Biotechnology 7 (1): 129-133, 2008
ISSN 1682-296X
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Antimicrobial Activities of the Extracts and Fractions of *Allanblackia floribunda*

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Abstract: *Allanblackia floribunda* is a tree employed in Nigeria and other countries to treat skin disease and other microbial diseases. The ethanol extract, n-hexane, chloroform, ethyl acetate and butanol fractions of the leaves, stem bark and root bark were evaluated for antimicrobial activities against *Staphylococcus aureus* NCIB 8588, *Bacillus subtilis* NCIB 3610, *Escherichia coli* NCIB 86, *Proteus vulgaris* NCIB 67, *Pseudomonas aeruginosa* NCIB 950, *Klebsiella pneumoniae* NCIB 418, *Candida albicans* and *Aspergilus flavus*, using agar diffusion method to validate the ethnobotanical uses of the plant. Among the extracts, the ethanol extract of the leaf gave the most significant antibacterial activity. However, no extract showed antifungal activity. Generally, the fractions obtained from the extracts elicited better activity, including antifungal activity against *C. albicans*. The highest inhibitory effect was exhibited by leaf extract against *Ps. aeruginosa* NCIB 950, while the ethyl acetate fraction of the stem bark gave the least inhibitory effect against *B. subtilis* NCIB 3610. The plant extract and fractions produced inhibition zone range between 5 and 35 mm.

Key words: Antimicrobial activities, ethanol extract, fractions, *Allanblackia floribunda*

INTRODUCTION

Allanblackia floribunda Oliver (Clausiaceae), commonly known as Tallow tree reproduces by seeds. Its occurrence is limited to tropical Africa, but centred mostly on the lowland rainforests (Van Rompaey, 2003).

The nuts of the plant produce fine oil taken by the members of local communities in Tanzania to relieve rheumatism (Anonymous, 2004). Also, in Cameroon, the stem bark of the plant mixed with *Capsicum frutescens* or *Solanum anguivi* is used for the treatment of cough (Betti, 2004). In Gabon, the bark is pounded and rubbed on the body to relieve painful conditions. There also, a decoction is taken for dysentery and as a mouthwash for toothache and, in Côte D'Ivoire, for stomach pains (Steentoft, 1988). In Ghana, the bark is used for medicinal purposes such as toothache, diarrhoea and as a pain reliever (Abiww, 1990). The bark decoction of the stem and root is also used in Central African Republic and West Africa to treat toothache, dysentery and as an analgesic (Lewis and Elvin-Lewis, 1977).

In Akwa Ibom State of Nigeria, the leaves as well as the bark of the stem and root of the plant are used by the local communities to treat dysentery, diarrhoea, skin diseases and some other microbial diseases.

The fruits and the seed kernels produce a hard white fat (Cunningham, 1993). The use of the fat in soap production has been suggested (Foma and Abdala, 1985). A new prenlated xanthone known as Allanxanthone A and some other known xanthones have been identified from the stem bark of *A. floribunda* and have also been shown to have cytotoxic activity against KB cell line (Nkengfack *et al*., 2002).

This study aimed at evaluating the leaves, stem bark and root bark of *A. floribunda* for antimicrobial activities against *Staphylococcus aureus* NCIB 8588, *Bacillus subtilis* NCIB 3610, *Escherichia coli* NCIB 86, *Proteus vulgaris* NCIB 67, *Pseudomonas aeruginosa* NCIB 950, *Klebsiella pneumoniae* NCIB 418, *Candida albicans* and *Aspergilus flavus*, in order to validate the ethnobotanical use of this plant for microbial diseases.

MATERIALS AND METHODS

Extraction: The fresh leaves (1 kg), stem bark (1 kg) and root bark (1 kg) of *Allanblackia floribunda* were collected in June, 2006, air-dried for a week and reduced to powder. The powder (300 g) was macerated in 1 L of EtOH-H_2O (1:1) for 72 h. The liquid extract obtained was concentrated to dryness *in vacuo* at 40°C to yield dry ethanol extract (40-50 g).

