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A Study Of The Antimicrobial Potency Of *Adenia Cissampeloides* Extracts On Bacteria And Fungi Of Clinical Importance.

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Abstract
The antimicrobial activity of extracts from *Adenia cissampeloides* were measured against two Gram negative bacteria, one Gram positive bacteria and two fungi. Ethanol, n-hexane and aqueous extracts of leaves and stem of the plant inhibited growth of the microorganisms. The phytochemical constituents obtained from the plant at varied appreciable proportions elicited the plant potential antimicrobial and antifungal properties. The highest antimicrobial and antifungal properties was observed in the stem extract 22.39mg/ml on *Staphylococcus aureus* while 42.66mg/ml and 41.69mg/ml were obtained on *Candida albicans* and *aspergillus niger* respectively The varied minimum inhibitory concentration range, obtained from the crude extract of the leaves and stem against the selected isolates of bacteria and fungi further established the antimicrobial and antifungal activity .
Keywords: *Adenia cissampeloides*, antimicrobial activity, antifungal activity

INTRODUCTION

The use of medicinal plants for treatment of microbial diseases is well known and has been documented since ancient times. Plants synthesize many components, which act as defensive agents, helping to protect them from microbial infection and other predators. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on the circumstances. Several plants species have been tested for antimicrobial properties but the vast majority have not yet been adequately evaluated(Gibbon,2003).

Investigations of African medicinal plants for their antimicrobial activity rank highest among biological tests carried out on plants and their isolates. This could be because the ethno medicinal plants are for diseases of microbial origin or because of ease of performing such tests. The increasing resistance to antibiotics has also resulted in search for leads from new organic molecules from plants with antimicrobial properties. However, one trend is emerging; whereas earlier tests were for general antibacterial activity using the common Gram positive and Gram negative organisms, a better selection of micro organism is now made to suit specific diseases. Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. This resistance is conferred by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell.
These protect microbial cells from both synthetic and natural antimicrobials (Stermitz *et al.,* 2000). Secondary metabolites resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to their recog-nition in potential target sites (Parekh *et al.,* 2005).

Effective antibacterial agents act by interfering with one or more of the following, synthesis, assembly or function of the macromolecular components of bacterial cells. The interference is brought about by :inhibition of cell wall synthesis, inhibition of nucleic acid synthesis, inhibition of protein synthesis, damaging of the cell membrane. (Ojo, 1998).

The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments and could help curb the problem of these multi-drug resistant organisms. In a study done with *Pseudomonas aeruginosa*, which is resistant to different antibiotics, its growth was inhibited by extracts from clove, jambolan, pomegranate and thyme (Nascimento et al., 2000).
Moreover, the synergistic effects of extracts with antimicrobial activity in association with antibiotics can provide effective therapy against drug resistant bacteria. *Adenia cissampeloides* is a climbing plant producing stems up to 30 metres long and 10cm in diameter at their base. The plant climbs into the surrounding vegetation, attaching itself by means of tendrils.

It is a popular medicinal herb in Africa, where it is often sold in local markets, the plant also supplies food and various commodities for the local population. It is sometimes cultivated or at least retained when land is cleared, and is also worthy of being grown as an ornamental in gardens. The plant has many uses in traditional medicine throughout tropical Africa. All parts of the plant are utilized, being used especially in treating a range of gastro-

intestinal complaints, various inflammatory ailments and for the relief of pain. The stem, leaves, fruit and roots all contain the cyanogenic glycosides.The leaves also contain gummiferol, a cytotoxic polyacetylenic di-epoxide, which has shown in vitro anti-cancer activity ,A diethyl-ether extract from the bark, formulated as an emulsifiable concentrate, is an effective anaesthetic for the African honeybee (Apis mellifera adansonii) Stem pulp showed a significant larvicidal effect on the beet armyworm Spodoptera exigua.

The plant is also used to treat a range of other complaints such as fevers and malaria; liver and gall bladder complaints; lung ailments such as bronchitis; cholera, intestinal worms, venereal diseases and sterility, An infusion of the root and leaves is drunk, and the powdered root and leaves eaten in porridge, to prevent a threatened abortion The leaves are rubbed on women's breasts to stimulate milk flow and the reddish sap is used as a facial cosmetic. This work was carried out to determine the antimicrobial potency of ***Adenia cissampeloides*** crude extracts on selected isolates of bacteria and fungi of clinical importance.

MATERIALS AND METHODS

Plant materials.

Samples of leaves, flowers, stem and root barks of *Adenia cissampeloides* were harvested in August 2014 from the premises of of Offin high school, Ayepe road, Sagamu, Ogun state and it was authenticated at Forestry Research Insitute Nigeria herbarium with voucher number FHI:109873 and voucher specimens are deposited at the location.

