Global Scientific JOURNALS
GSJ: Volume 9, Issue 11, November 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

PHYTOCHEMICAL AND ANTIBACTERIAL POTENTIALS OF *ARISTOLOCHIA RINGENS* ROOT EXTRACTS

[1]Olalekan Oderinde., [2]Andrew Onu., [3]Fatima Lawal Bilbis., [4]Egwuonwu Kingsley C. and [5]Olatunde Olaitan

. [1, 3, 4,] Department of Science Laboratory Technology, Umaru Ali Shinkafi Polytechnic Sokoto
[2]Department of Biochemistry, Usmanu Danfodiyo University Sokoto
[5]Department of Pharmaceutical and Medicinal Chemistry, Olabisi Onabanjo University Ago-Iwoye, Ogun State-Nigeria.

ABSTRACTS

Traditional medicine has gained prominence in the treatment of cancerous sore, lung inflammation, dysentery and dermatitis. The antibacterial activity of *A. ringens* was tested on two pathogenic bacteria viz., *Salmonella spp*, *Shigella spp* and *Escherichia coli*. Extracts from powdered root of *A. ringens* were prepared using sterile water, methanol and Ethylacetate. The extracts were tested on each of the pathogens for their antibacterial properties. The root was also screened for presence of secondary metabolites following standard procedures. The aqueous extracts of the stem and the root barks were effective against Salmonella spp and Escherichia coli at all concentration. The results showed that the aqueous extract of *A ringens* root was effective against *Salmonella spp* with highest inhibition of 2.5mm while *Escherichia coli* with highest inhibition of 2.3mm at concentration of 30 µg. The methanol extracts was effective against *Salmonella spp* and *Escherichia coli* with zone of inhibition of 2.4mm and 2.4mm respectively with concentration of 30µg. The methanolic extract of the root of *A ringens* was not effective against *Shigella spp* at the same concentration. The ethyl acetate extract was used on the microbial isolates. The ethyl acetate extract was non-effective on *Shigella spp* and *Escherichia coli* at all concentration while the extract was effective against *Salmonella spp* with zone of inhibition of 1.7, 2.1 and 2.8mm with concentration of 10 µg, 20 µg and 30 µg respectively. The root of *Aristolochia ringens* parts showed the presence of the following phytochemicals in methanol: phenols, tannins, terpenes and saponins. The aqueous extracts showed the presence of terpenes and flavonoids while ethylacetate revealed the presence of terpenes. However, alkaloids, steroids were absent in all the extracts of the plant. Conclusively, the results from this research shows that solvent extracts from the roots of *A. rigens* possess antibacterial properties. Hence, concerted efforts to produce safe and potent bacteriocide from its parts should be encouraged. From the results obtained in this study, it is recommended that pharmacological properties and the bioactive ingredients from these extracts should be studied, and scientifically evaluated

INTRODUCTION

Aristolochia ringens Vahl. is a perennial plant in the Aristolochiaceae family. In the south-western Nigeria (Yoruba), the plant is commonly known as 'Akogun'. It is an aromatic liane, scrambler, a climbing shrub or rhizome. The plant contains alkaloids and aristolochic acids. The plant is used locally in the treatment of wounds, dysentery, throat infections and skin problems. The antimicrobial potential and phytochemical composition of *A. ringens* root and bark have been investigated. In addition, the antidiabetic, antitrypanosomal and anticancer activities of the plant have been reported (Maberley, 2013).

A. ringens is used to treat various ailments such as wounds, dysentery, throat infections and skin problems of which are linked to microbial infestations. In Asian countries, especially India, over 2,500 plants have been studied to have provided alternative medicine and curative properties to the available synthetic drugs (Sarmiento et al., 2011; Thirumal et al., 2012). Extracts from Aristolochia sp, especially, phytochemicals and essential oil have been receiving earnest in-vitro investigations for their numerous activities. Among the documented activities traced to such phytochemical properties of *A. ringens* are antimicrobial, anti-inflammatory, anti-venom, antipyretic, antiseptic, abortifacient, emmenagogues, storage stability (preservative), foaming (lather), curative, taste, flavours and aroma on one hand and potent nephrotoxic, anti fertility and antispermatogenic on the other (AshokKumar *et al.*, 2010; Sinha and Choudhury, 2010; Tajkarimi *et al.*, 2010; Abhijit and Jitendra, 2011; Kumar *et al.*, 2011). In recent years, the traditional application of natural compounds of plant origin has been receiving a lot of attention as an alternative source of remedy for the treatment of diseases coupled with the belief of their better safety nature and of less or non toxicity.