Phytochemical screening: Applying the methods of Sofowora (1993) and Trease and Evans (2002), the dry

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ethanol extract of each part was subjected to phytochemical screening to reveal the presence of its secondary metabolites.

Partition chromatography: The dry crude ethanol extract (30 g) of each plant part was dissolved in 40 mL of distilled water and successively partitioned with n-hexane (50 mL ×3), chloroform (50 mL ×3), ethyl acetate (50 mL ×3) and n-butanol (50 ml ×3) to yield their respective fractions. The fractions were separately concentrated to dryness *in vacuo* to give dry residues.

Test organisms: The bacteria used in this study were typed cultures obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, while the fungi were clinical isolates collected from the same source. The bacteria: *Staphylococcus aureus* NCIB 8588, *Bacillus subtilis* NCIB 3610, *Escherichia coli* NCIB 86, *Proteus vulgaris* NCIB 67, *Pseudomonas aeruginosa* NCIB 950 and *Klebsiella pneumoniae* NCIB 418 were sustained on nutrient agar (Oxoid) slant at 4°C prior to use. However, the fungi *Candida albicans* and *Aspergilus flavus* were sustained on Sabouraud's Dextrose Agar (Oxoid) slants at 4°C before use.

Antimicrobial test: The dry ethanol extract and the dry fractions were evaluated against the test microorganisms using agar-gel diffusion method described by Alade and Irobi (1993). The ethanol extract and the fractions were redissolved in Dimethyl sulphoxide (DMSO). The ethanol extract and the fractions were tested at concentration levels of 40 and 80 mg mL^{-1}. Fixed volumes (150 μL) of the ethanol extract, fractions and DMSO were separately introduced into equidistant wells bored on the surface of the agar and Sabouraud's plates, which had been previously inoculated with one of the test organisms. A well containing a standard drug, chloramphenicol was made in the bacteria plates, while the fungal plates had a hole containing Nystatin as standard drug.

The bacteria were incubated at 37°C for 24 h, while the fungi were incubated at 25°C for seven days. The presence of zones of inhibition surrounding the wells was taken as an evidence of antimicrobial activity.

RESULTS

The extracts of the leaves, stem bark and root bark showed various classes of compounds (Table 1) inherent in the plant. The extracts of the three parts contained tannins and cardiac glycosides in high concentration, while flavonoids and terpenes occurred in moderate concentration. Anthraquinones, phlobatannins and alkaloids were absent in all the parts, while saponins were abundant in the leaves and absent in others.

Table 1: Phytochemical screening results of the plant parts of *Allanblackia floribunda*

Metabolites	Inference leaves	Stem bark	Root bark
Saponins	+++	-	-
Tannins			
(i) Bromine water test	+++	+++	+++
(ii) Ferric chloride test	+++	+++	+++
Anthraquinones			
Borntrager's test	-	-	-
Cardiac glycosides			
(i) Salkowski's test	+++	+++	++
(ii) Lieberman Burchard's test	++	++	++
(iii) Keller Kiliani's test	+++	+++	+++
Phlobatannins	-	-	-
Flavonoids	++	++	++
Terpenes	++	++	++
Alkaloids			
(i) Dragendorff's test	-	-	-
(ii) Mayer's test	-	-	-

+++: High concentration; ++: Moderate concentration; - : Absent

The extracts of the leaves, stem bark and root bark exhibited varying degrees of antimicrobial effects, with leaves showing the highest antibacterial activity and the root bark, the least (Table 2). It is noteworthy that none of the extracts was effective against the test fungi. Also, *E. coli* was resistant to all the extracts. The inhibitory effect of the leaf extract against *Ps. aeruginosa* was much better than that of Chloramphenicol. The purification of the extracts by partition chromatography showed some improvement on the antimicrobial activity of all the extracts. For instance, the ethyl acetate fraction of the leaf extract elicited an improvement on the activity of the extract, though fungal activity was still lacking. With the exception of aqueous fraction, all the other fractions generally showed improved activity than the stem bark extract. This improved activity included fungal effect against *C. albicans*. However, n-hexane fraction gave the highest activity. This pattern is similar for root bark extract.