Phytochemical analysis

The plant samples were dried at 55^{o}C and powdered using a grinder before 50g of powder from each sample was extracted with ethanol, N-Hexane and distilled water (220ml) for 3hours using soxhlet apparatus. The extract were evaporated to dryness under reduced pressure to give the crude unfractionated extract of each sample (leaf and stem) .

Test for Saponins :1g of the powdered leaves was boiled for 10 minutes. The Extract was then filtered while hot and allowed to cool and the frothing and emulsified test was performed on the extract. The same procedure was repeated for the powdered stems.

(a)Frothing test: 2.5 mls of the filtrates were diluted to 10mls each with distilled water and shaken vigorously for 2 minutes and the tubes was observed for frothing.

(b) Emulsifying test: 2.5 mls of the filtrate was added to two drops of liquid paraffin and shaken vigorously and was observed for fairly stable emulsion an indicative of saponin presence.

Test for Tannins

1g of the powdered leaves was boiled in 10mls of distilled water and cooled. The filtrates was adjusted to 20mls with distilled water.1ml of the filtrates was further diluted with distilled water to make 5mls. A few drops of 0.1% ferric chloride solution were added. The procedure was repeated for powdered stem.

Test for Alkaloids

The extract (1g) was dissolved in 10ml of purified water and filtered. To 5ml of the filterate, 2ml of 10 % HCL was added and boiled in a water bath for about 3 minutes. To 2ml of the solution, dragendroffs reagent was added. An orange or red precipitate indicates the presence of alkaloids. The same procedure was repeated for the stems

Test for Anthraquinone

0.5g of the powdered leaves is shaken with 10ml of benzene and filtered 5ml of the 10% ammonia is added to the filterate. The mixture is shaken and the presence of pink, red or violet colour indicates the presence of anthraquinone

Test for Steroids

1g of the powdered leaves was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface is indicative of the presence of a steroidal ring.

Test for Flavonoids

5mls of 10 % dilute ammonia solution was added to a portion of the aqueous filterate of the leaves extract followed by addition of conc. H_2SO_4.A yellow coloration observed in extract indicates the presence of flavonoids.

Biochemical characterization

The conventional biochemical test was conducted on the isolates of *Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* includes plating on selective media, Gram staining, catalase test, coagulase test, indole test, citrate test, lactophenol cooton blue test and Alexopholus manual was used for the identification of the selected fungi species.

Determination of Minimum Inhibitory Concentration

All the extracts were tested for antimicrobial and antifungal activity. This was determined by agar dilution method. Varying concentrations of extract were prepared by serial dilution and allowed to set in plates. Each plate containing different concentration was inoculated with test organism and incubated at 37^{o}C for 24hrs, chloramphenicol and griseofulvin were used as standards for antibacterial and antifungal activities respectively.

RESULTS

The unfractionated N-hexane, ethanol and aqueous extract of each sample (leaf and stem) gave a yield of 6.76, 3.26, 5.99,3.64,3.9,and 3.3 percent respectively as elicited in Table1,2 and 3 below. The phytochemical screening of leaf and stem of *Adenia cissampeloides* investigated in this study showed the presence of saponins, alkaloids, tannins, flavonoids and steroids while anthraquinone was absent in all the samples tested(Table 4) Broad spectrum of antibacterial activity was exhibited by the three extracts(n-hexane, ethanol and distilled water) of leaves and stem of *Adenia cissampeloides*. Growth was inhibited in all the isolates at relatively considerable concentration of 25-200mg/ml. The highest minimum inhibitory concentration was 22.39 was recorded against *Staphylococcus aureus* while *Candida albican* elicited the concentration of 42.66. Tests for antimicrobial inhibition that contained neither extract nor chloramphenicol had no zone of growth inhibition. Griseofulvin produced zone of inhibition of 18mm against *Aspergillus niger* and an inhibition zone of 12mm against the *Candida albicans.* Tests for antimicrobial inhibition that contain neither extract nor griseofulvin had no zone of growth inhibition.

Table 1. N-Hexane extraction yield of powdered sample of *Adenia cissampeliodes*

Extract	Weight of ground sample (g)	Weight of extract (g)	Percentage yeild (%)
Leaves	50	3.38	6.76
Stem	50	1.63	3.26

Table 2. Ethanolic extraction yield of powdered sample of *Adenia cissampeliodes*

Extract	Weight of ground sample (g)	Weight of extract (g)	Percentage yeild (%)
Leaves	50	2.995	5.99
Stem	50	1.82	3.64

Table 3. Aqueous extraction yield of powdered sample of *Adenia cissampeliodes*

Extract	Weight of ground sample (g)	Weight of extract (g)	Percentage yeild (%)
Leaves	50	1.95	3.9
Stem	50	1.68	3.36

Table 4. Phytochemical screening of the leaves and stem of *Adenia cissampeloides*

SN	Test	Leaves	Stem
1.	Saponins	+	+
2.	Alkaliods	+	+
3.	Tanninis	+	+
4.	Anthraquinone	-	-
5.	Flavonoids	+	+
6.	Steriods	+	+

Present(+) Absent(-)

Table 5. The Minimum Inhibitory Concentration(MIC) values of the different *Adenia cissampeliodes* crude extracts on selected test pathogens.