The search for new drugs has led to the increase in laboratory (in-vitro) research into herbal medicine to establish their acclaimed efficacy and their therapeutic applications. This study aimed to determine the antimicrobial property possessed by *A. ringens* as an effective therapeutic agent against pathogenic microorganisms and their associated infections and also to determine the active ingredients presents in the root extracts of *A. ringens.*

Materials and Methods

Materials:

Mueller Hinton Agar, weighing balance, filter paper, measuring cylinder, conical flask, cotton wool, Bunsen burner, autoclave, hot air oven, petridishes, incubator wire loop, alluminium foil, stainless plate, test tubes, triple stand, methanol, ethyl acetate, distilled water, ruler, cork borer, antibiotics (cefraxone and ciprofloxacine), bacteria pure culture (*Salmonella spp*, *Shigella spp* and *Escherichia coli*).

Collection of Plant Materials

The plant materials used for the work was the root of *Aristolochia ringens* (Ako-igun in Yoruba). The materials were purchased at Sokoto central market, North west Nigeria. They were thoroughly washed with sterile distilled water and air dried before milling into powder for antimicrobial in-vitro analysis.

Collection of bacterial isolates

The bacteria used in this study were obtained from the Microbiology Department, Umaru Ali Shinkafi Polytechnic Sokoto, Nigeria. They are: *Salmonella spp, Shigella spp* and *Escherichia coli*. Sub-culturing of both the bacterial isolates was done into sterile Petri plates and were stored in agar slants and kept in the refrigerator at 4^0C for subsequent use.

Plant Extract Preparation

Fifty grams (50g) of powdered root of *A. ringens* was measured into 400ml of each of the three solvents: Aqueous, methanol and Ethylacetate. These were allowed to soak for 24 hours. The supernatants were filtered into separate conical flasks using Whats'man No. 1 filter paper. The extracts were used immediately, and the remaining were stored in the refrigerator for further studies (Harrigan and McCane, 1986; Fawole and Osho, 1989).

Serial Dilution

Three test tubes were labeled with the Aqueous, methanol and Ethylacetate and the serial dilution were made using µg as follows: 10 µg, 20 µg and 30 µg respectively. The plates were later incubated for 24h at 37^oC for bacterial growth. The experiment was done in triplicates

Media Preparation

All the media prepared were according to manufacture's instruction. The media used were Mueller Hinton Agar. 17grams in to 500mls of distilled water The media were heat to dissolved for homogeneous mixture, and autoclaved at 121^0c for 15 minute; it was allowed to cool down for at least 30-35^0c before pouring.

Pouring

The media was poured in to a sterilized Petri-dish and allowed to solidified.

Sterility Test

The solidified media was incubated at 37^0c for 24hrs without any sample on it, in other to confirm the sterility of the media.

Inhibitory Tests on the *Aristolochia ringens* extracts

Sterile 8mm diameter size cork borer used for creating well cavity was used to cut and pick each bacteria culture with the agar. This was then inoculated into the 4mm deep well of Petri plates of Mueller Hinton Agar were also streaked with the bacterial isolates before the wells were bored and the prepared extracts in different concentrations poured into them.

Data collection

The diameter of growth inhibition for each bacterial colony was determined by direct meter rule measurement (mm). The average measurement of inhibition zone for each triplicate was then calculated. Diameter of inhibition zone for the commercially sold antibiotics disc set side-by-side was equally determined (Guptee, 2001; Nester et al., 2004).

Phytochemical Screening

The powdered plant was subjected to various chemical tests using standard procedures to identify the secondary metabolites as described by Harbone (1973), Trease and Evans (1989) and Sofowora (1993)

Results

The table below shows the Minimum Inhibitory Concentration (MIC) obtained for the various solvent extracts (Aqueous, methanol and Ethylacetate) of *A. ringens* root on the microbial isolates - *Salmonella spp, Shigella spp* and *Escherichia*.

Table 1 shows aqueous extract of *A ringens* root was effective against *Salmonella spp* with inhibition of 0.8, 2.5 and 2.5mm at concentration of 10 µg, 20 µg and 30 µg respectively and Escherichia coli with inhibition of 1.6, 2.2 and 2.3mm at concentration of 10 µg, 20 µg and 30 µg respectively while the aqueous extracts was slightly effective on *Shigella spp* with inhibition of 0.0, 0.0 and 2.0mm at concentration of 10 µg, 20 µg and 30 µg respectively. The aqueous extract from the root of *A ringens* was found to be effective only against *Salmonella spp* and *Escherichia coli.*

Table 2 showed that methanol extracts was effective against *Salmonella spp* and *Escherichia coli* with zone of inhibition of 1.8, 2.1 and 2.4mm and 0.0, 2.2 and 2.4mm respectively with concentration of 10, 20 and 30µg. The methanolic extract of the root of *A ringens* was not effective against Shigella spp at the same concentration.

