ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Biological Sciences 4 (7): 570-574, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.570.574

Antimicrobial Properties of Methanolic Extracts of *Anogeissus leiocarpus* (Guill and Perr)

[1]T. Ichor and [2]E.E. Ekoja

[1]Department of Biological Sciences, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria
[2]Department of Crop and Environmental Protection, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria

Corresponding Author: T. Ichor, Department of Biological Sciences, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria

ABSTRACT

Methanol extracts of leaves of *Anogeissus leiocarpus* were screened for antimicrobial activity against *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Shigella* sp. at the Biological Science Laboratory of the University of Agriculture, Makurdi. The disc diffusion method was used to assay for the antimicrobial property on the test isolates. The extracts at different concentrations inhibited the growth of *S. typhi, E. coli* and *Shigella* sp. The minimum inhibitory concentration of the extraction for *S. typhi, E. coli* and *Shigella* sp. were 49.73, 30.62 and 33.17 mg mL^{-1}, respectively. The antibacterial activity may likely be due to the presence of some active compounds present in the plant extract. Further, preliminary phytochemical screening of the leaves extract of this plant is suggested.

Key words: Antimicrobial, extract, inhibitory concentration, phytochemical, screening

INTRODUCTION

Anogeissus leiocarpus belongs to the family Cambretaceae (Hennenberg *et al*., 2005). It is a deciduous tree that is sparsely distributed and highly searched for in Otukpo Local Government Area of Benue State due to its tradomedical importance. It can grow to a height of up to 30 m, but typically 15-18 m with light green foliage and a wider tank at the base which is sometimes striped. Commonly called African Birch, it is known as Otra in Idoma, Kwankila in Hausa, Atara in Ibo and Orin-odan in Yoruba (Amali, 2007).

Traditionally, the roots of the plant when used as chewing stick are known to have antibacterial effect on *Lactobacillus* sp. (Owoseni and Ogunnusi, 2006). The extracts of the plant in combination with that of *Xanthoxhyim gilletti* mixed with citrus juice revealed efficacy on HIV-associated opportunistic infections and complications (Kwame *et al*., 2005). It has also demonstrated antimicrobial activity against a variety of viruses, malaria parasite and some bacteria (Taiwo *et al*., 1999). Personal interactions with some Herbalists revealed that leaf extracts of this tree is used commonly in the treatment of typhoid fever, diarrhea, malaria fever, rheumatism, cough and skin infections whether administered singly or in combination with other herbs even when no scientific studies have been conducted on it to confirm its antimicrobial properties.

Furthermore, the difficulty in the acquisition of new compounds of therapeutic benefits from microbial sources, the resurgence of several infectious diseases that appeared to have been controlled and the increase in bacterial resistance resulting from development of mutant strains,

recent efforts have been directed towards the development of new antimicrobials from plant sources. This study was therefore undertaken to assess the antimicrobial potentials of the plant.

MATERIALS AND METHODS

Plant collection and Identification: Fresh leaves of *Anogeissus leiocarpus* were collected from Otukpo Local Government Area of Benue State, Nigeria. The plant was identified by Mr. Ekwuno Patrick of the Department of Forestry, Federal University of Agriculture, Makurdi.

Sample preparation and extraction procedure: The leaves were dried at room temperature for one week within which a uniform weight was attained. The dried leaves were further ground into fine powder using a mechanical grinder. About 40 g of the pulverized leaves were weighted into 250 mL of methanol (95%) in a conical flash. The suspension was covered and shaken vigorously at 30 min intervals for 8 h and was allowed to stand for about 48 h. The suspension was subsequently shaken and filtered using Whatman's filter paper No. 1. The filtrate was evaporated to dryness using a rotary evaporator (Model Type 349/2 Corning Ltd.). A yield of 1.2 g was obtained and stored below ambient temperature.

Test microorganism: The test organisms; *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Shigella* sp. were isolated from clinical specimens obtained from Microbiology Section of the Federal medical centre, Makurdi. They were subcultured on nutrient agar and the pure cultures re-subcultured on *Salmonella* and *Shigella* Agar, Eosin Methylene Blue Agar (EMBA) and Mackonkey Agar. They were characterized biochemically, culturally and microscopically and were stored at 4°C until required for the study.

