients form complexes with these nutritionally important minerals such as Zn^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} thereby preventing efficient absorption by the body systems.[45]

Table 3: Results of Phytochemical Screening of *C. aconitifolius* leaves

Tests	Results
Alkaloids	+ve
Tannins	+ve
Saponin	+ve
Steriods	-ve
Phlobatannin	-ve
Flavonoids	+ve
Terpenoids	-ve
Cardiac glycoside	+ve

+ve = Presence of constituent
-ve = Absence of constituent

The results of phytochemical analysis of leaves of *C. aconitifolius* are shown in Table 3. It showed that the plant contained alkaloids, tannins, saponin, flavonoids and cardiac glycoside while steroids, phlobatannins and Terpenoids were absent. This is similar to the findings of Okoli *et al*.,[46] who detected the bioactive compounds such tannins, saponin, flavonoids, cardiac glycoside, alkaloids in *Euphorbia hirta*. Alkaloids present have been reported as one of the largest group of phytochemicals in plant with amazing effects on humans[47] and have been used for treatment of intestinal infections associated with AIDS and hypertension[48,49]. Another constituent of leaves of *C. aconitifolius* was tannin. Parekh and Chanda[50] reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment ailment of inflamed or ulcerated tissues. Studies have shown that saponin which was also detected have been used for treatment of hyperglycaemia and that dietary source of saponins offer preferential chemopreventive strategy in lowering the risk of human cancer[51,52]. Flavonoids, another constituent of *C. aconitifolius* leaves extract exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic and antioxidant properties.[53] The antimicrobial attributes of these bioactive constituents have been associated with their abilities to inhibit cell wall formation in fungi[54], intercalate with DNA[55] and inactivate microbial adhesions and enzyme[56].

The antibacterial effects of the extract are shown in Table 4. *Klebsiella pneumonia* had a zone of inhibition that varied from 1.0mm (125mg/ml) to 4.5 (500mg/ml), *P. aeruginosa* had a zone of inhibition of 1.5mm (125mg/ml)to 5.0mm (500mg/ml), similarly, *E. coli* had a zone of inhibition of 1.0mm (125mg/ml) to 3.5mm (500mg/ml), *S. aureus* had a zone of inhibition of 1.0mm (62.5mg/ml) to 6.5mm (500mg/ml). This is similar to the findings of Kalyoneu *et al*.,[57] who reported that various extracts of *Rubia tinctorum* L. showed inhibitory effects against the test bacteria (*E. coli, Bacillus subtilis, Micrococcus luteus, S. aureus, P. aeruginosa*) with zones of inhibition. Similarly, Nkomo and Kambizi[58] reported that *Gunnera perpensa* extracts from both methanol and water were inhibitory to all gram positive bacteria (*S. aureus*, *S. epidermidis*, *Bacillus cereus*, *Micrococcus kristinae* and *Streptococcus faecalis*) tested. However, all the test bacteria were resistant to the extract at lower concentrations of 31.25mg/ml and 62.5mg/ml without zones of inhibitions except *S. aureus*. This is similar to the findings of Matheshwari and Kumar[59] who reported that aqueous extract of *Abelmoschus moschatus* did not exhibit any antibacterial activity against *E. coli, Bacillus megaterium, Bacillus subtilis, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumonia, Corynebacterium diphtheriae, S. typhii, P. aeruginosa, Shigella flexneri*. The extract had a weak antibacterial activity on the test bacteria at 31.25mg/ml and 62.5mg/ml with each test organism showing no zone of inhibition. Similarly, it had a strong bacteriostatic effect on *S. aureus* with zone of inhibition of 6.5mm at concentration of 500mg/ml while the control was 0.0mm.

Table 4: Antibacterial activities of the methanolic extract of *C. aconitifolius* leaves using paper disc method

Concentration of the Extract (mg/ml)	Test Bacteria			
	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*
Control	0.0	0.0	0.0	0.0
31.25	0.0	0.0	0.0	0.0
62.50	0.0	0.0	1.0	0.0
125	1.0	1.5	3.0	1.0
250	3.0	2.0	4.0	2.0
500	4.5	5.0	6.5	3.5

Table 5: Effect of the Methanolic extract of *C. aconitifolius* leaves on Radial Mycelial Growth (in mm) of *Aspergillus niger* and *Aspergillus tamarii*.

Test Fungi	Time of incubation in hours	500 mg/ml	% Inh.	250 mg/ml	% Inh	125 mg/ml	% Inh	62.5 mg/ml	% Inh	31.25 mg/ml	% Inh.	C
Aspergillus tamarri	24	0	100	2	78	3	67	5	44	7	22	9
	48	2	83	5	58	6	50	9	25	11	11	12
	72	6	76	12	52	14	44	20	20	22	12	25
Aspergillus niger	24	1	91	3	73	5	55	8	27	10	9	16
	48	4	75	7	56	10	38	13	9	15	6	16
	72	6	72	10	55	14	36	20	9	22	0	22

The results of the effect of the extract on radial mycelial growth of the test fungi under varied hours of incubation are shown on Table 5. *Aspergillus tamarii* had a percentage inhibition that varied from 22% (31.25mg/ml) to 100% (500mg/ml) at 24hrs, similarly, it had a percentage inhibition of 11% (31.25mg/ml) to 83% (500mg/ml) at 48hrs and at 7hrs of incubation, A. *tamarii* had a percentage inhibition of 12% (31.25%) to 76%

(500mg/ml) while *A. niger* had a percentage inhibition of 9% (31.25mg/ml) to 91% (500mg/ml) at 24hrs, it had a percentage inhibition of 6% (31.25mg/ml) to 75% (500mg/ml) at 48hrs and after 72hrs, *A. niger* exhibited a percentage inhibition of 0% (31.25mg/ml) to 72% (500mg/ml). The result of this study showed that radial mycelial growths of both test fungi were inhibited as the concentration of the extract increased. This is similar to the findings of Shailini and Rachana[60] who reported that increased concentrations of methanolic crude extract of *Tectona grandis, Shilajit and Valeriana wallachi* inhibited the spore germination of *Alternaria cajani*, *Helminthosporium spp., Bipolaris spp., Curvalaria lunata* and *Fusarium* spp. similarly, Donlaporn and Suntornsuk[61] reported the fungistatic effects of *Jatropha curcas* seeds extract on *Phythium aphanidermatum* and *Fusarium semitectum*. However, this result differed greatly from the findings of Erturk *et al*.,[62] who found that the essential oils from *Coriandum sativum* did not show inhibitory effect against *A. niger*. In this present, the extract of *C. aconitifolius* leaves was more effective against *A. tamarii* than *A. niger* at different incubation periods.

The antimicrobial activity exhibited by this leaf was due to the presence of certain phytochemicals such as alkaloids, saponin, tannin, flavonoids and cardiac glycoside (Table 3). This is similar to the findings of Sokomba *et al*.,[63] who reported that methanolic leaf extract of *Synclisia scabrida* exhibited significant activity against the pathogens tested due to the presence of high amount of flavonoids and alkaloids that are also known to possess antimicrobial activity.

CONCLUSION

This research work showed that *C. aconitifolius* leaves could be a potential antimicrobial agent for treatment of diseases and ailments and could also be a good source of minerals. However, it is unwise to eat raw *C. aconitifolius* leaves because it contains hydrocyanogenic glycoside which is toxic in nature. Therefore, cooking before consumption is encouraged.

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