Table 3 revealed that a contrary result when the ethyl acetate extract was used on the microbial isolates. The ethyl acetate extract was non-effective on Shigella spp and Escherichia coli at all concentration while the extract was effective against *Salmonella spp* with zone of inhibition of 1.7, 2.1 and 2.8mm with concentration of 10 µg, 20 µg and 30 µg respectively.

The root of *Aristolochia ringens* parts showed the presence of the following phytochemicals in methanol: phenols, tannins, terpenes and saponins. The aqueous extracts showed the presence of terpenes and flavonoids while ethylacetate revealed the presence of terpenes. However, alkaloids, steroids were absent in all the extracts of the plant (Table 4).

Table 1: Aqueous extract of *A ringens* root

Sample	Media	Isolate	Extracts		Control (Antibiotics) mg/ml	
			Conc. (µg)	Zone of inhibition (mm)	Antibiotics (mg)	Zone of inhibition (mm)
Akogun Aqueous Extract	Mueller Hinton Agar	*Salmonella spp*	10 20 30	0.8 2.5 2.5	Cefraxone Ciprofloxacine	5.7 5.3
Akogun Aqueous Extract	Mueller Hinton Agar	*Shigella spp*	10 20 30	0.0 0.0 2.0	Cefraxone Ciprofloxacine	3.9 4.5
Akogun Aqueous Extract	Mueller Hinton Agar	*Escherichia coli*	10 20 30	1.6 2.2 2.3	Cefraxone Ciprofloxacine	5.9 5.5

Table 2: Methanol extract of *A ringens* root

Sample	Media	Isolate	Extracts		Control (Antibiotics) mg/ml	
			Conc. (µg)	Zone of inhibition (mm)	Antibiotics (mg)	Zone of inhibition (mm)
Akogun Methanolic Extract	Mueller Hinton Agar	*Salmonella spp*	10 20 30	1.8 2.1 2.4	Cefraxone Ciprofloxacine	5.7 5.3
Akogun Methanolic Extract	Mueller Hinton Agar	*Shigella spp*	10 20 30	0.0 0.0 2.0	Cefraxone Ciprofloxacine	3.9 4.5
Akogun Methanolic Extract	Mueller Hinton Agar	*Escherichia coli*	10 20 30	0.0 2.2 2.4	Cefraxone Ciprofloxacine	5.9 5.5

Table 3: Ethylacetate extract of *A ringens* root

Sample	Media	Isolate	Extracts		Control (Antibiotics) mg/ml	
			Conc. (µg)	Zone of inhibition (mm)	Antibiotics (mg)	Zone of inhibition (mm)
Akogun Ethylacetate Extract	Mueller Hinton Agar	*Salmonella spp*	10 20 30	1.7 2.1 2.8	Cefraxone Ciprofloxacine	5.7 5.3
Akogun Ethylacetate Extract	Mueller Hinton Agar	*Shigella spp*	10 20 30	0.0 0.0 2.0	Cefraxone Ciprofloxacine	3.9 4.5
Akogun Ethylacetate Extract	Mueller Hinton Agar	*Escherichia coli*	10 20 30	0.0 0.0 0.0	Cefraxone Ciprofloxacine	5.9 5.5

Table 4: A preliminary phytochemical screening of *Aristolochia ringens* root

Phytochemical Compound	Extracts		
	Aqueous	Methanol	Ethylacetate
Alkaloid	-	-	-
Phenol	-	+	-
Tannins	-	+	-
Terpenes	++`	++	+++
Steroids	-	-	-
Saponins	-	+	-
Flavonoids	+	-	-

Key: + = slightly present; ++ = moderately present; +++ = highly present; - = absent

DISCUSSION

The research conducted by Tajkarimi *et al.* (2010) on in- vitro analysis of plant extract for antimicrobial activities opined that nowadays being expressed by researchers in two ways: Minimum Inhibitory Concentration (MIC) and Minimum Antimicrobial Concentration (MAC). The MIC required to inhibit < 90% inoculums viability is a measure of extract "effectiveness" determined by inhibitory clearing zone of 10mm, the wider the zone of inhibition the better the potency according to Tajkarimi et al. (2010) and Nand *et al.* (2012). The results showed that the aqueous extract of *A ringens* root was effective against *Salmonella spp* with highest inhibition of 2.5mm while *Escherichia coli* with highest inhibition of 2.3mm at concentration of 30 µg. This might be due to the presence of active components which was sufficient to show activity at the dose level employed as reported by Kamal *et al.* (2010). In a similar report, Akharaiyi and Boboye (2010) revealed the presence of bioactive properties in plant parts at various degrees that also reflects in their therapeutic efficacy. The methanol extracts was effective against *Salmonella spp* and *Escherichia coli* with zone of inhibition of 2.4mm and 2.4mm respectively with concentration of 30µg. The methanolic extract of the root of *A ringens* was not effective against *Shigella spp* at the same concentration. The ethyl acetate extract was used on the microbial isolates. The ethyl acetate extract was non-effective on *Shigella spp* and *Escherichia coli* at all concentration while the extract was effective against *Salmonella spp* with zone of inhibition of 1.7, 2.1 and 2.8mm with concentration of 10 µg, 20 µg and 30 µg respectively.

