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Auspicious Antimicrobial Potentials of Ethanol Extracts of Stem and Root of Cnidoscolus Aconitifolius

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Abstract: *More than 50,000 lives are claimed by antimicrobial-resistant (AMR) infections annually across Europe and the US alone, with numerous hundreds of thousands more dying in other parts of the world. The destructive effects of this growing worldwide menace have led to a search for suitable and affordable alternative antimicrobial agents of plant origin. This study aimed at examining antimicrobial activity and phytochemical constituents of C. aconitifolius (CA). The antimicrobial screening of the plant extracts at different instances showed higher activity (ranging from 42% to 343%) against the test organisms than the commercial antibiotics used as standards. Phytochemical assessment showed the presence of secondary metabolites of high repute in both prophylactic and remedial activities in medicine.*

Keywords: *antimicrobial potentials, Cnidoscolus aconitifolius, auspicious, ethanol extracts*.

1. INTRODUCTION

Over the years in different parts of the world (developing countries inclusive) there have been reports of increasing prevalence of resistance in many pathogens [1]. To guarantee their survival against the arsenal of antimicrobial agents to which they were being barraged, microorganisms were progressively becoming resistant. The resistance mechanisms are multifaceted depending on which specific pathways are inhibited by the drugs. Sosa et al., (2010) reported that it is primarily based on modification of the structure and the reaction pathway of the antimicrobial agent so as to circumvent the inhibition by the agent in order to survive [2]. Cooper and Shlaes reported the limited ability of the pharmaceutical sector in the 21st century to dependably provide new drugs to the clinic to address the resistance-mediated desuetude of old drugs [3]. In answering the question of where the new antibiotics needed to address the growing problem of resistance will come from, Fernebro [4] prescribed that both conventional and non-conventional approaches are desirable to address this persistent medical problem.

As part of the non-conventional approach, this study is conducted to explore the antimicrobial activity of CA, a medicinal plant with reputation for wide variety of claims for therapeutic effectiveness [5]. *Cnidoscolus aconitifolius* (Miller) I.M. Johnston of the family Euphorbiaceae is a fast growing perennial plant. It is commonly known as tree spinach (English). Its probable origin is the Yucatán Peninsula of Mexico [6]. The plant has a stretched history of use for both therapeutic and comestible purposes. The Cnidoscolus genus consists of more than 40 species [7] and has a wide range of distribution spreading from temperate to tropical zones [8]. It is of high medicinal significance; it has numerous claims for treatment of insomnia, gout and alcoholism [9, 10], it serves also in boosting low blood volume, lowering blood cholesterol, management and treatment of Diabetes mellitus [11].

Despite the high therapeutic significance of this plant there is yet a supposed dearth of scientific information on its antimicrobial potentials. The aim of this study therefore, is to investigate the antimicrobial activities of CA with a view to exploring its potentials for possible development of highly potent, non-toxic, broad-spectrum, and inexpensive antibiotic drugs.

2. MATERIALS AND METHODS

Fresh samples of *C. aconitifolius* (leaves, stems and roots) were collected from a farmland in Gwagwalada Area Council, FCT-Abuja, Nigeria. Taxonomical identification and authentication was carried out at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Herbarium specimen (NIPRD/H/6549) was deposited at the Institution's herbarium. The air-dried plant materials (leaf, stem and root) were pulverized for subsequent use.

2.1. Extraction of Plant Material

Extraction of the powdered air-dried leaves of *C. aconitifolius* was carried out using the method described by [12], by macerating 500 g of the sample in 2 litres of absolute ethanol at room temperature for 48 h. This procedure was repeated three times and the combined extract evaporated under vacuum at 40°C.

2.2. 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 Trease & Evans and Sofowora [13, 14].

2.3. Antimicrobial Test

Using the agar diffusion method as described by Villanueva *et al.* [15], the antimicrobial activities of ethanol extracts of the leaf, stem and root of CA were investigated. The cooled molten agar was inoculated with 0.2 ml of an overnight broth culture of test micro-organisms and mixed properly. Into the set poured plates were cups (9 mm diameter) aseptically bored? The test solutions of the extracts (20 mg/ml) were then introduced into each of the selected cups on each plate. Equivalent amount of the standard antimicrobial agents (positive control) and solvent (negative control) were introduced into the remaining cups on each plate. For proper diffusion of the test solutions, the plates were left at room temperature for 1 h, followed by incubation at 37°C for 24 h. Resulting clear zones of inhibition were recorded. The experiment was carried out in triplicate.

