African Journal of Biotechnology Vol. 6 (15), pp. 1785-1787, 6 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*

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Accepted 2 February, 2007

Compounds of pharmacological interest (tannins) were isolated from *Dichrostachys cinerea* and assayed against *Staphylococcus aureus, Shigella boydii, Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa* using the agar diffusion method. Tannins exhibited antibacterial activities against all the test microorganisms. *Sh. flexneri* was the most resistant to tannins isolated from the plant material followed by *Sh. boydii, E. coli, Staph. aureus* and *P. aeruginosa* respectively. Minimum inhibitory concentration of the tannins ranged between 4.0 and 5.5 mg/ml while the minimum bactericidal concentration ranged between 4.5 and 6.0 mg/ml.

Key words: Pharmacological, agar diffusion, antibacterial.

INTRODUCTION

Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compounds (Sofowora, 1986). The use of higher plants and shrubs including some vegetables were originally recognized as antiseptic. For example, thymol, a simple phenol present in essential oil of plants like *Thymus vulgaris* and *Monanda punctoda* have both antibacterial and antiviral properties (Baton and Hatfield, 1982). *Acalypha indica* has acalyphine used in the treatment of sore gum; it has expectorant and emetic properties (Bedon and Hatfield, 1982). *Sida acuta* contains alkaloids (Banzouzi et al., 2004; Karou et al., 2003). In Central America, the plant is used to treat asthma, renal inflammation, cold, fever and headache (Caceres et al., 1987; Coee and Anderson, 1996). Extract of the plant is very active on pathogenic bacteria (Karou et al., 2005). Tannins have been isolated from some plants (Mitcher et al., 1988; Hasfermaria et al., 1993). Egwim et al. (2000) demonstrated the presence of tannins in *Euphobia hirta*. The plant exhibited antimicrobial activity against *Salmonella typhi in vitro.* Tannins are general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gellatin from solution. Tannin can be toxic to bacteria, filamentous fungi and yeast (Harborne, 1973).

Dichrotachys cinerea is commonly called “dundu” among the Hausa speaking people of northern Nigeria and “Kora” among the Yoruba speaking people of Western Nigeria (Gill, 1992). The plant is a shrub, usually attaining a height of up to 5 – 10 m. The leaves are compound and pinnate. The inflorescence consists of a penduculate spike. The flowers have two sets of colours – pinkish white basally and yellow terminally (Mann et al., 2005). This study was carried out to evaluate the antibacterial properties of tannins isolated from *D. cinerea*.

MATERIALS AND METHODS

Source of plant material

The root of the plant was collected from Bida, Nigeria. The plant was duly authenticated at the Forestry Research Institute, Jos, Nigeria as *Dichrotachys cinerea.*

Microorganisms

Clinical isolates of *Staphylococcus aureus, Shigella boydii, Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa* used in this study were obtained from School of Medical Laboratory Technology, National Veterinary Research Institute, Vom, Nigeria.

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Table 1. Antibacterial effect of tannin isolated from *Dichrostachys cinerea.*

Concentration (mg/ml)	Mean diameter of zone of inhibition (mm ± SD)				
	Staph. aureus	***Sh. boydiii***	***Sh. flexneri***	***E. coli***	***P. aeuruginosa***
0.5	0	0	0	0	0
1.0	0	0	0	0	0
1.5	7.5 ± 0.1	9.5 ± 0.02	12.0 ± 0.1	8.5 ± 0.1	6.5 ± 0.1
2.0	10.0 ± 0.02	11.0 ± 0.05	15.0 ± 0.2	10.5 ± 0.1	8.0 ± 0.02
2.5	13.5 ± 0.5	14.0 ± 0.2	19.0 ± 0.01	14.0 ± 0.1	11.0 ± 0.01
3.0	15.0 ± 0.08	17.0 ± 0.1	22.0 ± 0.02	16.5 ± 0.2	13.5 ± 0.2
3.5	18.0 ± 0.02	21.0 ± 0.1	24.0 ± 0.05	18.5 ± 0.3	16.0 ± 0.1

Preparation of plant extract

Ethanolic extract of the root of the plant was prepared according to the method described by Okogun (2000) with slight modifications. A 50 g sample of the plant root was air-dried, ground into powder using an electric blender (National MX 4911W, Matsushita Electronics). The blended material was transferred into a beaker and 10 ml of absolute ethanol added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48 h. The extract was decanted and the solvent removed by evaporation at ambient temperature (28 ± 2°C) to obtain the extract.

