

Full Length Research Paper

Phytochemical constituents, antimicrobial and antioxidant potentials of tree spinach [*Cnidoscolus aconitifolius* (Miller) I. M. Johnston]

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Ethanol extracts of leaves, stem and root of *Cnidoscolus aconitifolius* (Miller) I. M. Johnston, were screened for their phytochemical constituents and also investigated for their antimicrobial and antioxidant activities. Phytochemical screening revealed the presence of secondary metabolites of both preventive and curative importance in medicine. All the plant parts (leaf, stem and root) showed activity against *Escherichia coli* and *Bacillus subtilis* while the stem showed the broadest spectrum of activity against the different strains of bacteria used. Although the antioxidant activity of the extract [determined using 2,2-Diphenyl-picryl-1-hydrazyl radical (DPPH)] is less potent in comparison with vitamin C, however, it showed increase in activity with increased concentration.

Key words: Phytochemical, antimicrobial, antioxidant and *Cnidoscolus aconitifolius*.

INTRODUCTION

In developing countries, millions of people depend on wild resources, including wild medicinal and edible plants for their healthcare and to meet dietary needs (Balick and Cox, 1996; Balemie and Kebebew, 2006). The tendency of populations in developing countries to favor traditional medicinal plants is mainly due to the inaccessibility of modern medical care, as well as economic and cultural factors (Abbiw, 1996). Rural and urban populations in some parts of West Africa use certain plant species for therapeutic and dietary purposes. Among the plants traditionally used by people with scarce economic resources, is a cultivated plant belonging to the Euphorbiaceae family (Baustica-Cruz et al., 2011); this family includes *Cnidoscolus aconitifolius* (Miller) I. M. Johnston, which is also frequently consumed. *C. aconitifolius*, known as tree spinach (English), *efo iyana ipaja*, or *efo Jerusalem* (Yoruba) is commonly found growing in the Western part of Nigeria. It is an ornamental, evergreen, drought deciduous

shrub of 3 to 5 m tall (Kuti and Torres, 1996). The palmate lobed leaves are large, 32 cm long and 30 cm wide alternately arranged on chartaceous and succulent petioles (Figure 1). The crop originated as a domesticated leafy green vegetable in the Maya region of Guatemala, Belize and Southeast Mexico during pre-Cambrian period (Ross-Ibarra and Molina-Cruz, 2002). It is cultivated in domestic gardens rather than in agricultural fields, and as such can be used throughout the year.

Despite the widespread use of this plant across the states, scientific literature is yet to fully investigate the traditional uses and nutritional values of these species. However, there are some reports that describe the nutritive values of *C. aconitifolius* (Kuti and Konoru, 2004). *C. aconitifolius* was found to be mainly valued as a food source; nonetheless, it was and continues to be an important medicinal plant (Ross-Ibarra and Molina-Cruz, 2002).

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Figure 1. Aerial view of *Cnidocolus aconitifolius*.

It is a widely distributed annual plant, ranging from temperate to tropical zones, and has a long history of use as both a medicinal and an edible plant (Nebel and Heinrich, 2009). It has certain antibacterial properties, as well as a contraceptive effect (Dong et al., 2010). It has been observed in use as diuretic, circulation and lactation stimulants, and has also been recommended for diabetes, obesity, acne, kidney stones and eye problems (Rowe, 1994).

Research has shown that *C. aconitifolius* is rich in natural antioxidant (Kuti and Konoru, 2004), which scavenges free radicals. Many chronic diseases and causes of food spoilage are linked to pro-oxidants. Antioxidant components are therefore useful in food preservation and drug formulations (Loliger, 1991). Synthetic antioxidants

like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are suspected to be tumorigenic (Ito et al., 1985). Therefore, there is a need to search for potential antioxidant compounds, especially from herbs, that can replace their synthetic counterparts. An expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified compounds with antioxidant properties have substantial protective effects against free radical associated diseases (Tsao et al., 2004).

In view of the reputed efficacies of this plant, this study aims at evaluating the phytochemical components present in the plant, the antioxidant (radical scavenging) and antimicrobial activities with a view to exploiting its activities

for improving of human health.

MATERIALS AND METHODS

Plant

Fresh leaves, stem and root of tree spinach (*C. aconitifolius*) were collected from Gwagwalada in Gwagwalada Area Council of the Federal Capital Territory (FCT) of Nigeria. Botanical identification was carried out at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD/H/6549) was deposited at the Institute's herbarium. The samples were washed with water and air dried under controlled conditions to avoid too many chemical changes occurring. The dried leaves, stem and root were separately pulverized and the resulting powdered samples used for further analysis.

Extraction procedure

The pulverized plant samples (250 g of each) were macerated with absolute ethanol in an aspirator bottle at room temperature for 48 h. The extracts were obtained following concentration of the filtrates in a rotary evaporator under reduced pressure at 40°C. The extracts were allowed to dry at atmospheric pressure until constant weight was achieved thus obtaining yields between 6.68 and 19.72%.

