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Short Communication

The effect of *Cnidoscolus aconitifolius* on multi-drug resistant micro-organisms

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This study was carried out to investigate possible bacteriostatic and bactericidal activities of *Cnidoscolus aconitifolius* leaf extract on multi-drug resistant micro-organisms. The antimicrobial property of *C. aconitifolius* leaf extract was carried out on the following multi-drug resistant micro-organisms; *Escherichia coli*, *Klebsiella species, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enterica* Gallinarum and *Candida albicans.* The results from this study show that none of the multi-drug resistant micro-organisms was sensitive to the leaf extract of *C. aconitifolius*.

Key words: *Cnidoscolus aconitifolius*, bioactivity, multi-drug resistant micro-organisms.

INTRODUCTION

Cnidoscolus aconitifolius is a perennial shrub belonging to the family *Euphorbiaceae*. It is commonly found in the tropic and sub tropical regions worldwide, including Africa, North and South America, India, etc. It is commonly eaten as vegetable in soup (Ganiyu, 2005) in Southwestern Nigeria where it is called 'Iyana Ipaja'. The edible parts of the plant, which taste like spinach when cooked, serve as important nutritional source of protein, vitamin (A and C), minerals (calcium, iron, and phosphorus), niacin, riboflavin and thiamine for populations that cannot afford expensive foods rich in these nutrients (Yang, 1979). *C. aconitifolius* shoots and leaves have been taken as laxative, diuretic and circulatory stimulant, to improve digestion, stimulate lactation, and harden the fingernails (Rowe, 1994).

The high fibre content and antibacterial activities of this plant have been reported by various authors including Sarmiento-Franco et al. (2002) and Awoyinka et al. (2007). The anti-diabetic properties of leaf extract of *C. aconitifolius* in inbred type 2 diabetic mice has also been reported (Oladeinde et al., 2007). Recently, the ameliorative effects of *C. aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition have been reported (Oyagbemi et al., 2008). The current study was carried out to determine the antimicrobial activities of the aqueous leaf extract of *C. aconitifolius* on some multi-drug resistant clinical bacteria isolates and a laboratory stock fungus.

MATERIALS AND METHODS

Collection of plant materials

Fresh matured leaves of *C. aconitifolius* were collected at the University Teaching Hospital, College of Medicine, Ibadan, Nigeria and were identified and authenticated at Department of Botany and Microbiology, University of Ibadan. The leaves were air-dried, reduced to powder and were kept separately in airtight containers until the time of use.

Extraction of plant material

Air-dried powder (1 kg) of fresh matured *C. aconitifolius* leaves was extracted by percolation at room temperature with 70% ethanol (EtOH) and later concentrated under reduced pressure (bath temperature 50°C) and finally evaporated to dryness. The dried mass yield obtained was 62.5 g.

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Table 1. Antimicrobial activities of leaf extracts of *C. aconitifolius* to the micro organisms tested.

Organism	Source	Resistant to	Antimicrobial activities of the extract
Escherichia coli	UCH091	Ci, cot, amx, tet, ca, chl, cf, gen, nal, aug and cxc	No activity
Escherichia coli	VTH0919	Cf, cef, nor, tet, amp, gen and ofl	No activity
Escherichia coli	VTH0911	Cef, tet, amp and nal	No activity
Klebsiella species	UCH092	Amp, amx, cot, nit, nal, ofl, aug and tet	No activity
Staphylococcus aureus	UCH093	Chl, amx, ery and gen	No activity
Pseudomonas aeruginosa	UCH 094	Aug, nal, cf, ca, gen and sf	No activity
Salmonella enterica	VTH096	Cf, gen, ery, pen, cxc, amx and tet	No activity
Candida albicans	PMUI lab. stock		No activity

Ci = Ceftriaxone; cot = cotrimoxazole; amx = amoxylin; tet = tetracycline; ca = ceftazidime; chl = chloramphenicol; cf = ciprofloxacin; gen = gentamycin; nal = nalidixic acid; aug = augumentin; Cxc = cloxacillin; cef = cefuroxime; amp = ampicillin; nit =nitrofurantoin; ofl = ofloxacin; ery = erythromycin; pen = penincillin G; sf =sulfisoxazole. UCH = University College Hospital, Ibadan; VTH = Veterinary Teaching Hospital, University of Ibadan; PHUI = Pharmaceutical Microbiology, University of Ibadan.

Microorganism used for the study

The microorganisms used for the study include three strains of multidrug resistant *Escherichia coli*, one *Klebsiella species*, one *Staphylococcus aureus*, one *Pseudomonas aeruginosa*, one *Salmonella enterica* Gallinarum and one laboratory stock of *Candida albicans.*