Determination of inhibitory activity: Inhibitory activity of methanol extract was determined using disc diffusion method as reported by Andrews (2001). Five millimeter diameter disc were made from Whatman No. 3 fitter paper using a punching device. The discs were then impregnated with 500 mg mL^{-1} of the methanol extract and when fairly dried were placed on solid culture medium freshly inoculated with the test microorganisms by streaking. The culture plates were incubated (37°C, 24 h) and zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC): Minimum Inhibitory Concentration (MIC) was determined by dilution method. Single and double strength nutrient broths were prepared. The broth was inoculated with a loopful of each test organism separately and thoroughly swirled to ensure uniformity. Three sterile test tubes labeled 1 to 3 were placed on the rack in which the first test tube contained 1 mL of double strength broth while the rest had 1 mL single strength nutrient broth. One mililiter of 500 mg mL^{-1} of the extract was added to test tube 1 an serially diluted to test tube 3, to give a corresponding concentration of 500 mg mL^{-1}, 250 and 125 mg mL^{-1} in test tubes 1, 2 and 3, respectively. The test tubes were incubated (37°C, 24 h). MIC was estimated for the test tubes with the lowest concentrations in which no growth was observed (Andrews, 2001). A negative control tube contained a broth culture of the microorganisms without the extract.

Determination of bacteriostatic and bactericidal properties: This was carried out according to the methods adopted by Wongkham *et al.* (2001).

Statistical analysis: Data obtained from the experiments were subjected to the analysis of variance (ANOVA) separately using (SAS software) and significantly ($p<0.05$) different mean values were separated using Duncan Multiple Range Test (DMRT).

RESULTS

Table 1 shows the results of antimicrobial effects of the extracts on the test isolates. In general, the zones of inhibition decreased with a reduction in the concentration of the extract. The extract did not inhibit the growth of *S. aureus* at any of the concentrations used. The higher zone of the growth inhibition was exhibited on *S. typhi* with a zone diameter of 17.5 mm at a concentration of 250 mg mL^{-1} and *Shigella* sp. with a zone diameter 14.5 mm at 500 mg mL^{-1} while the lowest zone of growth inhibitions (8.6 m) was exhibited by the 125 mg mL^{-1} extract concentration on *E. coli*.

Table 2 show the MIC of the extract on the test isolates. The lowest MIC was obtained with *E. coli* which had 30.62 mg mL^{-1} while *S. typhi* had an MIC of 49.73 mg mL^{-1}.

Table 3 shows the bacteriostatic and bactericidal properties of the methanol extract of the plant. The methanolic extract was bactericidal on *Salmonella typhi* in all the concentrations. The plant extract exhibited bacteriostatic activity on *E. coli* and *Shigella* sp. A sterile wire loop was rubbed around a clear zone of inhibition resulting from an extracts' inhibition of the bacteria and then streaked on the freshly prepared nutrient agar in a Petri-dish and incubated (37°C, 24 h). Bactericidal property was determined when the media had no growth on them whereas, the media that had growth indicated that the resulting bacterial inhibition was only bacteriostatic.

Table 1: Sensitivities of different concentrations of the methanolic extract of *Anogeissus leiocarpus* against some bacteria

	Zone of inhibition (mm)			
Extract concentration (mg mL^{-1})	*S. typhi*	*S. aureus*	*E. coli*	*Shigella* spp.
500	13.2[a]	NI	14.0[b]	14.5[c]
250	17.5[a]	NI	11.5[b]	9.2[c]
125	9.4[a]	NI	8.6[b]	8.8[c]

Mean values followed by the same letter in a column are not significantly different from one another (DNMRT: $p<0.05$). Values are means of triplicate readings, NI: No inhibition

Table 2: Minimum inhibitory concentrations of the methanolic extract of *A. leiocarpus* (mg mL^{-1})

Organism	MIC (mg mL^{-1})
S. typhi	49.73[a]
E. coli	NI
E. coli	30.62[b]
Shigella sp.	33.17[b]

Mean values followed by the same letter in a column are not significantly different from one another (DNMRT: $p<0.05$). NI: No inhibition

Table 3: Determination of bacteriostatic and bactericidal properties of methanolic extract on test organisms

	Organisms			
Plant	*S. typhi*	*S. aureus*	*E. coli*	*Shigella* sp.
A. leiocarpus	-	NI	+	+

-: No growth, +: Growth, NI: No inhibition

DISCUSSION

The results obtained from this study showed that the methanolic extract of the plant inhibited the growth of *Salmonella typhi*, *Escherichia coli* and *Shigella* sp. isolates to varying degrees. This implies that the leaf of the plant possesses some active phytochemicals that can inhibit the growth of some microorganisms. This result is consistent with the findings of Esimone *et al.* (1998), Ntiejumokwu and Alemika (1991), Osadebe and Ukwueze (2004) and Nweze *et al.* (2004) who found that various extracts of the plants inhibited the growth of some hospital isolates. The results also corroborated the findings of Zumbes *et al.* (2007). The low MIC of the extract on *S. typhi*, *E. coli* and *Shigella* sp. is of great importance to the health care system since it can be used as an alternative to orthodox antibiotics in the treatment of infections due to these isolate especially as they are becoming resistant to known antibiotics (Singleton, 1999; El-Shemy *et al.*, 2007; Nascimento *et al.*, 2000). This will also reduce the cost of obtaining health care since the plant is readily available and the cost of preparation is relatively cheaper.

