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Phytochemical and anti-microbial screening of Crude Ethanolic Extract of *Aristolochia repens*

Onawumi O.O.E, Oladoye S.O*, Oladipo M.A and Abidakun K.T.
Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria
*Corresponding author (e-mail: oluoladoye@yahoo.co.uk)

Abstract
Aristolochia repens was extracted with 80% ethanol and the extract concentrated *in vacuo* to obtain the crude ethanolic extract. Phytochemical screening of the crude extract revealed the presence of steroids, terpenoids, flavonoids, anthraquinones, tannins, saponins and reducing sugar. The crude extract showed excellent to moderate inhibitory activity on *Pseudomonas aeruginosa*, *Staphlococcus aureus*, *Proteus spp* , *Aspergillus flavus* and *Candida albican*.
Key words: *Aristolochia repens*, phytochemical, anti-microbial, extract.

Introduction:

The genus *Aristolochia* consists of between 450 and 600 species growing in temperate and tropical climates worldwide (Wanke, 2007). They are mostly cultivated as ornamentals but most species are also popular medicaments. Some *Aristolochia* species have been used traditionally as antidote against snake bite, treatment of fever, diarrhea, hypertension and malaria (Pacheco *et al*., 2009; Kumar *et al*., 2003). A number of the species have also been used in traditional medicine as anti – inflammatory agents, analgesics, treatment of arthritis, wound, skin diseases and rheumatism (Martinez *et al*., 2002; Sosa *et al*., 2002, Heinrich *et al*., 2009).
Aristolochia repens is locally called Akogun in South Western part of Nigeria. Its' dried stem and root are used locally as a cure for heamorroids. While the other species have been extensively studied for their chemical constituents and biological activities (Wu *et al*., 2004; Hashimoto *et al*., 1999), little has been done in respect of *Aristolochia repens*, hence this study.

Experimental:

Plant collection:

Aristolochia repens was collected in the month of August 2013 in Ogbomoso, Oyo state, Nigeria and was taxonomically identified in the department of Pure and Applied Biology , Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria.

Extraction of Plant material: The air dried stem and root of the plant (265 g) was pulverized and soaked in 80% ethanol at room temperature and left to stand for one week. After one week, the extract was filtered and filterate concentrated *in vacuo* to give a syrupy liquid which was left to dry in a dessicator, prior to analysis.

Phytochemical Screening:

Phytochemical tests were carried out on the extract following standard procedure (Trease&Evans, 1983; Sofowora, 1993).

Test for Alkaloids:
0.2 g of the extract was warmed with 2% H_2SO_4 for two minutes, thereafter; two to three drops of dragendorff reagent were added. The absence of Alkaloids was indicated by absence of a precipitate.

Test for steroids:
2 ml of acetic anhydride was added to 0.2 g of the extract, followed by addition of 2 ml H_2SO_4, the changing of colour from violet to green was taken as indication for the presence of steroids.

Test for Tannins:
0.2 g of the extract was dissolved in water and heated on a water bath, few drops of ferric chloride was added. A dark green colour developed indicating the presence of tannins.

Test for Saponins:
0.2 g of the extract was shaken with 5 ml distilled water and then heated to boil. Persistent frothing was observed showing the presence of saponins.

Test for flavonoids:
0.2 g of the extract was dissolved in dilute NaOH to give a yellow coloured solution. Addition of dil HCl turned the solution colourless, indicating the presence of flavonoids.

Test for glycosides:

0.2 g of the extract was hydrolyzed with HCl and the mixture neutralized with NaOH solution. Fehling solutions A&B were added and the mixture heated. Absence of precipitate was taken as indicating absence of glycosides.

Microorganisms and Materials:

Two gram negative bacteria, *Pseudomonas aeruginosa* and *Proteus* specie, one gram positive bacteria, *Staphylococcus aureus* and two fungi, *Aspergillus flavus* and *Candida albican* were used in the study. Nutrient agar was used as the growth medium for the bacteria while Potato dextrose agar was used as the growth medium for the fungi.

Inoculation of the Plate:

28 g of Nutrient Agar was dissolved in 1 L of water, sterilized at 15 mins at 121^{o}C, allowed to cool, poured into a plate and left to solidify. Potato Dextrose Agar was similarly prepared by dissolving 39 g in 1 L of water following the same procedure for Nutrient Agar.

Anti microbial bioassay:

The disc agar diffusion method (Salie *et al*., 1996, NCCLS, 2006) was adopted to evaluate antimicrobial activities of the crude extract. Each of the bacteria isolate was prepared by inoculating test tubes containing 5 ml of sterilized nutrient broth with a loopful of each of the bacterial isolate and incubated for 24 hrs. Fungi inoculums were prepared by dissolving 0.2g of yeast extract of sucrose in distilled water. After sterilization, a loopful of each of the fungus was inoculated into the cooled medium and incubated for 72 hrs. After A slight turbidity in the suspension of the fungi and bacteria culture media have been observed, sterilized swap sticks were used to inoculate the solidified NA and PDA plate for bacteria and fungi isolate respectively.

Each of the sterile filter paper disc (Whatman No. 1) of 5 mm diameter was uniformly impregnated with each of the concentration of the extract prepared (5, 25, 50, 100 and 250 µg/ml), air dried and then placed on the labeled inoculated bacterial and fungi plates aseptically using forceps. Fungi plates were incubated at 30^{o}C and allowed to grow for 3 days in order to check the zone of inhibition while bacterial plates were incubated at 37^{o}C and zone of inhibition checked after 24 hrs. The zones of inhibition were measured with a transparent ruler in mm.