2.4. Comparison of Antimicrobial Activity

The antimicrobial activity of each of the extracts in relation to that of each of the standard drugs against each of the test organism was calculated using the formula below:

$$\text{Relative activity} = \frac{\text{Zone of inhibition by plant extract}}{\text{Zone of inhibition by standard drug}} \times 100\%$$

This comparison excludes situations where either the extract or the standard drug fails to show antimicrobial activity against an organism and are represented by ES in Tables 2 & 3.

3. RESULTS AND DISCUSSION

Table1. *Zone of inhibition (mm) of the antimicrobial activity of the extracts of CA to the test organisms at 20mg/well and of standard antibiotics at specified µg/well*

ORGANISMS	EXTRACTS			STANDARD ANTIBIOTICS	
	Leaves	Stem	Root	Gent. (25µg)	Chlo. (30µg)
B. subtilis	14.5	21	32	15	22.5
E. coli	12	23	24	NI	7
K. oxytoca	12	12	NI	12	10.5
Proteus spp	NI	NI	15	8	NI
P. aeruginosa	NI	14.5	NI	23	24

Note: *NI = No inhibition, Gent. = Gentamycin, Chlo. = Chloramphenicol*

Table2. *Percentage antimicrobial activity of each of the extracts in relation to activity of Gentamycin (%)*

	Bs	Ec	Ko	Ps	Pa
Leaf	96.7	ES	100	ES	ES
Stem	139.9	ES	100	ES	63.0
Root	213.3	ES	ES	187.5	ES

Key: *Bs = B. subtilis, Ec = E. coli, Ko = K. oxytoca, Ps = Proteus spp., Pa = P. aeruginosa, ES = Excluded situation*

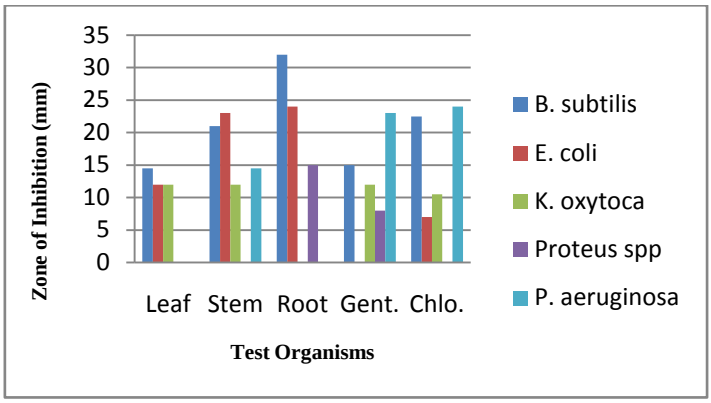

Fig1. Showing antimicrobial activity of the extracts of *CA* and standard antibiotics

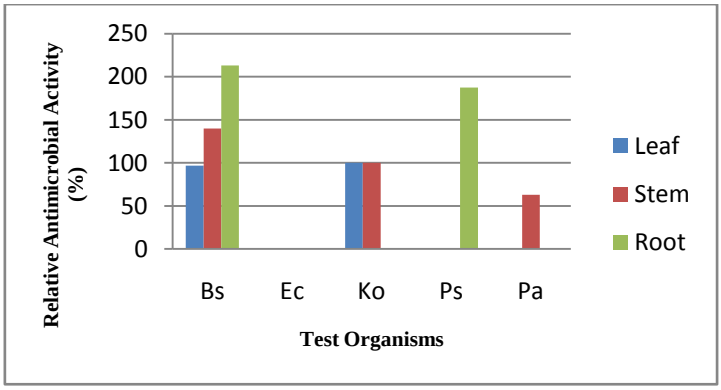

Fig2. *Percentage antimicrobial activity of each of the extracts in relation to activity of Gentamycin (%)*

Table3. *Percentage antimicrobial activity of each of the extracts in relation to activity of Chloramphenicol (%)*