Extracts from *A. ringens* root using various solvents were noted to differ significantly in their biocides activities. Water extracts have been noted to have very poor activity by some workers (Nzeako et al., 2006; Kamal et al., 2010). Corroborating these findings, this study found that the aqueous and methanolic extracts from the root of A. ringens were observed to be very potent against *Salmonella spp and Escherichia coli.* While the Ethylacetate was slightly potent against the tests organisms. *Aristolochia ringens* may therefore be used for the production of herbal medicine that will serve as an effective medicinal plant drug against some pathogenic microorganisms and their associated infections.

The root of *Aristolochia ringens* parts showed the presence of the following phytochemicals in methanol: phenols, tannins, terpenes and saponins. The aqueous extracts showed the presence of terpenes and flavonoids while ethylacetate revealed the presence of terpenes. However, alkaloids, steroids were absent in all the extracts of the plant. The traditional use of these plant parts for treatment of various illnesses and curative properties against several diseases can be traced to these bioactive compounds. The importance of tannins, terpenes and saponins in various antibiotics used in treating common pathogenic strains has been reported by Kubmarawa et al. (2007) and Mensah et al. (2008). Del-Rio et al. (1997) and Okwu (2004) reported that flavonoids and phenols are potent water soluble anti-oxidants which prevent oxidative cell damage having antiseptic, anti-cancer and anti-inflammatory effects with mild anti-hypertensive properties.

Conclusion

Conclusively, *A. ringens* is used to treat various ailments such as wounds, dysentery, throat infections and skin problems of which are linked to microbial infestations. The results from this research shows that solvent extracts from the roots of *A. rigens* possess antibacterial properties.

Hence, concerted efforts to produce safe and potent bacteriocide from its parts should be encouraged.

Recommendations

From the results obtained in this study, it is recommended that pharmacological properties and the bioactive ingredients from these extracts should be studied, and scientifically evaluated.

REFERENCES

Abhijit, D and Jitendra, N. D. (2011). Aristolochia indica L.: A review. Asian Journal of Plant Sciences, 10(2):108-116.

Abubakar, E.M. (2010). Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria. African Journal of Plant Science Vol. 4(6), pp. 202-209.

Akharaiyi, F. C and Boboye, B. (2010). Antibacterial and phytochemcial evaluation of three medicinal plants. Journal of Natural Products. 3: 27-34

Alviano, W.S; Alviano, D.S; Diniz C.G; Antoniolli A.R; Aviano C.S; Farias, L.M; Carvalho, M. A; Souza, M. M and Bolognese, A. M. (2008). In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch Oral Biol., 53(6):545-52.

Ashokkumar, P; Rajkumar, M and Kanimozhi, M. (2010). Phytochemical Screening and Antimicrobial Activity from 5-Indian medicinal plants against human pathogens. Middle-East Journal of Scientific Research, 5(6):477-82

Das, R; Kausik, A and Pal, T. K. (2010). Anti-Inflammatory Activity Study of Antidote Aristolochia indica to the Venom of Heteropneustes fossils in rats. Journal chemical pharmacology Res., 2:554-562

Del-Rio, A; Obdulio B.G; Gastillo J; Marrin R.R and Ortuno A. (1997). Uses and Properties of Flavanoids. J. Agric. Food Citrus Chem., 45: 4505-4515.

Edeoga, H.O.; Okwu, D.E and Mbuebie, B.O.(2005). Physiochemical Constituent of some Nigeria Medicanal Plants. Fr. Journal Biotechnology, 4: 655-688.

Fawole, M.O and Osho, B.A. (1989). Laboratory Manual of Microbiology. Spectrum Publisher, Ibadan, Nigeria. ISBN-987-246-032-N

Guptee, S. (2001). Practical Microbiology. Jaypee Brothers Medical Publishers (P) Limited 2nd Edition. Jammu, New Delhi. India.

Harborne, J.B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman A &Hall.London; 279p.