Antibiotic sensitivity test

The *in vitro* antibiotic sensitivity tests were carried out using conventional antimicrobial agents, namely: Sparfloxacin (5 mcg), chloramphenicol (30 mcg), gentamycin (10 mcg), amoxicillin (30 mcg), cloxacillin (10 mcg), co-Trimoxazole (25 mcg), cefuroxime (30 mcg), nalidixic acid (30 mcg), penincilin (5 units), ampicillin (25 mcg), tetracycline (30 mcg), ofloxacin (5 mcg), ciprofloxacin (5 mcg), norfloxacin (10 mcg), nitrofurantoin (100 mcg), ceftazidime (30 mcg), ceftriaxone (30 mcg) and sulfisoxazole (300 mcg) as described by Walton (1972) and modified by Adetosoye (1984). The isolates were multidrug resistant clinical isolates from University College Hospital, Ibadan and from Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ibadan having the resistant patterns shown in Table 1. Each of the isolates was inoculated into 5 ml sterile nutrient broth and incubated at 37°C for 8 h. A 1:2000 dilution of the culture was made with sterile nutrient broth, while the mixture was shaken vigorously. Subsequently, a diagnostic sensitivity test agar plate was inoculated by flooding with the 1:2000 diluted mixture of the culture. The excess fluid was drained off and the plate was allowed to stand on the bench for 1 h after which antibiotic sensitivity discs of the respective antibiotic discs (Fidson Healthcare LTD.6 Ilupeju Bypass, Ilupeju, Lagos, Nigeria) were aseptically applied. The test plates were allowed to stand on the bench for 1 h to allow the antimicrobial agents to diffuse into the agar. The plates were then incubated at 37°C for 18 h after which the results were recorded. The zone of inhibitions around each antibiotic disc in each test was compared with the corresponding zone of inhibition around the antibiotic disc for the control organism.

Determination of antimicrobial activities of leaf extracts of *C. aconitifolius*

The antimicrobial activity of the extracts was determined using agar diffusion technique as earlier described by Adeniyi et al. (1996). Mueller Hinton agar plates were seeded each with 0.1 ml of a 1:100 dilution of tryptose soy broth (TSB) overnight culture of each clinical bacterial isolates. Sabouraud agar was carpeted with the fungi isolates. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 6 mm diameter was used to cut uniform well on the surface of the agar, 0.2 ml of the extract re-suspended in distilled water at a concentration of 20 mg/ml was added to the bored hole on the agar. The Mueller Hinton agar seeded with the bacteria, were incubated at 37°C for 24 h, while the Sabouraud agar was carpeted with the fungi isolates were incubated at 250C for three(3) days. Subsequently, the diameter of the zone of inhibition was measured. The distilled water used as solvent was included in each of the plates as solvent control, while conventional antibiotic discs were used as chemo-therapeutic control.

RESULTS AND DISCUSSION

Results from Table 1 show the resistant patterns of *E. coli*, *Klebsiella* species*, Staph. aureus, P. aeruginosa* and *Salm. enterica* Gallinarum to the conventional antimicrobial agents. Also, the leaf extracts of the *C. aconitifolius* did not show any activity to the multidrug resistant micro organisms tested as shown in Table 1.

In an earlier work, Awosika et al. (2007) reported the antimicrobial activities of the various extracts of *C. aconitifolius*. They observed that the control drug chloramphenicol showed the largest and the most significant ($P < 0.05$) zone of inhibition (11.5 ± 0.1 mm) for *Staph. aureus* and (17 ± 0.1 mm) for *Salm. enterica*, compared to all other extracts on the two bacteria investigated in the study. The ethanolic extract was able to inhibit *Salm. enterica* within 1.5 ± 0.5 mm, while the dry and fresh leaf water extract showed no sensitivity on the organism.

It was further reported that, the fresh leaf water and ethanolic extracts produced zones of inhibitions for *Staph. aureus*, while the dried leaf water extracts did not. The ethanolic extract was found to produce an inhibition zone of 3.00 mm ± 0.1 mm for the *Staph. aureus* isolate

tested. Based on the 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 with better efficacy from the ethanolic extract of the plant.

The problem of drug resistance is a current global public health problem. For example, an increase in antimicrobial resistance to conventional antimicrobial agents in non-typhoidal *Salmonella* serotypes has been demonstrated since the early 1990's and has grown to become a global problem (Alcaine et al., 2007). Recently, Ogunleye et al. (2008), reported occurrence of multidrug resistant *E. coli* of poultry origin in two Southwestern states of Nigeria. Observation from the work revealed that thirty nine *E. coli* isolates were recovered from the two hundred and fifty samples of intestine, kidneys, lungs, hearts, ovary, spleen and colorectum from diseased chickens that were examined. Each of the thirty nine isolates was resistant to 5 - 12 antimicrobial agents.

It has been noted that occurrence of antimicrobial resistance among bacteria pathogens usually makes the right choice of empirical antimicrobial treatment by clinicians more difficult (Hubert et al., 1998; Walker, 2000).

The continued search for new antimicrobial agents from natural products like plants is thus very relevant. Based on the earlier report of antimicrobial activities of the ethanolic fresh leaf extract of *C. aconitifolius* by Awosika et al. (2007), this study was carried out on the multidrug resistant isolates. The results from the current work however, showed that the aqueous suspension of the ethanolic leaf extracts of *C. aconitifolius* did not produce antimicrobial activity for any of the seven multidrug resistant clinical bacteria isolate and one laboratory stock fungus used for this study. This finding is contrary to the report of Awosika et al. *(*2007). The observation in the current work may be attributed to the multidrug resistance nature of the clinical bacteria isolates obtained from both human and animals used for this study. From Table 1, it is observed that some of the isolates were resistant to wide range of conventional antimicrobial including the cephalosporin and fluoroquinolones group of drugs. It is imperative to take a decisive step in terms of policy and its enforcement in Nigeria to curtail indiscriminate abuse and misuse of conventional antibiotics that usually results in development of antimicrobial resistance. This becomes important because of the cost and long processes involved in the development of new, acceptable and safe antimicrobial agent.

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