Phytochemical screening

The ethanol extracts of leaf, stem and root of *C. aconitifolius* were screened for the presence of phytochemical constituents such as alkaloids, terpenoids, anthraquinones, flavonoids, tannins, saponins, steroids and glycosides using qualitative phytochemical screening tests described by Sofowora (1993) and Trease and Evans (1989). To test for alkaloids, 0.5 g aqueous extract in 5 ml 1% HCl was boiled, filtered and Mayer's reagent was added to the filtrate (Harborne, 1973; Trease and Evans, 1989). The presence of flavonoids was determined using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution (Kapoor et al., 1969; Earnsworth et al., 1974). The extract was subjected to Frothing test as a preliminary test for the identification of saponin and haemolysis test was carried out on the frothed extracts for confirmation (Sofowora, 1993). The test for tannins was carried out by adding ferric chloride solution to the filtrate obtained from extracting 3 g of each plant extract in 6 ml of distilled water. For cardiac glycosides, Killer- Killani test (Trease and Evans, 1989) was adopted whereby 0.5 g of the extract was treated with 2 ml glacial acetic acid containing a drop of ferric chloride solution and 1 ml of concentrated H₂SO₄. Test for phlobatannins was carried out when about 0.5 g of the plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. To test for steroid, 2 ml of acetic anhydride was added to 0.5 g of methanol extract of the sample with 2 ml H₂SO₄.

Antimicrobial test

Antimicrobial activities of ethanol extracts of the leaf, stem and root of *C. aconitifolius* were carried out using the agar diffusion method. To 20 ml of cooled molten agar was added 0.2 ml of an overnight broth culture of test micro-organisms (the stock was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing). It was well mixed, then poured into a sterile Petri-dish and allowed to set. Thereafter, cups (9 mm diameter) were bored aseptically into the solid nutrient agar

using a sterile cork borer. The test solutions of the extracts at concentration of 20 mg/ml were then introduced into each of the designated cups on each plate (using sterile hypodermic syringes for each test solution) ensuring that no spillage occurred. The same amount of the standard antimicrobial agents and solvent were introduced into the remaining cups on each plate to act as positive and negative controls, respectively. The plates were left at room temperature for 1 h, to allow the test solutions diffuse into the medium, turned upside-down and thereafter incubated at 37°C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimetre (Villanueva et al., 2009).

Antioxidant activity

The free radical-scavenging activity of tree spinach's stem extract was evaluated by assessing its discoloration of 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) in methanol by a slightly modified method of Brand-Williams et al. (1995). The following concentrations of the extract were tested (0.05, 0.1, 0.5, 1.0, 2.0, and 5.0 mg/ml). The decrease in absorbance was monitored at 514 nm. Vitamin C was used as the antioxidant standard at concentrations (0.02, 0.05, 0.1, 2.0, 0.5, and 0.75 mg/ml).

One milliliter of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM of DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100$$

where A = the absorption of the blank sample without extract; B = the absorption of the extract.

RESULTS

Qualitative analysis of phytochemicals

Phytochemical analysis carried out on the ethanol extracts of tree spinach leaf, stem and root showed the presence of tannins, saponins, cardiac glycosides, terpenoids and alkaloids in all the plant parts; steroid is present only in the leaf extract while phlobatannins and flavonoids were not detected (Table 1).

Antibacterial activity

The antibacterial activity was assessed by the measurement of inhibition zones according to the parameters suggested by Alves et al. (2000): inhibition zones < 9 mm, inactive; 9 to 12 mm, less active; 13 to 18 mm, active; > 18 mm, very active.

The leaf extract was less active against *Klebsiella oxytoca* and *Escherichia coli* while the stem extract is less active against *K. oxytoca*; the root, leaf and stem extracts were active against *Proteus* species, *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. The stem and root extracts were very active against both *B. subtilis* and *E. coli* (Table 2). Although, the minimum

Table 1. Phytochemical screening of *Cnidoscopus aconitifolius* (Miller) I.M. Johnston.

Test	Leaf	Stem	Root
Steroids	+	-	-
Phlobatannins	-	-	-
Flavonoids	-	-	-
Tannins	+	+	+
Alkaloids	+	+	+
Cardiac glycoside	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+

(+) Present; (-) absent.

Table 2. Zone of inhibition (mm) of the antimicrobial activity of the extracts of *Cnidoscopus aconitifolius* (Miller) I. M. Johnston to the test organisms at 20 mg/well.

Extract	<i>K. oxytoca</i>	<i>Proteus</i> spp.	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Roots	NI	15	32 (static)	24	NI
Leaves	12	NI	14.5 (static)	12	NI
Stems	12	NI	21 (static)	23	14.5

NI= No inhibition

Table 3. Zone of inhibition (mm) of the antimicrobial activity of commercial antibiotics to the test organisms at specified µg/well.

