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ANTIBACTERIAL AND ERYTHROCYTIC GENERATION POTENTIAL OF *CNIDOSCOLUS ACONITIFOLIUS* IN ALBINO RATS

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ABSTRACT

This study was conducted to determine the antibacterial effects using agar diffusion technique and erythrocytic generation potential of Cnidoscolus aconitifolius in albino rats. The phytochemical screening of C. aconitifolius revealed the presence of six phytochemicals in methanolic and aqueous crude extracts namely; alkaloid, phenol, flavonoid, tannin, steroid and saponins. Methanol and aqueous crude extracts of C. aconitifolius showed low activity (1.5-12 mm) against Salmonella typhi and Escherichia coli. The minimum bacteriocidal concentration (mBc) of both extracts was found to be 100 mg/ml while the minimum inhibitory concentration (MIC) was 200 mg/ml. However, 30 g of C. aconitifolius supplemented diet had a significant ($p<0.05$) effect on hematological parameters (such as packed cell volume, white blood cell count and monocytes) examined. Similarly a significant ($p<0.05$) increase in body weight was recorded for group of rats fed with C. aconitifolius supplemented diet.

1. INTRODUCTION

Cnidoscolus aconitifolius, commonly known as tree spinach, is a cultivated, fast growing perennial plant that belongs to the family *Euphorbiaceae*. It has succulent stems which exude a milky sap when cut. The genus *Cnidoscolus* comprises at least 40 species with a range of distribution extending from temperate to tropical zones (Everitt et al., 2007; Nebel and Heinrich, 2009). It is an ornamental, evergreen, drought deciduous shrub of 3 to 5 m tall. The palmate lobed leaves are large, 32 cm long and 30 cm wide, alternately arranged on chartacious and succulent petioles (Kuti and Konoru, 2004).

Its origin is not known but it is thought to be from the Yucatán Peninsula of Mexico, where the plant is mainly cultivated as food, therapeutic and as edible plant. Its recent spread into new areas may not be unconnected with its medicinal significance; having a wide variety of claims made for its medicinal efficacy as a treatment for numerous ailments such as insomnia, gout and alcoholism. It also has the potential of boosting low blood volume, lowering blood cholesterol, management and treatment of diabetes mellitus (Jensen, 1997; Atuahene et al., 1999; Grubben and Denton, 2004; Adolfo and Michael, 2005).

The levels of *C. aconitifolius* leaf nutrients are two- to threefold greater than any other land-based leafy green vegetable. It is a good source of protein, vitamins, calcium, and iron; and it is also a rich source of antioxidants. However, raw *C. aconitifolius* leaves are toxic as they contain a glycoside that can release toxic cyanide similar to that found in cassava. Some varieties also have stinging hairs and require gloves for harvesting. In respect of these and for safe consumption; cooking time of 5-15 minutes is required (Kuti and Torres, 1996; Kuti and Konuru, 2004).

C. aconitifolius is easy to grow, suffers little insect damage, tolerant of heavy rainfall and exhibits some drought tolerance. About 6-12 inches woody stem cut is required for propagation, as seeds are rarely produced. Therefore, early growth is slow as roots are slow to develop on the cuttings, so the leaves are not harvested until the second year. Thereafter, *C. aconitifolius* leaves can be harvested continuously as long as no more than 50 % of the leaves are removed from the plant, which guarantees healthy new plant growth (Kuti and Torres, 1996).

Due to the adverse effects of the most common antibiotics, increase in the emergence of multidrug resistant strains of pathogens and the economic predicament of most developing countries, the search for new, cheap and less toxic antimicrobials has become necessary. This study attempts to determine the influence of the leaves of *C. aconitifolius* supplemented diet on hematological parameters of albino rats and in-vitro antibacterial potentials of leaves crude extracts of *C. aconitifolius* against *Salmonella typhi and Escherichia coli.*

2. MATERIALS AND METHODS

2.1. Collection and Identification of the Plant Materials

Fresh samples of the leaves were collected from the NECO Staff quarters, Minna, Niger State, Nigeria. The plant materials were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification by a taxonomist. A Voucher specimen number (CA 258BS) was given and the specimen was deposited in the herbarium.

