

## Short Communication

# Phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae)

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Eight principal bioactive compounds were investigated in the dry leaf water and ethanolic extracts of *Cnidoscolus aconitifolius*. In all, three active components were positive for both extracts. These include phenols, saponins and cardiac glycosides. Phlobatannin was detected in the water extract while alkaloids occurred in the ethanolic extract. Flavonoids, anthraquinones and combined anthraquinones were absent in both extracts. The antimicrobial activity of the plant was carried out on *Salmonella typhi* and *Staphylococcus aureus* using ethanolic. *S. typhi* showed some sensitivity to the ethanolic extract ( $1.5 \pm 0.5$  mm) unlike the dry and fresh water extracts but much more sensitive ( $P < 0.05$ ) to chloramphenicol ( $17 \pm 0.1$  mm). However, fresh leaf water extract, dry leaf ethanolic extract and chloramphenicol showed  $2.0 \pm 0.5$ ,  $3.0 \pm 0.1$  and  $11.5 \pm 0.1$  mm bioactivity respectively against *S. aureus*. There was no indication of antimicrobial activity in the dry leaf water extract for both bacteria strains.

**Key words:** *Cnidoscolus aconitifolius*, *Salmonella typhi*, *Staphylococcus aureus*, phytochemical, bioactivity.

## INTRODUCTION

Diseases that remain most challenging in today's health-care system tend to be complex involving multiple mechanisms, targets and drugs for effective disease management. In contrast to current combination therapies, however, plant based drugs contain a mixture of multiple components thereby saving considerable time and expense (Karnath, 2002)

*Cnidoscolus aconitifolius* belong to a group of arbrescent shrubs. It is an evergreen, drought deciduous shrubs up to 6 m in height with alternate palmate lobed leaves, milky sap and small flowers on dichotomously branched cymes. The leaves are large, 32 cm long and 30 cm wide on chartaceous and succulent petioles. The crop originated as a domesticated leafy green vegetable in the Maya region of Guatemala, Belize, Southeast Mexico during pre-Cambrian period (Ibarra and Cruz, 2002). It has continued to be used as food, medicine and ornamental plant till date. Due to its ease of cultivation, potential productivity and above all its substantial nutritional value, the plant has spread all over the world including the tropics. Colloquially the plant is referred to as Chaya (Donkoh et al., 1990). In the western part of Nigeria

it is called different names such as efo lyana lpaja and efo Jerusalem.

Although the plant is mainly cultivated as food it has continued to be an important medicinal plant. Much of its recent spread into new areas may likely be attributed to its medicinal value. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from its ability to strengthen fingernails and darken gray hair to cure for alcoholism, insomnia, gout, scorpion stings, brain and vision improvement (Jensen, 1997, Atuahene et al., 1999).

In view of the reputed efficacies of this vegetable plant, this present study investigates its phytochemical constituents in an attempt to establish its most active form and antimicrobial activities against *Salmonella typhi* and *Staphylococcus aureus*.

## MATERIALS AND METHODS

### Plant material

Fresh sample of *C. aconitifolius* were collected from Staff Quarters of Babcock University, Ilisan-Remo, Ogun State Nigeria. Botanical identification was carried out at the herbarium (FHI) Forestry Research Institute of Nigeria, Ibadan, Oyo state Nigeria.

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**Table 1.** Phytochemical constituents of *Cnidioscolus aconitifolius*.

Bioactive agent	Dry Leaf water Extract	Ethanolic Extract
Alkaloids	-	+
Tannins	+	++
Phlobatannin	+	-
Saponins	+	++
Free Anthraquinone	-	-
Combined Anthraquinone	-	-
Flavonoids	-	-
Cardiac glycosides	+	+

Key: - Absence; + Presence.

### Preparation of plant extracts

Extraction was carried out with a modified method of Swain (1966). The water extractions were carried on both dried and freshly cut leaf with the use of mortar and pestle. Ethanolic extraction was carried out on the dried leaf with 96% ethanol in soxhlet extractor (Model No. 3567, Austria) for 3 h. At the end of each respective extraction, extract was filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 30°C for 25 min using a rotary evaporator (Gallenkamp UK). The resulting residue called the dried leaf extract; fresh leaf water extract and ethanolic extract respectively were transferred to a hot air oven where it was dried to a constant weight at 45°C. A portion of the residue was used to test for plant constituents while the rest was used for the bacterial susceptibility test.

### Phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Sofowora (1993) method. The test for tannins was carried out by subjecting 3 g of each plant extract in 6 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For cardiac glycosides, Keller-Kiliani test (Trease and Evans, 1989) was adopted (0.5 g of extract was added to 2 ml acetic anhydride plus H<sub>2</sub>SO<sub>4</sub>). The test for alkaloids was carried out by subjecting 0.5 g aqueous extract in 5 ml 1% HCl, boiled, filtered and Mayer's reagent added (Harborne, 1973, Trease and Evans, 1989). The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the frothed extracts in water to remove false positive results (Sofowora, 1993). The extract was also tested for free glycoside bound anthraquinones (Wall, 1952; Sofowora, 1993). Five grams of extract was added to 10 ml benzene, filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnins, and potassium hydroxide solution (Kapoor, 1969; Earnsworth et al., 1974).

### Test organisms

Pure cultures of bacteria were collected from the University College Hospital Ibadan (UCH), Ibadan. They were confirmed and standardized as *S. typhi* and *S. aureus*.

### Bacterial susceptibility testing

The sensitivity testing of the extracts were determined with slight modification using disc diffusion method (Bauer et al., 1966). A

standardized inoculum ( $1-2 \times 10^7$  cfu/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. A sterile paper disc previously soaked in a known concentration of extract (20 mg/ml per disc) was carefully placed at the centre of the triplicate labeled seeded plate. The plates were later incubated at 37°C for 24 h after which they were observed for zones of inhibition. The inhibition zones were measured with a ruler and compared with the control disc containing standard antibiotic chloramphenicol at a concentration of 30 µg.

## RESULTS AND DISCUSSION

The phytochemical analysis carried out on the dry leaf water extract and ethanolic extract showed the presence of some bioactive compounds in the plant. In the two forms of extract, eight bioactive constituents were tested for, out of which only three were present in the two extractions (Table 1). Analysis of tannins in the two extracts was positive but higher colour intensity was observed in the ethanolic extract than the dry leaves water extract. Presence of tannins suggests the ability of this plant to play a major role as antidiarrhoeic and antihaemorrhagic agent (Asguith and Butler, 1986). Saponins though positive for both extracts, persistent frosting was intense in the ethanolic extract than the dry leaf water extract. This compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1985., Price, 1987). Hence this plant could be suitable for these purposes. Cardiac glycosides showed positive results for both the ethanolic and dry leaf water extracts with no clear intensity indication in both extracts. The cardiac glycosides have been used for over two

**Table 2.** Sensitivity of *Salmonella typhi*.

Extract	Zone of Inhibition
Ethanolic Extracts	1.5±0.5
Dry Leaf Water Extract	-
Fresh Leaf Water Extract	-
Chloramphenicol	17±0.1

**Table 3.** Sensitivity of *Staphylococcus aureus*.

Extracts	Zone of inhibition (mm)
Ethanollic	3.0 ± 0.1
Dry leaf water extract	-
Fresh leaf water extract	2.0 ± 0.5
Chloramphenicol	11.5 ± 0.1

centuries as stimulants in cases of cardiac failure (Trease and Evans, 1985; Olayinka et al., 1992). This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension.

It was also found that alkaloids were only present in ethanolic extracts while absent in the dried leaf water extract. The main reason that can be adduced for this is the mode of extraction. On this premise it will be advisable to extract the leaf of *C. aconitifolius* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for these purposes (Trease and Evans, 1978; Zee-cheng, 1997). However phlobatannin was found to be positive only in the dried leaf extracts while in the ethanolic extract, this test was negative. This is also an indication that this compound can only be derived with the dry leaf water extract of the plant. The presence of phlobatannins suggests the diuretic property of the plant (Okuda, 1991). The foregoing would suggest the possible utilization of *C. aconitifolius* as diuretic agent. It was not really surprising that flavonoids were found absent for both forms of extracts. The sole greenish appearance of this leafy vegetable substantiates the fact that flavonoids contribute to the brilliant multi color for most plants (Sofowora, 1993). Also, free anthraquinone and combined anthraquinone were also not found in all the forms of extracts. Though the therapeutic applications of these metabolites are vaguely understood (Trease and Evans, 1985; Sofowora, 1993). Thus the absence may not be a minus for the medicinal efficacies of *C. aconitifolius*.

During the antibacterial susceptibility test it was observed (Table 2 and 3) that the control drug chloramphenicol showed the largest and significance ( $P < 0.05$ ) zone of inhibition compared to all other extracts on the two bacterial investigated in this study. The ethanolic extract was only able to inhibit *S. typhi*, while the dry and fresh leaf water extract showed no sensitivity on the organism. However, the fresh leaf water and ethanolic extracts showed inhibitions on *S. aureus* while the dried leaf water extracts showed no inhibition. The ability of *C. aconitifolius* showing sensitivity to two different strains of bacteria (gram positive and gram negative bacteria) shows its application as a broad spectrum antimicrobial agent with the largest efficacy being the ethanolic extract form in this study.

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