DISCUSSION

The result of this study showed that the extracts and fractions of the leaves, stem bark and root bark of *Allanblackia floribunda* gave good inhibitory effects against all the test microorganisms except *A. flavus*.

The result also revealed that the leaf extract gave more significant inhibitory effects than those of stem bark and root bark. However, all the extracts exhibited only antibacterial effects.

The fractions obtained from the extracts through partition chromatography gave improved activity against

test organisms, including activity against *C. albicans* which the extracts failed to inhibit. A similar result has been observed for the leaves of *Heinsia crinita* (Ajibesin *et al*., 2003). This suggests that antimicrobial activity was increased by purification of active constituents of the plant. Generally, all the extracts and the fractions failed to inhibit *A. flavus*. However, aqueous fraction did not show significant inhibitory effect against all the test organisms except *B. subtilis*. This may be due to the fact that virtually all the antimicrobial principles had been extracted from the aqueous phase during fractionation. The best inhibitory effect was elicited by the leaf extract against *Ps. aeruginosa*, while the least was by ethyl acetate fraction of the stem bark against *B. subtilis*. Furthermore, the antimicrobial activities of the extracts and the fractions were dose-dependent.

The presence of terpenes observed in the phytochemical screening may be responsible for the enhanced effect of n-hexane fraction, since terpenes are non-polar compounds located in non-polar fractions. In a similar fashion, tannins, flavonoids and saponins may account for the improved activity of the ethyl acetate and butanol fractions. The antimicrobial activity of phenolics (tannins, flavonoids) and saponins has been established in some plants (Burapadaja and Bunchoo, 1995; Adesina *et al*., 2000; Binutu and Cordell, 2000;

Table 2: Antimicrobial activity of the extracts and fractions of the plant parts on the test organisms

	Zones of inhibition of organisms (mm) Concentration (mg mL^{-1})													
	L1		L2		L3		L4		L5		L6		B1	
Microorganisms	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Bacteria														
Escherichia coli (NCIB 86)	-	-	15±3.74	6±2.45	30±7.48*	12±2.82	11±1.41	-	-	-	16±2.82	11±2.82	-	-
Proteus vulgaris (NCIB 67)	14±2.82	12±0.00	-	-	-	-	11±3.74	-	-	-	-	-	12±0.00	-
Pseudomonas aeruginosa (NCIB 950)	35±2.82*	19±1.41	-	-	-	-	14±2.45	6±0.00	23±1.41*	15±1.41	-	-	12±2.82	5±1.41
Staph. aureus (NCIB 8588)	9±0.00	-	-	-	11±2.82	6±2.45	20±1.41*	9±3.74	21±4.89*	10±0.00	-	-	-	-
Bacillus subtilis (NCIB 3610)	-	-	18±3.74*	6±2.82	13±0.00	7±1.41	28±5.09*	14±2.45	19±3.74*	6±2.82	32±2.45*	15±4.73		
Klebsiella pneumoniae (NCIB 418)	11±3.74	7±1.41	18±1.4	-	11±1.41	-	20±2.45*	10±1.41	15±2.82	11±0.00	-	-	-	-
Fungi														
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Zones of inhibition of organisms (mm) Concentration (mg mL^{-1})											
	B2		B3		B4		B5		B6		R1	
Microorganisms	A	B	A	B	A	B	A	B	A	B	A	B
Bacteria												
Escherichia coli (NCIB 86)	26±2.45*	11±3.74	16±3.74	7±0.00	11±1.41	-	15±0.00	-	-	-	-	-
Proteus vulgaris (NCIB 67)	5±0.00	-	-	-	-	-	11±3.74	6±2.82	-	-	-	-
Pseudomonas aeruginosa (NCIB 950)	-	-	-	-	11±2.82	6±0.00	-	-	-	-	18±0.00	14±3.74
Staph. aureus (NCIB 8588)	19±2.45*	-	28±4.89*	10±3.74	-	-	16±0.00	-	-	-	-	-
Bacillus subtilis (NCIB 3610)	9±2.82	-	14±2.45	9±1.41	5±2.82	-	27±1.41*	7±2.45	18±3.74	10±1.45	-	-
Klebsiella pneumoniae (NCIB 418)	26±2.45*	16±1.41	-	-	17±3.74*	10±2.45	-	-	-	-	-	-
Fungi												
Candida albicans	17±0.00	-	6±0.00	-	14±2.45	7±0.00	19±2.82*	8±2.45	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Continued