2.2. Drying Procedure

The leaves were air dried at room temperature (28 ^{o}C) for about three weeks, under controlled (aseptic) conditions.

2.3. Preparation of Plant Extracts

After the leaves were air dried and blended in a mixer grinder, extraction was carried out with a modified method of Swain (1988) in which 400 g of powder-dried leaves were macerated in 2 litres of methanol (in the ratio of 1 g : 5 ml). Methanol extraction was carried out on the dried leaves with 96 % methanol in a soxhlet extractor for 3 h. The mixture of the solvent and the plant material was filtered using Whatman filter paper No. 1. The resulting residue called the methanol extract, was transferred to a hot air oven where it was dried to a constant weight, at 45 ^{o}C. Three grams (3 g) of the crude extract was dissolved in 2 ml of methanol after which 2 ml of Tween 80, and 26 ml of distilled water were added to obtain a solution of 30 ml, which was the stock solution for the phytochemical and antimicrobial test (Swain, 1966).

2.4. Phytochemical Screening of the Leaf Extract

The extracts were screened for phytochemical properties according to the methods described by Trease and Evans (1989) and Sofowora (1993).

2.4.1. Test for saponins
A measured amount (5 ml) of the crude extract was shaken vigorously for 2 minutes with 10 ml of distilled water. Observation for frothing was done and the result recorded.

2.4.2. Test for flavonoids
A measured amount of the extract (0.2 g) was diluted with a few drops of dilute 0.2M sodium hydroxide. Observation for the appearance of a yellow solution was done. Few drops of dilute hydrochloric acid were again added and observation for colour change was done and the result was recorded.

2.4.3. Test for steroids
A measured amount (1 ml) of the extracts was dissolved in 10 ml of chloroform and an equal volume of concentrated sulphuric acid was added by the sides of the test tube. The presence of steroids is usually indicated by the development of a red colour by the upper layer and yellow with green fluorescence by the acid layer.

2.4.4. Test for phenol
An amount (2 ml) of extract solution was dissolved in 5 ml of distilled water. Few drop of neutral 5 % ferric chloride solution were added. A dark green color indicated the presence of phenolic compound.

2.4.5. Test for alkaloids
A measured amount (0.5 g) of the crude extract was stirred with 5 m of 1 % hydrochloric acid in a water bath for thirty minutes and then filtered. A portion (1 ml) of the filtrate was put in a test tube. A few drops of Dragendorff's reagent were added to the test tube. Observation for an orange-red precipitate was done and the result recorded.

2.4.6. Culture Media

A measured amount (6.3 g) of Salmonella Shigella agar was dissolved in 100 ml of water through heating at 60^0 C for 5 minutes and was used as a selective medium for the confirmation of the test organisms. A portion (2.8 g) of the nutrient agar dissolved in 100 ml of water was sterilized and used for susceptibility testing (Idu et al., 2014).

2.5. Identification of the Test Organisms

The test organisms (*S. typhi* and *E. coli*) were obtained from the stock culture in the Microbiology Laboratory, General Hospital, Minna, Niger State, Nigeria. The isolates were identified using the method of Cheesbrough (2010).

2.6. Standardization of the Test Organisms

The McFarland standard was employed in the standardization of the test organisms. Morphologically similar colonies of each test organisms were transferred aseptically from an agar plate culture into a tube containing 4 to 5 ml of a suitable broth medium (nutrient broth). The broth was shaken and incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards (usually in 2 to 6 hours). The turbidity of the actively growing culture in the broth was adjusted with sterile saline or broth to obtain turbidity that was optically comparable to that of the 0.5 McFarland standards (Lalitha, 2004).