Test organism	TET (10 µg)	GEN (25 µg)	COT (10 µg)	CHL (30 µg)
<i>K. oxytoca</i>	NI	12	NI	10.5
<i>Proteus</i> spp.	NI	8	NI	NI
<i>B. subtilis</i>	NI	15	NI	22.5
<i>E. coli</i>	NI	NI	NI	7
<i>P. aeruginosa</i>	20	23	27	24

NI = No inhibition; TET =Tetracycline (10 µg); GEN = Gentamycine (10 µg); COT = Contrimoxazole (25 µg); CHL= Chloramphenicol (30 µg).

Table 4. Antioxidant activity of the stem extract of *Cnidoscopus aconitifolius* (Miller) I. M. Johnston.

Concentration (mg/ml)	Inhibition (%)
5.0	57.13
2.0	24.87
1.0	15.39
0.5	8.71
0.1	8.23
0.05	2.92

inhibitory concentration (MIC) was not determined; the root extract showed the highest activity while the stem extract showed the broadest spectrum of activity against the strains of bacteria used. All the extracts showed broader spectrum of activity than two (Tetracycline and Contrimoxazole) of the commercial antibiotics (Table 3).

Antioxidant activity

The results of the qualitative antioxidant analysis of the stem extract showed that *C. aconitifolius* contained natural antioxidants. The sample showed increasing level of antioxidant activity with increased extract concentration (Table 4).

DISCUSSION

Phytochemical analysis carried out on the tree spinach extracts showed the presence of tannins, saponins, cardiac glycosides, terpenoids and alkaloids; this is in consonance with previous reports (Awoyinka et al., 2007; Yakubu et al., 2008). The absence of flavonoids is in complete contrast to recent reports by Peixoto Sobrinho et al. (2012), of high frequency of flavonoids especially in aerial parts of *Cnidoscopus* species and Yuan et al.

Table 5. Antioxidant activity of vitamin C (ascorbic acid).

Concentration (mg/ml)	Inhibition (%)
0.75	91.41
0.50	91.32
0.20	91.50
0.10	90.47
0.05	90.19
0.02	85.00

(2007) who reported isolation of fifteen flavonoids from *Cnidocolus texanus*; however, it is in agreement with the report by Awoyinka et al. (2007). Similarly, phlobatanins were not detected in all the plant parts probably due to solvent effect as reported by Awoyinka et al. (2007). The healing and anti-inflammatory activities popularly attributed to *Cnidocolus* spp. are strongly associated with its tannin content (Araújo et al., 2008). Hence, this plant could be suitable for these purposes. Trease and Evans (1989) and Olayinka et al. (1992) reported the use of cardiac glycosides for over two centuries as stimulants in cases of cardiac failure which perhaps justifies the already locally established function of the plant in the treatment and management of hypertension. Price (1987) and Trease and Evans (1989), have shown saponins to have immense significance as antihypercholesterol, hypotensive and cardiac depressant agent suggesting the suitability of this plant in this respect.

The antimicrobial investigation carried out on the crude ethanol extracts of all the plant parts (root, stem and leaves) showed a broad spectrum of activity as presented in Table 2. All the extracts showed appreciable activity against *B. subtilis* and *E. coli* while the stem exhibited the broadest spectrum of activity against the different strains of bacteria used.

All the extracts showed more effectiveness than some of the commercial antibiotics (Tetracycline and Cotrimoxazole) and compete favourably with others (Gentamycin and Chloramphenicol) 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 a very broad spectrum of activity. The result of qualitative antioxidant activity shows that the plant exhibit potent antioxidant activity; the presence of tannins according to Polterait (1997) is likely to be responsible for the free radical scavenging effects observed. Similarly, saponins have been implicated to exhibit antioxidant activity by reducing the levels of lipid hydroperoxides (Rodrigues et al., 2005). The saponins present in the plant extract may thus be responsible for the reduction in the level of malondialdehyde (MDA). The DPPH test provides information on the reactivity of the test compound with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of

free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the degree of reduction in absorbance measurement is an indication of the radical scavenging [antioxidant] power of the extract. The percent inhibition of the extract increases as the concentration of the extract increases. However, the extract is less potent [8.71% at 0.5 mg/ml (Table 4)] when compared with vitamin C [91.32% at 0.5 mg/ml (Table 5)].

Conclusion

This study revealed the ability of *C. aconitifolius* to inhibit both Gram positive and Gram negative bacteria; thereby suggesting that it could serve 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. It was also shown that there is increase in antioxidant activity of the extract with concentration; this can serve as a guide to search for compounds with antioxidant activity. Thus, identification of the chemical constituents responsible for the antioxidant activities of the plant species may lead to the development of new excellent alternative natural antioxidant.

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