	Zones of inhibition of organisms (mm)												
	Concentration (mg mL^{-1})												
	R2		R3		R4		R5		R6				
Microorganisms	A	B	A	B	A	B	A	B	A	B	C	N	DM
Bacteria													
Escherichia coli (NCIB 86)	21±1.41*	11±2.82	18±0.00	11±2.45	12±0.00	6±2.45	-	-	-	-	24±0.00	NA	-
Proteus vulgaris (NCIB 67)	18±0.00	14±2.45	22±1.41*	16±0.00	-	-	-	-	-	-	14±3.74	NA	-
Pseudomonas aeruginosa (NCIB 950)	22±1.41*	16±3.74	-	-	27±0.00*	18±1.41	21±2.45*	12±2.82	-	-	15±1.41	NA	-
Staph. aureus (NCIB 8588)	-	-	-	-	12±3.74	11±2.82	-	-	-	-	12±1.41	NA	-
Bacillus subtilis (NCIB 3610)	-	-	-	-	22±3.74*	18±1.41	-	-	26±2.82*	12±4.89	21±2.45	NA	-
Klebsiella pneumoniae (NCIB 418)	18±2.82	-	31±1.41*	-	-	-	8±2.82	-	-	-	27±3.74	NA	-
Fungi													
Candida albicans	16±2.82	11±0.00	15±3.74	-	-	-	8±2.45	-	-	-	NA	15±2.82	-
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	NA	14±2.45	

L1 = Leaf ethanol extract
L2 = Leaf n-hexane fraction
L3 = Leaf chloroform fraction
L4 = Leaf ethyl acetate fraction
L5 = Leaf butanol fraction
L6 = Leaf aqueous fraction
A = 80 mg mL^{-1}
B = 40 mg mL^{-1}
C = Chloramphenicol (2 μg mL^{-1})
N = Nystatin (2 μg mL^{-1})
NA = Not applicable
DM = DMSO

B1 = Stem bark ethanol extract
B2 = Stem bark n-hexane fraction
B3 = Stem bark chloroform fraction
B4 = Stem bark ethyl acetate fraction
B5 = Stem bark butanol fraction
B6 = Stem bark aqueous fraction
Values are mean± SD (n = 4).
*: $p<0.01$ with respect to control

R1 = Root ethanol extract
R2 = Root n-hexane fraction
R3 = Root chloroform fraction
R4 = Root ethyl acetate fraction
R5 = Root butanol fraction
R6 = Root aqueous fraction
- = No zone of inhibition.

Pistelli *et al*., 2002; Mandal *et al*., 2005). The extracts of the plant and their fractions gave significant zones of inhibition against the test organisms, thereby validating the ethnobotanical claims on this plant as remedy for the treatment of infections and diseases caused by these organisms. This result corroborates the reports on the validation of claims from ethnobotanical survey on other plants used to treat microbial diseases (Rajakaruna *et al*., 2002; Khan and Omoloso, 2002; Idowu *et al*., 2005; Rene *et al*., 2006; Ekpo *et al*., 2007). From the result, the plant was found to be effective against bacterial infections, but weak against fungal diseases. Further, the leaves whose extract and fractions exhibited the best activity are recommended for use in preference to other parts of the plant. Since activity improved with purification, this encourages further investigation to be carried out on the active fractions of the plant in order to isolate and characterize the active constituents responsible for the improved activity recorded in this study. The active constituents if isolated may show much better activity, leading to the production of effective antibiotics.