2.7. Bacterial Assay of the Crude Extracts

The antibacterial assay of the crude extracts was done using a punch well method described by Idu et al. (2014). The plates were prepared by dispensing 20 ml of nutrient agar into sterile petri plates and allowed to set. A 4 mm cork borer was used to punch holes in the medium. Four cup well were made on each petri plate, and adequately spaced out after inoculation. Thereafter, 0.2 ml of the different 150, 200 and 250 mg/ml concentrations was introduced into each well. The petri plates were incubated at a temperature of 37 ºC for 24 hours, after which observation for the zones of inhibition was conducted, measurement of the zones of inhibition were carried out and the results recorded in comparison with the effect of the standard antibiotic (Chloramphenicol) as the control (Idu et al., 2014).

2.8. Minimum Inhibitory Concentration (MIC)

The MIC of the antimicrobial compounds was determined by broth dilution methods. A measured amount (5 ml) of nutrient broth was dispensed into each test tube and 0.5 ml of bacteria suspension (1.0×10^6) was used to inoculate each test tube containing the broth. This was followed by the introduction of different concentrations (150 mg/mL and 200 mg/mL) of the crude extract into the test tubes. The antimicrobial compounds were not added to the control test tubes. The uninoculated test tube was used to check the sterility of the medium and as the negative control while the positive control tube (which was the inoculated test tube) was used to check the suitability of the medium for growth of the microorganisms and the viability of the inoculums (Idu et al., 2014). The final volume in all the test tubes was adjusted to 10 ml using distilled water. All the test tubes were properly shaken and then

incubated at 37 ^{o}C for 24 hrs. The MIC was determined by the lowest concentration of the extract that inhibited visible growth (Idu et al., 2014).

2.9. Minimum Bactericidal Concentration (MBC)

The MBC of the extracts was determined by sub culturing the contents of the tube(s) that showed inhibition or no turbidity on nutrient agar plate. The absence of growth on incubation for 24 hours was confirmatory for MBC.

2.10. Experimental Animals

Twenty (20) rats were randomly divided into four groups of 5 rats each: Each group was separately housed as indicated as follows: group A served as control and received no treatment, group B were fed with 10 g/feed *C. aconitifolius* leaf inclusion diet, group C were fed with 20 g/feed *C. aconitifolius* leaf inclusion diet and group D were fed with 30 g/feed *C. aconitifolius* leaf inclusion diet.

2.11. Determination of body weight

The body weights of the rats were determined on a weekly bases and the weight gain was computed using Equation (1) (Edet et al., 2010)

$$\text{Weight gain (g)} = \text{final weight of rat - initial weight of rat} \quad (1)$$

2.12. Determination of Hematological Parameters

Five milliliter (5 ml) of blood sample was collected from each rat for haematological analysis. The hematological indices analyzed include haemoglobin (Hb), haematocrite (HCT), red blood cells (RBC), red cell distribution weight (RDWt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), granulocyte count (GRA) lymphocytes (LY), platelet count (PLT), mean platelet volume (MPV). Platelet count and platelet distribution weight were determined using the automated hematologic analyzer (model number: SYSMEX KX21), a product of SYSMEX Corporation, Japan. The methods described by Dacie and Lewis (1991) were adopted for the analysis.

2.13. Statistical Analysis

The data were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± standard error of mean. Comparisons between different groups was done using Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). Values of $P<0.05$ were considered as statistically significant as prescribed by Mahajan (1997).

3. RESULTS AND DISCUSSION

The phytochemical screening of *C. aconitifolius* revealed the presence of alkaloids, phenols, flavonoids, tannin in both methanolic and aqueous extracts, of which saponins and steroids were only present in the aqueous extract (Table 1).

Table 1: Phytochemical constituents of *Cnidoscolus aconitifolius*

Phytochemicals	Methanol	Aqueous
Alkaloids	++	++
Saponins	- -	++
Phenols	++	++
Flavonoids	+	++
Tannin	++	++
Steroids	- -	++

Key: + = Presence of the phytochemical compound; -- = Absence of the phytochemical compound

The presence of phytochemicals in appreciable quantity may be attributed to the use of polar solvents for extraction. The phytochemicals identified in this study agree with the report of Adeniran et al. (2011) but contrary to the report by Mordi and Akanji (2011).