The observed antibacterial effect corroborates its traditional uses. The plant leaves are traditionally used in the treatment of Typhoid fever, Malaria fever, Diarrhea, Cough, Rheumatism and Skin infections. In this study, the extracts of the plant leaves demonstrated bactericidal activity on *S. typhi* to a high degree and also inhibited the growth of *E. coli* and *Shigella* sp. which are responsible for enteric illnesses (Adams and Moss, 1999).

CONCLUSION

The findings of this study have validated the traditional use of *Anogeissus leiocarpus* in the treatment of typhoid fever. Identifying the active ingredient of *Anogeissus leiocarpus* responsible for antimicrobial activities may be the focus of further research activities.

ACKNOWLEDGMENT

I acknowledge Prof. Olusi, T.A. for the opportunity he granted me to co-supervisor our pioneer work of Edache Daniel, Amali.

REFERENCES

Adams, M.R. and M.O. Moss, 1999. Food Microbiology. The Royal Society of Chemistry Cambridge, USA., pp:181-186, 192-203.

Amali, D.A., 2007. Identification of medicinal plants used for treatment of common infections in Benue-State. An Undergraduate Project Submitted to Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria.

Andrews, J.M., 2001. Determination of minimum inhibitory concentration. J. Antimcrob. Chemother., 48: 5-16.

El-Shemy, H.A., A.M. Aboul-Enein, K.M. Aboul-Enein and K. Fujita, 2007. Willow leaves extracts contain anti-tumor agents effective against three cell types. Plos One, 2: e178-e178.

Esimone, C.O., M.U. Adikwu and J.M. Okonta, 1998. Preliminary antimicrobial screening of the ethanolic extract from the *Lichen usnea subfloridans* [L.]. J. Pharma. Res. Dev., 3: 99-101.

Hennenberg, K.J., D. Goetze, V. Minden, D. Traore and S. Porembski, 2005. Size-class distribution of *Anogeissus leiocarpus* (Combretaceae) along forest-savanna ecotones in Northern ivory coast. J. Trop. Ecol., 21: 273-281.

Kwame, T.A., A. Stepehn, K.W. Anthony, F.K. Yaw, D.W. Rita, K.W. Jeffrey and A. Elnora, 2005. Compositions comprising natural agents for the treatment of HIV-associated opportunistic infections and complications and methods for preparing and using compositions comprising natural agents. http://www.freepatentsonline.com/y2005/0266105.html

Nascimento, G.G.F., J. Locatelli, P.C. Freitas and G.L. Silva, 2000. Antibacterial activity of plants extracts and phytochemical on antibiotic-resistant bacteria. Braz. J. Microbiol., 31: 247-256.

Ntiejumokwu, S. and T.O.E. Alemika, 1991. Antimicrobial and photochemical investigation of the stem bark of *Boswellia dedziella*. West Afr. J. Pharmacol. Drug Res., 10: 100-104.

Nweze, E.I., J.I. Okafor and O. Njoku, 2004. Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and morinda lucida benth used in Nigerian Herbal Medicinal practice. J. Biol. Res. Biotechnol., 2: 39-46.

Osadebe, P.O. and S.E. Ukwueze, 2004. A comparative study of the phytochemical and anti-microbial properties of the Eastern *Nigeria* species of African Mistletoe (*Loranthus micranthus*) sourced from different host trees. J. Biol. Res. Biotechnol., 2: 18-23.

Owoseni, A.A. and T. Ogunnusi, 2006. Antibacterial effect of three selected chewing stick extracts on *Lactobacillus* spp. Int. J. Trop. Med., 3: 103-106.

Singleton, P., 1999. Bacteria in Biology. Biotechnology and Medicine. 4th Edn., John Wiley and Sons Ltd., Chichester, USA.

Taiwo, O., H. Xu and S. Lee, 1999. Antibacterial activities of extracts from Nigerian chewing sticks. Phyt. Res., 13: 675-679.

Wongkham, S., P. Laupattarakasaem, K. Pienthaweechai, P. Areejitranusorn, C. Wongkham and T. Techanitiswad, 2001. Antimicrobial activity of *Streblus asper* leaf extract. Phytother. Res., 15: 119-121.

Zumbes, H.J., T.O. Belenu and F.C. Onwuliri, 2007. *In vitro* antibacterial activity of *Anogeissus leiocarpus* leaf extracts on some bacteria associated with diarrhea. Int. J. Natl. Applies Sci., 3: 53-56.