Standard antibiotics (Amoxycillin, Steptomycin, Chloranphenicol, Augumentin and Septrin) were used as control for bacteria while Griseofulvin and nystatin were used for fungi.

Results and Discussion:

Table 1: Phytochemical constituents of *Aristolochia repens* extract

Phytochemicals Results
Alkaloids -
Anthraquinones +
Glycosides -
Flavonoids +
Phlobatannins -
Reducing sugar +
Saponins +
Steroids +
Tannins +
Terpenoids +

Key: + = present - = absent

Table 2: Antibacterial activity of crude ethanolic extract of *Aristolochia repens* (Zones of inhibition in mm)

Organism	Crude ethanol extract (ppm)					Control (30 µg/ml)					
	250	100	50	25	5	AM	ST	CH	AU	SXT	
Psedomonas aeruginosa	19	15	14	12	12	15	25	29	18	20	
Proteus spp	15	12	11	-	-	20	19	20	15	14	
Staphylococcus aureus	12	12	-	-	-	19	17	19	19	20	

AM = Amoxylin ST = Streptomycin CH = Chloramphenicol AU = Augumentin SXT = Septrin

Table 3: Antifungal activity of crude ethanolic extract of *Aristolochia repens* (Zones of inhibition in mm)

Organism	Crude ethanol extract (ppm)					Control (30µg/ml)	
	250	100	50	25	5	GR	NST
Aspergillus flavus	17	13	10	-	-	18	18
Candida albican	16	11	10	-	-	18	18

Table 1 shows that *Aristolochia repens* is a very rich source of different classes of phytochemicals , Steroids, terpenoids flavonoids, anthraquinones, tannins, saponins and reducing sugar were found present in the extract of this plant. The presence of these varied phytochemicals has lent credence to the resourcefulness of this plant extracts and its use in the treatment of many diseased conditions as most of these phytochemicals have been shown to contain biologically active compounds that may serve as drugs.

Tables 2 and 3 showed the antibacterial and antifungal effects of the crude extracts. It is observed from the tables that the drug showed remarkable inhibitory activity on two bacterial viz: *Pseudomonas aeruginosa* and the Proteus specie comparable to the inhibitory activity of the standard antibiotics used as control especially at high concentrations and moderate activity on Staphyloccocus aureus . Table 3 also showed that this drug inhibited the growth of the two fungi (*Aspergillus flavus* and *Candida albican*) used to a significant extent. The import of these is that this drug has proved to be a potential source of valuable antimicrobial drugs to ameliorate diseased conditions and to validate the use of this plant in treatment of infections.

Conclusion:

In conclusion, this study has established that steroids, terpenoids, flavonoids, anthraquinones, tannins, saponins and reducing sugars are the main phytochemical constituents of *Aristolochia repens* ethanolic extract. The extract also exhibited remarkable inhibitory activity on the bacteria and fungi used in this study in a concentration dependent manner. This study has therefore been able to validate the use of this plant in the treatment of microbial infections and is therefore a potential source of broad spectrum antibiotics.

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References:

Hashimoto, K., Higuchi, M., Makino, B., Sakakibara, I., Kubo, M., Komatsu, Y., Maruno, M and Okada M. (1999). Quantitative analysis of Aristolochic acids toxic compounds contained in some medicinal plants. *Journal of Ethnopharmacology*, 64, 185 - 189

Heinrich M, Chan J, Wank S, Neinhuis C.H, and Simmonds M.S.S (2009). Local uses of *Aristolochia* species and content of Aristolochic Acids I&II – a global assessment based on bibliographic sources. *Journal of Ethnopharmacology*, 125, 108 - 144

Kumar V.P, Chauhan N.S, Padh H and Rajani M (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. *Journal of Ethnopharmacology*, 107, 182 – 188

Pacheco A.G, de Oliveira P.M, Pilo-Veloso D,de Carvalho Alcantara A.F. (2009). 13C-NMR data of diterpenes isolated from *Aristolochia* species. *Molecules*, 14, 1245 – 1262

Salie F., Eagles P.F.K and Leng H.M.J (1996). Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of Ethnopharmacology,* 52, 27 - 33

Sofowora A (1993). Medicinal plants and Traditional Medicine in Africa. 2nd Edition, spectrum Books Limited, Ibadan, Nigeria, pp. 1-153

Martinez, M.C., Nortier, J., Vereerstraeten, P., Vanherweghem, J.L (2002). Progression rate of Chinese herb nephropathy: Impact of *Aristolochia fangchi* ingested dose. *Nephrology Dialysis Transplantation*, 17, 408 – 412

NCCLS (National Committee for Clinical Laboratory Standards), (2006). Performance standards for antimicrobial disk susceptibility tests. 4th ed. Approved standard M2 – A4, Villanova.

Sosa S, Balick, M.J, Arvigo, R., Esposito, R.G., Pizza, C, Altinier, G and Tubaro A (2002). Screening of the anti – inflammatory activity of some Central American plants, *Journal of Ethnopharmacology*, 81, 211-215

Trease G.E and Evans W.C (1983). Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. Pp. 343-383

Wanke, S (2007). Evolution of the genus Aristolochia: Systematics, Molecular Evolution and Ecology. Ph.D Thesis, Technical University, Dresden, Germany.

Wu, T.S, Damu, A.G, Su, C.R, Kuo P.C (2004). Terpenoids of Aristolochia and their biological activities. *Natural Products Reports*, 21, 594 – 624.