The methanol and aqueous crude extracts at 200 mg/ml showed appreciable antibacterial activity with zones of inhibition ranging from 5-12 mm (Tables 2 and 3). Similarly, the minimum bacteriocidal concentration of both methanol and aqueous extracts was between 100-200 mg/ml (Tables 4 and 5).

Table 2: Antibacterial effects of the methanol crude leaf extract of *Cnidoscolus aconitifolius*

Organism	Methanol extract concentration (mg/ml)			Control (mg/ml)
	000150	200	250	100
Salmonella typhi	1.5 mm	5 mm	8.5 mm	23 mm
Escherichia coli	1.8 mm	8 mm	10.0 mm	20 mm

The methanolic crude extract of *Cnidoscolus aconitifolius* leaves exhibited low activity, even at high concentration (Table 2). This could be attributed to antagonistic effect of the phytochemicals, as methanol is polar and can extract multiphytochemicals. This finding corroborates the finding of Oyagbemi et al. (2011) who observed a similar trend, but with ethanolic extract of the same plant at high concentration.

Table 3: Antibacterial effect of the aqueous leaf extract of *Cnidoscolus aconitifolius*

Organism	Aqueous extract concentration (mg/ml)			Control (mg/ml)
	150	200	250	100
Salmonella typhi	0.5 mm	5 mm	8.5 mm	23 mm
Escherichia coli	2.8 mm	9 mm	12.0 mm	20 mm

The aqueous extract of *Cnidoscolus aconitifolius* showed low activity against *Salmonella* typhi and *Escherichia coli.* This could be attributed to resistance peculiar with *S.* typhi and *E. coli.* However, as the concentration of the extract increased, activity also increased, though not comparable to that observed with the standard drug (control) (Table 3).

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of the methanol extracts

Organisms	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	100	0.00
Escherichia coli	100	200

Table 5: Minimum inhibitory concentration and Minimum bactericidal concentration of the aqueous extracts

Organisms	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	200	0.00
Escherichia coli	100	200

The methanol extract of *Cnidoscolus aconitifolius* had an inhibitory effect at 100 mg/ml on *Salmonella typhi* and *Escherichia coli*. Also, has a bactericidal effect on *Escherichia coli* at 200mg/ml though not bactericidal for *Salmonella typhi*. The MIC and MBC of aqueous extracts recorded was 100 mg/ml and 200 mg/ml respectively on *E. coli*. More so, aqueous extract was not bactericidal on the *Salmonella typhi* even at higher concentration (Table 4 and 5). This could also be attributed to resistance.

Body weight gain of the rats fed with 10 – 30 g *Cnidoscolus aconitifolius* leaf containing feed were significantly ($p < 0.05$) higher than those of the control rats. The groups of rat fed with leaf containing feed at 10, 20 and 30 g had body weight gain of 32.4± 3.24, 22.92± 4.11 and 34.53± 6.52 respectively, while the control group had body weight gain of 18.02± 3.24 (Table 6).

Table 6: Change in body weight in rats fed with *C. aconitifolius* leaf supplemented diet

Groups	Week 1	Week 2	Week 3	Week 4	Weight gain (g)
10 g of *C. aconitifolius*	159.05 ± 4.56	179.56 ± 4.67	189.04 ± 5.67	191.45 ± 4.56	32.4 ± 3.24^{c}
20 g of *C. aconitifolius*	145.75 ± 5.67	162.35 ± 5.67	167.56 ± 4.67	168.67 ± 5.45	22.92 ± 4.11^{b}
30 mg of *C. aconitifolius*	199.90 ± 5.32	220.43 ± 4.56	238.53 ±14.56	234.43 ± 4.56	34.53 ± 6.52^{c}
Control	159.54 ± 3.56	169.42 ± 3.46	174.32 ± 5.67	177.56 ± 5.65	18.02 ± 3.20^{a}

Values are mean ± SEM of 5 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$)

The ability of the *C. aconitifolius* feed to improve the body weight of the rats in four weeks study period suggest high caloric content of the *C. aconitifolius* supplemented feed. Similar finding has been reported by Edet et al. (2010) (Table 6).