	Minimun inhibitory concerntration values (mg/ml)					
	Leaf extract			Stem extract		
Test organism	n-Hexane	Ethanolic	Aqueous	n-Hexane	Ethanolic	Aqueous
Staphlococcus aureus	22.39	6.31	20.89	6.31	20.89	20.89
Escherchia Coli	6.31	10.47	20.89	6.31	20.89	41.69
Pseudomonas aeruginosa	6.31	20.89	41.69	10.47	20.89	41.69
Candida albicans	42.66	20.89	41.69	20.89	41.69	41.69
Aspergillus niger	20.89	20.89	10.23	41.69	41.69	41.69

Discussion

Although in vitro activity can not be directly translated into effective use of the plant in herbal medicine, observation of bioactivity do give some indication of possible curative effects. Considerable quantities of antimicrobial concentration was localized in the stem and leaves

The data obtained from this study elicited the overall yield of *Adenia cissampeloides leaves* as the highest compared to the yield obtained from the stem, in which n-hexane leaf extract gave 6.76%, and ethanolic leaf extract was 5.99%, while the aqueous leaf extract gave the yield 3.9%.. The extraction yield obtained from the stem were recorded as follows , n-hexane stem extract gave 3.26%, and ethanolic stem extract which gave 3.64% while aqueous stem extract which gave 3.36%.The phytochemical screening carried out, in the leaf and stem of *Adenia cissampeloides* revealed the presence of saponins, alkaliods, tannins, flavonoids and steriods and negative for anthraquinone which coincides with result obtained from other work (Njoku et al. 2000).

In most medicinal plants, the therapeutic value is due to mostly to the presence of one or more secondary metabolites like tanins, phenols, phenolic acids, quinones, flavnones, flavonoids, coumarins, alkaliods which are synthesized by plants in response to microbial infection and activity produced is due to concentration in the plant part used(Gibbon,2003).

The antimicrobial potency varied at different plant loci, 200mg/ml concentration the ethanolic extract of the leaf showed higher activity compared to n-hexane extract of the leaf and the aqueous extract of the leaf. The antimicrobial potency of the n-hexane stem extract at 200mg/ml elicited high potency compared to the ethanolic stem extract, and the aqueous stem extract which agreed with the findings on the antimicrobial potency of *Adenia cissampeloide*(Morah, 1985) .

The n-hexane leaf extract elicited the highest MIC value of 22.39mg/ml, followed by the aqueous leaf extract value of 20.89mg/ml and the ethanolic leaf extract MIC value of 6.31mg/ml. However, the ethanolic stem extract and aqueous stem extract showed the highest MIC value of 20.89mg/ml while MIC value of 6.31mg/ml of n-hexane extract were recorded against *Staphylococcus aureus*. The aqueous leaf extract elicited the highest MIC value of 20.89mg/ml followed by the ethanolic leaf extract with MIC value of 10.47mg/ml, then and the n-hexane leaf extract with MIC value 6.31mg/ml. While the Aqueous stem extract also recorded the highest value of 41.69mg/ml and MIC values of 20.89mg/ml and 6.31mg/ml for ethanol then followed by the n-hexane stem extract respectively for *Escherichia coli*(Acamovic, 2001)

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The aqueous leaf extract to *Pseudomonas aeruginosa* showed the highest MIC value of 41.69mg/ml while the ethanolic leaf extract gave the MIC value of 20.89mg/ml, and the n-hexane leaf extract having a MIC value of 6.31mg/ml. Futhermore, the aqueous stem extract elicited the highest MIC value of 41.69 mg/ml and the ethanolic stem extract of MIC value of 20.89mg/ml while the n-hexane stem extract elicited a MIC value of 10.47mg/ml

The *Candida albicans* and *Aspergillus niger* showed a varied MIC values of n-hexane leaf extract value of 42.66mg/ml, the aqueous leaf extract MIC value of 41.69mg/ml, and the ethanolic leaf extract of MIC value 0.89mg/ml. Both the ethanolic stem extract and aqueous stem extract having the MIC value of 41.69mg/ml while the n-hexane stem extract had 20.89mg/ml..Both the n-hexane leaf extract and ethanolic leaf etxract possesed the highest MIC value of 20.89mg/ml and the aqueous leaf extract having the MIC value of 10.23mg/ml.