	Bs	Ec	Ko	Ps	Pa
Leaf	64.4	171.4	114.3	ES	ES
Stem	93.3	328.6	114.3	ES	60.4
Root	142.2	342.9	ES	ES	ES

Key: *Bs = B. subtilis, Ec = E. coli, Ko = K. oxytoca, Ps = Proteus spp., Pa = P. aeruginosa, ES = Excluded situation*

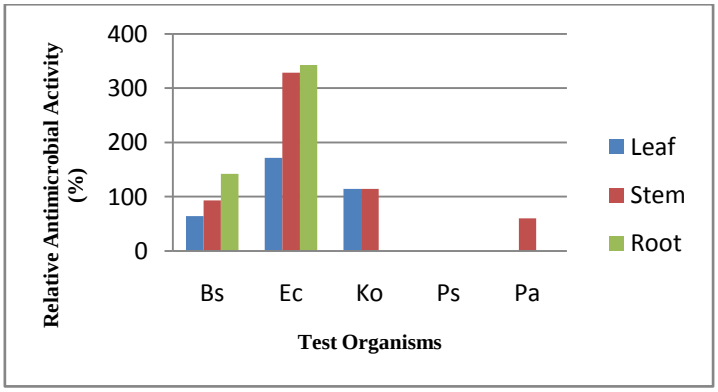

Fig3. *Percentage antimicrobial activity of each of the extracts in relation to activity of Chloramphenicol (%)*

Table4. *Phytochemicals from C. aconitifolius*

	Extracts		
Phytochemicals	**Leaf**	**Stem**	**Root**
Alkaloids	+	-	+
Cardiac glycosides	+	-	+
Flavonoids	-	-	-
Phlobatanins	-	-	-
Saponins	+	+	+
Steroids	+	-	-
Tannins	+	-	-
Terpenoids	+	+	+

Key: + = *present,* - = *absent*

From table 1 and figure 1, the root extract in particular showed greater activity against *B. subtilis, E. coli* and *Proteus spp*. than the two standard antibiotics used. This higher activity ranges from 42% to 343% (Tables 2 and 3). The leaf and stem extracts showed activity similar to that of Gentamycin (Fig. 2) and greater than that of Chloramphenicol (Fig. 3) against *K. oxytoca* which the root extracts showed no inhibition. The trend of the antimicrobial activity of the extracts isroot > stem > leafas shown in Table 1. It may probably be concluded from this result that the concentration of the antimicrobial agent decreases as one move from the root to the leaf in the plant. Also it might be possible to experience a synergistic effect if whole plant extract instead of individual plant parts have been used. This supposition can be supported by the fact that *K. Oxytoca* and *P. aeruginosa* against which the root extract showed no activity were inhibited by other plant parts' extracts. The growth of *K. oxytoca* was inhibited by extracts from both the leaf and stem extracts while the stem extract showed activity against *P. aeruginosa* (Table 1).

The therapeutic properties of plants are perhaps due to the presence of various secondary metabolites which are the non-nutritive plant compounds. These classes of compounds (such as alkaloid, tannin, anthraquinone and flavonoid) are known to have curative activity against several pathogens and therefore could suggest their use traditionally for the treatment of various ailments[16, 17].Tannin has been found to possess astringent properties, hasten the healing of wounds and inflamed mucous membranes [18]. Tannin and flavonoid are believed to be responsible for anti-diarrhea activity [19]. Some phytochemicals are known to have antimicrobial activity. It has been reported that tannins have antibacterial property [20].In contrast to this previous report, tannins were not detected in the present study in the stem and root extracts of the plant yet they exhibited higher antimicrobial activity than the leaf extract (Tables 1 and 4). This probably implies that the antimicrobial potential of this plant does not primarily depend on the presence of tannin alone. Furthermore, this study showed the absence of flavonoids and anthraquinone. The absence of these metabolites may not have any negative implication on the therapeutic applications or the medicinal efficacies of *C. aconitifolius.*

4. CONCLUSION

The antimicrobial screening of *C. aconitifolius* revealed the propitiousness of developing highly potent, non-toxic, broad spectrum and cheap antibiotic drugs from the plant. Some of the extracts showed higher antimicrobial activity than the two commercial antibiotic drugs used as standard in the study. This initial study has emerged a trailblazing work expected to culminate in appropriate collaborations for achievement of dream drugs.