Table 7 shows the changes in hematological indices following 10 – 30 g inclusion of *C. aconitifolius* leaf in the feed of albino rats. The WBC, red cell distribution weight and Neutrophil counts were significantly ($p < 0.05$) lowered while Monocytes, lymphocytes and Eosinophils were significantly ($p < 0.05$) higher in rats fed with *Cnidoscolius aconitifolius* leaf (10 -30%) as compared with the control value. The rats fed 30 g *C. aconitifolius* leaf had a significant ($p < 0.05$) increasing effect on the red blood cell count (RBC), packed cell volume (PCV), haemoglobin (Hb) when compared to the control rats. These improved values of PCV, RBC and Hb indicate a haematinic and blood enhancer effects of *C. aconitifolius*. The results of the present study agree with the findings of Idu et al., (2014),

who reported that aqueous extract of *C. aconitifolius* improves the level of some hematological parameters in phenyl hydrazine-induced anaemic rabbits. Similarly, the elevation of the counts for lymphocytes and eosinophils (Table 7) in the blood of rats fed with supplemented diet is an indication that *C. aconitifolius* has potential to boost immunity. This is because lymphocytes, monocytes and eosinophils are scavenger immune cells that participate actively in the phagocytosis of foreign particles to the body of an organism.

Table 7: Hematological parameters

Parameters	Control	10g/feed *C. aconitifolius*	20g/feed *C. aconitifolius*	30g/feed *C. aconitifolius*
PCV (%)	34.00 ± 3.35^{a}	34.00 ± 3.65^{a}	35.00 ± 4.67^{a}	41.00 ± 3.76^{b}
WBC (x 10^{6})	7.80 ± 2.71^{c}	5.55 ± 2.36^{a}	6.10 ± 1.57^{b}	5.65 ± 3.21^{a}
HB (g/L)	10.30 ± 0.26^{a}	9.70 ± 2.20^{a}	9.10 ± 2.13^{a}	12.35 ± 2.56^{b}
RBC (x 10^{12})	6.70 ± 1.53b	5.65 ± 1.22^{a}	6.30 ± 1.90^{b}	7.40 ± 1.56^{c}
RDWt	20.70 ± 2.51^{c}	18.20 ± 2.75^{b}	17.70 ± 0.21^{ab}	16.90 ± 2.24^{a}
Platelets (x 10^{6})	633.00 ± 10.32^{b}	487.00 ± 7.47^{a}	2487.00 ± 121.38^{d}	801.00 ± 5.42^{c}
MCHC (g/l)	30.00 ± 4.45^{a}	31.50 ± 4.21^{a}	29.00 ± 2.10^{a}	30.00 ± 6.46^{a}
MCV	53.00 ± 2.73^{a}	53.50 ± 5.48^{a}	56.00 ± 4.46^{a}	56.00 ± 3.21^{a}
MCH (pg)	15.50 ± 4.13^{a}	16.50 ± 2.21^{a}	14.00 ± 1.11^{a}	17.00 ± 1.44^{a}
Neutrophil (%)	24.00 ± 2.28^{c}	14.00 ± 0.45^{a}	20.00 ± 4.49^{b}	21.50 ± 3.02^{b}
Lymphocyte (%)	51.50 ± 4.68^{b}	54.00 ± 6.02^{b}	27.00 ± 3.70^{a}	61.50 ± 7.46^{c}
Monocyte (%)	24.50 ± 4.76^{a}	29.00 ± 5.303^{c}	31.00 ± 2.21^{c}	27.00 ± 2.11^{b}
Eosinophil (%)	24.55 ± 1.38^{a}	58.00 ± 3.12^{d}	31.00 ± 2.43^{c}	27.46 ± 5.38^{b}

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

4. CONCLUSION

C. aconitifolius contain six phytochemical constituents, namely alkaloid, phenol, flavonoid, tannin, steroid and saponins. However, its antimicrobial effect was lower as compared with standard drug used. There was increase in hematological parameter of the rat fed supplemented diet. Similarly, there was significant increase in body weight of the rats fed supplemented diet.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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