However, all the crude stem extracts had the same MIC value of 41.69mg/ml was recorded in this study. The noted antibacterial and antifungal activity of *Adenia cissampeloides* against *Staphylococcus aureus*, *Esherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. suggest potential use of the

plant extracts for treatment of gastroenteritis and other associated infections. Further investigations, however, will be needed to demonstrate medicinal application.

REFERENCES

Angeh JE (2006). Isolation and characterization of antibacterial compounds present in members of *Combretum* section, *Hypocrateropsis*. PhD Thesis. University of Pretoria.

Acamovic T, Brooker JD (2005). Biochemistry of plant secondary metabolites and their effects in animals. Proc. Nutr. Soc. 64: 403-412.

Basri DF, Fan SH (2005). The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. IndianJ. Pharmacol. 37(1):26-29

Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M, Ozbek T (2006). Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (Asteraceae). Turk. J. Biol. 30: 65-73.

Cheesbrough M (2006) Disrtict Laboratory practise in tropical countries (part 2), Cambridge university press 62-70: 135-140

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582.

Gibbons S (2003). An overview of plant extracts as potential therapeutics. Expert Opin. Ther. Pat. 13(4): 489-497

Rojas JJ, Ochoa VJ, Ocampo SA, Monoz JF (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in treatment of nonnosocomial infections. BMC Complement. Alternat. Med. 6: 2.

Rukangira, E. (2004) The African Herbal Industry and Chalanges W.W.W. Conserveafrica. Org

Ojo (1998) Microbes, humas, and animals, During ventures publishers pp 3,26,27,31

Freixa B, Vila R, Vargas L, Lozano N, Adzet T, Caniguera S (1996). Screening for antifungal activity of nineteen Latin American plants. Phytother. Res. 12(6): 427-430.

Gibbons S (2003). An overview of plant extracts as potential therapeutics. Expert Opin. Ther. Pat. 13(4): 489-497.

Ghani A (1990). Introduction to Pharmacognosy. Ahmadu Bello University Press, Ltd. Zaria, Nigeria. pp. 45-47, 187-197.

Green RJ (2004). Antioxidant Activity of Peanut Plant Tissues. Masters Thesis. North Carolina State University. USA.

Hammer K.A, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., 86(6): 985.

Iwu M.W, Duncan A.R, Okunji CO (1999). New antimicrobials of plant origin. In: Janick J (editor), Perspectives on new crops and new uses. ASHS Press. Alexandria, pp. 457-462

Morah, F.N.I. (1985) The constituents of the stem of *Adenia cissampeloides. Jour. Sci. Educat.* 1:117-122.

Morah, F.N.I. (1988) Tetraphyllin B from *Adenia cissampeloides. Phytochemistry* 27:2985-2986.

Morah, F.N.I. (1993a) Toxic constituents of *Adenia* species. *Discovery and Innovations* 5:111-113.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 31: 247-256.

Njoku PC, Ezeibe AU (2007). Phytochemical and elemental analyses of *Helianthus annus* and its use as blood clotting agent. J. Chem. Soc. Niger. 32(2): 128-132.

Ogukwe CE, Oguzie EE, Unaegbu C, Okolue BN (2004). Phytochemical screening on the leaves of *Sansevieria trifasciata*, J. Chem. Soc. Nigeria, 29(1): 8-10.

Ogunleye DS, Ibitoye SF (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. Trop. J. Pharm. Res. 2(2): 239-241

Parekh J, Jadeja D, Chanda S (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk. J. Biol. 29: 203-210.

Rojas J.J, Ochoa VJ, Ocampo SA, Monoz JF (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in treatment of nonnosocomial infections. BMC Complement. Alternat. Med. 6: 2.

Rukangira, E. (2004) The African Herbal Industry and Chalanges W.W.W. Conserveafrica. Org.

Scheck A.C, Perry K, Hank NC, Clark WD (2006). Anticancer activity of extracts derived from the mature roots of *Scutellaria baicalensis* on human malignant brain tumor cells. Biomed Centr. Complement. Alternat. Med. 6: 27.

Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K (2000). Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc. Natl. Acad. Sci. USA. 97(4): 1433-1437.

Tanaka JCA, da Silva CC, de Oliveira AJB, Nakamura CV, Dias Filho BP (2006). Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum.* Braz. J. Med. Biol. Res. 39(3): 387 391.

Wallace JR (2004). Antimicrobial properties of Plant Secondary Metabolites. Proc. Nutr. Soc. 63: 621-629.

W.H.O. (1991) Traditional medicine and modern health care progress reported by the Director General Document No A44110 W.H.O General