Extraction of tannins

Sample of the powdered root (3 g) was boiled in 5 ml of distilled water for 3 min on a hot plate. The mixture was filtered while hot and the resulting filtrate was used to carry out ferric chloride test. (Trease and Evans, 1983) sample of the filtrate (1.0 g) was weighed into a beaker and 10ml of distilled water added. This was boiled for 5 min. Two drops of 5% ferric chloride ($FeCl_2$) was then added. Production of greenish precipitate indicated the presence of tannins (Trease and Evans, 1983).

Antimicrobial test

The antibacterial test was performed using the agar diffusion method of Collins et al. (1995). The test microorganisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm in diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized by flaming. To each well was introduced different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 or 3.5 mg/ml) of tannins isolated from the plant extract. Control experiments with no plant extracts were set up.

The plates were allowed to stand for one hour at room temperature for diffusion of the substances to proceed before the growth of microorganisms commenced. The plates were incubated at 37°C for 24 h. The zones of inhibition were then recorded.

Determination of minimum inhibitory concentration

Various concentrations of tannins from *D. cinerea* ranging between 4.0 and 6.0 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of *Staph. aureus, Sh. boydii, Sh. flexneri, E. coli* and *P. aeruginosa* diluted to give a final concentration of 10^6 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of tannin that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case (Collins et al., 1995).

Determination of minimum bactericidal concentration

After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of alkaloid that does not yield any colony growth on the solid medium after the incubation period was regarded as minimum bactericidal concentration (MBC) (Alade and Irobi, 1995).

RESULTS AND DISCUSSION

The tannins isolated in this study exhibited anitibacterial activity against *Staph. aureus, Sh. boydii* and *Sh. flexneri, E. coli* and *P. aeruginosa* (Table 1). The inhibitory activities exhibited by the tannins tends to agree with the report that antibacterial properties of plants is due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils (Scherbouvaskii, 1971; Leven et al., 1979; Bassole et al., 2003; Erasto et al., 2004; Viljoen et al., 2003).

The increase in antibacterial effectiveness observed with increase in concentration of tannins isolated in this study is in agreement with the work of Kurosaki and Nishi (1983) who reported that higher concentrations of antimicrobial substances showed appreciable growth inhibition to microorganisms. Several plants which are rich in tannins have been shown to possess antimicrobial activeties against a number of microorganisms. For example Adebayo et al. (1983) investigated the antimicrobial activity of leaf extract pf *Eugenia uniflora* and reported that tannins, alkaloids and glycosides were detected (Adebajo et al., 1983).

African medicinal plants have been found to exert good *in vivo* antimicrobial activities and some active principles have been isolated (Damintoti et al., 2005). Examples are muziyadial isolated from *Warburgia salutaris* (Rabe and

Staden, 2000) and vemodalin from *Vernonia coloratus* (Reid et al., 2001).

The MIC of tannins isolated in this study against the test organisms ranged between 4.0 and 5.5 mg/ml while the MBC ranged between 4.5 and 6.0 mg/ml (Table 2). Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. The results of the present study support the traditional medicinal use of *D. cinerea.* The present study suggests that the tannins isolated from the test plant possess remarkable bacteria toxic activity. Thus there is possibility of developing the plant as a source of antimicrobial agent.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tannins isolated from Dichrostachys cinerea.

Organism	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	5.5	6.0
Sh. boydii	4.5	5.0
Sh. flexneri	4.0	4.5
E. coli	5.0	5.5
P. aeruginosa	5.5	5.5

REFERENCES

Adebajo AO, Adewumi CO, Essein EE (1983). Antiinfective agents of higher plants. International symposium of Medicinal Plants. 5th edn. University of Ife, Nigeria. pp. 152–158.

Alade PI, Irobi ON (1995). Antifungal activities of crude leaf extract of *Acalypha wikesiana*. J. Ethnopharmacol. 39: 171–174.

Bedon E, Hatfield GM (1982). An investigation of the antiviral activities of *Podophyllum Peltatun.* Lloydia 45(6): 725.