REFERENCES

[1] Byarugaba D. K., Antimicrobial resistance and its containment in developing countries. In Antibiotic Policies: Theory and Practice, ed. I. Gould and V. Meer, 2005, pp 617–646. New York: Springer.

[2] Sosa A de J., Byarugaba D.K., Amabile C., Hsueh P. R., Kariuki S. and Okeke I. N., Antimicrobial resistance in developing countries. (Eds.) XXIII, 2010, p 554.

[3] Cooper M. A., Shlaes D.,Fix the antibiotics pipeline. *Nature*472: 32, (2011).

[4] Fernebro J., Fighting bacterial infections-future treatment options. *Drug Resist Updat;* 14: 125–39, (2011).

[5] Adeniran O. I. and Abimbade S.F.,Characterization of compounds from leaf extracts of tree spinach –Cnidoscolus aconitifolius (Miller) I. M. Johnston *Int. J. Sci. Res. in Chem. Engr.*, 1(5), pp. 82-86,(2014).

[6] Grubben G. J. H. and Denton O. A., Plant Resources of Tropical Africa 2: Vegetables. PROTA Foundation, 2004, pp.200–201. ISBN 978-90-5782147-9.

[7] Everitt J. N., Lonard R. L. and Little C.R., Weeds in south Texas and Northern Mexico. Luburk: Texas Tech. University press ISBN 0-89672614-2, (2007).

[8] Nebel S., and Heinrich M., Ta Chòrta: A comparative ethno botanical linguistic study of wild food plants in a Graecanic area in Calabria Southern Italy. *Econ. Bot.* 63(1): 78-92,(2009).

[9] Jensen S.A.,Chaya, the Mayan miracle plant. *J. Food Sci*, 51: 234 – 244, (1997).

[10] Atuahene C. C., Poku-Prempeh B. and Twun G., The nutritive values of chaya leaf meal (Cnidoscolus aconitifolius) Studies with broilers chickens. *Animal Feed Sci Technology*, 77: 163-172, (1999).
[11] Adolfo A. and Michael H., Mexican plants with Hypoglycaemic effect used in the treatment of diabetes. *J. Ethno harm*, 99: 325-348, (2005).
[12] Adeniran O.I., Olajide O. O., Igwemmar N.C. and Orishadipe A. T., Phytochemical constituents, antimicrobial and antioxidant potentials of tree spinach [Cnidoscolus aconitifolius (Miller) I.M. Johnston]. *J. Med. Plants Res.,* 7(19): 13171322, (2013).
[13] Trease G. E. and Evans W. C., Pharmacognosy. 15th Edition. Saunders publishers, London, 2002, pp 221 – 229.
[14] Sofowora A., Medicinal Plants and Traditional Medicines in Africa. John Wiley & Sons New York, 1993, pp. 97-145.
[15] Villanueva R. D., Hilliou L. and Sousa-Pinto I., Postharvest culture in the dark: an eco-friendly alternative to alkali treatment for enhancing the gel quality of K/L-hybrid carrageen an from Chondrus crispus (Gigartinales, Rhodophyta). *Bioresour. Technol*. 100:2633-2638, (2009).
[16] Hassan M. M., Oyewale A. O., Amupitan O., Abdullahi M. S. and Okonkwo E. M., Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of Detarium microcarpum. *J. Chem. Soc.* Nigeria. 29: 26-29, (2004).
[17] Usman H. andOsuji J. C., Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldialeavis*. *Afr. J. Trad. CAM*. 4(4): 476-480, (2007).
[18] Okwu D. E., Phytochemicals and vitamin contents of indigenous species of South Eastern Nigeria. *J. Sustain Agric. Environ*. 6: 30-34. 149-153, (2004).
[19] Enzo A. P., Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Qwais M, Modern Phytomedicine Turning Medicinal Plants in to drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007, pp.248-260.
[20] Elmarie V.W. and Johan C. P., Purification and identification of active antibacterial component in *Carpobrotus edulis* L. *J. Ethnopharmacol*. 76: 87-91, (2001).

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