REFERENCES

Abiww, D.K., 1990. Useful plants of Ghana. Intermediate Technology Publication and Royal Botanic Gardens, Kew, pp: 1-10.

Adesina, S.A., O. Idowu, A.O. Ogundaini, H. Oladimeji, T.A. Olugbade, G.O. Onawunmi and M. Pais, 2000. Antimicrobial constituents of *Acalypha wilkesiana* and *A. hispida*. Phytother. Res., 14: 371-374.

Ajibesin, K.K., J.B. Ekpo and N.D. Bala, 2003. Comparative pharmacognostic and antimicrobial studies on leaves of two varieties of *Heinsia crinita*. Global J. Med. Sci., 2: 49-57.

Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. J. Ethnopharmacol., 39: 171-174.

Anonymous, 2004. African tree piques European interest. Spore, No. 111, World Agro Forestry Centre, pp: 7.

Betti, J.L., 2004. An ethnobotanical study of medicinal plants among the *Baka pygmies* in the Dja Biosphere Reserve, Cameroon. Afr. Stud. Monogr., 25: 1-27.

Binutu, O.A. and G.A. Cordell, 2000. Gallic acid derivatives from *Mezoneuron benthamianum* leaves. Pharm. Biol., 38: 284-286.

Burapadaja, S. and A. Bunchoo, 1995. Antimicrobial activity of tannins from *Terminalia citrina*. Planta Medica, 61: 365-366.

Cunningham, A.B., 1993. African medicinal plants. Setting priorities at the interface between conservation and primary healthcare. People and Plants Working Paper, pp: 1-50.

Ekpo, B.A.J., K. Ajibesin, D.N. Bala and P. Nwafor, 2007. Antimicrobial activities of the extracts and fractions of *Triumfetta cordifolia* A. Rich (Tiliaceae). Nig. J. Bot., 20: 181-186.

Foma, M. and T. Abdala, 1985. Kernel oils of seven plant species of Zaire. J. Am. Oil Chem. Soc., 62: 910-911.

Idowu, P.A., J.O. Moody and H.A. Odelola, 2005. Phytochemical and antimicrobial screening of three Nigerian medicinal plants used to treat infectious diseases traditionally. J. Pharm. Bioresour., 2: 116-119.

Khan, M.R. and A.D. Omoloso, 2002. Antibacterial, antifungal activity of *Harpullia petiolaris*. Fitoterapia, 73: 331-335.

Lewis, W.H. and M.P.H. Elvin-Lewis, 1977. Medical Botany. John Wiley and Sons Ltd., New York, pp: 232-252.

Mandal, P., S.P. Sinha Babu and N.C. Mandal, 2005. Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterapia, 76: 462-465.

Nkengfack, A.E., G.A. Azebaze, J.C. Vardamides, Z.T. Fomum and F.R. van Heerden, 2002. A prenylated xanthone from *Allanblackia floribunda*. Phytochemistry, 60: 381-384.

Pistelli, L., A. Bertoli, E. Lepori, I. Morelli and L. Panizzi, 2002. Antimicrobial and antifungal activity of crude extract and isolated saponins from *Astragalus verrucosus*. Fitoterapia, 73: 336-339.

Rajakaruna, N., C.S. Harris and G.H.N. Towers, 2002. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharm. Biol., 40: 235-244.

Rene, N., O.H. Oladimeji, P.M.E. Ubulom and B.A.J. Ekpo, 2006. Larvaecidal and antimicrobial potential of *Anthocleista djalonensis* L. (Gentianaceae). African J. Pharm. Res. Dev., 2: 97-101.

Sofowora, A., 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp: 150-153.

Steentoft, M., 1988. Flowering plants in West Africa. Cambridge University Press, Cambridge, pp: 1-352.

Trease, G.E. and W.C. Evans, 2002. Pharmacognosy. 15th Edn., Saunders, pp: 214-252, 336-337.

Van Rompaey, R., 2003. Distribution and ecology of *Allanblackia* species (Clusiaceae) in African rain forests with special attention to the development of a wild picking system of the fruits. Unilever Research Ecosystem, Vlaardingen, pp: 1-39.