Banzouzi JT, Prando R, Menan H, Valentin A, Roumestan C, Mallic M, Pelissier U, Blache Y (2004). Studies on medicinal plants of Ivory Coast: Investigation of *Sida acuta* for *in vitro* antiplasmodial activeties and identification of an active constituent. Phytomedicine 11: 338-341.

Bassole INH, Ouattara AS, Nebie R,Ouottara CAT, Kabore ZI, Traore AS (2003). Chemical composition and anitimicrobial activities of the essential oil of *Lippia chevelieri* and *Lippia mutiflora* from Burkina Faso Phytochemistry 62: 209-212.

Caceres A, Giron LM, Martinez AM (1987) Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. J. Ethnopharmacol. 19: 23-245.

Coee, FG, Anderson GJ (1996). Ethnobotany of the Garifuna of the eastern Nicaragua. Eon. Bot. 50: 71-107.

Collins GH, Lynes PM, Grange JM (1995). Microbiological Methods (7th edn) Butterwort – Heinemann Ltd, Britain pp. 175–190.

Damintoti, K, Aly S, Antonella C, Saydou U, Carla M, Jacques S, Vittorie C, Alfred ST (2005) Antibacterial activity of alkaloids from *Sida acuta*. Afr. J. Biotechnol. 4(12): 1452 –1457.

Egwim CE, Banso A, Adeyemo SO (2002). Efficacy of some Nupe medicinal plants against *Salmonella typhi*: an *in vitro* study. J. Ethnopharmacol. 80: 21-24.

Erasto P, Bojase–Moleta G, Majinda RRT (2004). Antimicrobial and antioxidant flavonoids from the roots wood of *Bolusathus spesiosus*. Phytochem. 65: 875-880.

Gill LS (1992). Ethnomedical uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria. p. 100.

Hebtermariam S, Gray AI, Waterman RG (1993). A new antibacterial sesquiterpene from *Premma oliyotricha* . J. Nat. Prod. 56(1): 140–143.

Harborne JB (1973). Photochemical Methods. A guide to modern techniques of plant analysis. Chapman and Hall, London. p. 279.

Karou D, Dicks MH, Sanon S, Simpore J, Traore A S (2005). Antimalarial activity of *Sida acuta,* Burm F. (Malvacease) and *Pterocarpus erinaceus* poir. *(Fabaceae)* J. Ethnopharmacol. 89: 291-294.

Karou D, Dicko MH, Simpore J, Traore AS (2005). Antioxidant and antibacterial activities of polyhphenols from ethnomedicinal plants of Burkina Faso. Afr. J. Biotechnol. 4: 823–825.

Kurosoki F, Nishi A (1983). Isolation and antimicrobial activity of the phytoalexin–6-methoxymellein from cultured carrot cells. Phytochemistry 22(3): 669-672.

Leven M, Vanden Berghe DA, Morteu I, Vilientnick A, Lomweas EC (1979). Screening of higher plants for biological activity. Planta medica 36: 311-312.

Mann A, Gbate M, Umar A (2003). Medicinal and Economic plants. Jube Evans Books and Publications, Bida Nigeria. p. 161.

Mitcher LA, Okwute SK, Gollapudie SR, Drake S, Anova E (1988). Antimicrobial Pterocarpans of Nigeria. *Erythrina mildbeadii*. Photochemistry 27(11): 3449-3452.

Okogun JI (2000). Methods of Medicinal Plant extract preparation. National Institute for Pharmaceutical Research and Development (NIPRD) Idu - Abuja, Nigeria.

Rabe T, Van Staden J (2000). Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris* J. Ethonopharmacol. 73: 171-174.

Reid K, Jager AK, Van Staden J (2001). Isolation of the antibacterial vernodalin from traditionally used *vernonia* colorata South Afr. J. Bot. 67: 73-74.

Sofowora A (1986). The state of medicinal plant research in Nigeria. University of Ife Press Ife, Nigeria.

Trease GE, Evans IC (1983). Pharmacognosy (12th edn) Bailliere Tindall London. pp. 21-22.

Viljoen A, Van Vuuren S, Ernest E, Klepser M, Demirci B, Basser H, Van Wyk BE (2003). *Osmitopsis asteriscoides (Asteraceae)* – the antimicrobial and essential oil composition of cape – Dutch remedy. J. Ethnopharmacol. 88: 137-143.