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Preliminary phytochemical and antimicrobial screening of the leaves of *Byrsocarpus coccineus* Schum & Thonn (Connaraceae)

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Abstract

The leaf extracts of *Byrsocarpus coccineus* was evaluated for antimicrobial activity using agar diffusion assay. The results of the antimicrobial screening showed that the ethyl acetate fraction at 200mg/ml produced zones of inhibition ranging from 22.5 to 35mm against the test organisms while the minimum inhibitory concentration of the fraction were 1.75mg/ml, 1.75mg/ml, 0.88mg/ml and 0.44mg/ml against *Escherichia coli, Salmonella typhi, Candida albicans* and *Staphylococcus aureus* respectively. The *n*-butanolic fraction gave MIC of 7.0mg/ml, 7.0mg/ml, 1.75mg/ml and 1.75mg/ml against *E. coli, Staph. aureus, C. albicans* and *S. typhi* respectively. The extracts exhibited good antimicrobial activity with the ethyl acetate fraction showing more activity than the n-butanol fractions. The gram positive bacteria are more susceptible to the extracts than the gram negative bacteria. Results of the phytochemical screening revealed the presence of flavonoids, tannins on both fractions while saponin was present only in the n-butanol fraction. The microbial activity of the two fractions can be explained by the presence of these secondary metabolites.

Keywords: *Byrsocarpus coccineus*; Antimicrobial studies; Phytochemical screening

Introduction

About half of the world medicinal compounds are probably derived or obtained from plants (Akerele, 1988). Important drugs of the past 50 years or so that revolutionized modern medical practice were mostly isolated from plants that have established history from which for one purpose or the other have been employed in ancient civilizations (Schultes, 1986). Systematic screening of plans for bioactive compound is now a routine in many laboratories devoted to biomedical research, of particular interest is the search for compounds with antimicrobial activities. This has gained importance in recent years because of the alarming increase in the rate of resistance of micro-organism to existing antibiotics. The plant *Byrsocarpus coccineus* is indigenous to tropical Africa especially Togo, and Nigeria (Dalziel, 1955). Folklore uses of the plant include: the leaf decoction for treatment of sexually transmitted diseases,

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sores and skin infection and also as antidote to arrow poisoning. The whole plant decoction is applied to swelling and tumours and also to arrest bleeding (Irvine, 1961).

The uterotonic and molluscidal activities of the ethanol leaf extracts have been reported (Amos *et al.*, 2002, Okunji and Iwu, 1988), while a coumaryl derivative and flavonoid glycoside were isolated from the leaves of this plant (Vickery and Vickery, 1980). The present study seeks to investigate the antimicrobial activity of leaf extracts of the plant.

Experimental

Plant material. The leaves and stem were collected in the month of August in Samaru, Zaria and authenticated at the herbarium section of the Biological Science Department, Ahmadu Bello University, Zaria where a voucher specimen (864) has been deposited.

Extraction. The leaves of the plant were air dried and powdered with pestle and mortar. The powdered leaves (250g) was extracted exhaustively with 95% ethanol for 14 days. The combined ethanolic extract was concentrated at reduced pressure to afford a greenish mass (40g) referred to as ethanolic extract. 30g of the extract was suspended in water and filtered. The water soluble part was extracted with ethyl acetate (2 x 500ml) and N-butanol (5 x 500ml) to give a yield of 1.2g and 1.9g coded EA and N-BT respectively.

Preliminary phytochemical screening. The crude ethanolic extract (EE), the ethyl acetate soluble fraction (EA) and the N-butanol soluble fraction (N-BT) were subjected to preliminary phytochemical screening using standard procedures (Sofowora, 1993; Silva *et al.*, 1998).

Antimicrobial screening. The ethyl acetate (EA) and the *n*-butanol fraction (NBT) were reconstituted in 10% aqueous methanol to a concentration of 200mg/ml respectively. The test organisms: *Staphylococcus aureus, E. coli, Salmonella typhi* and *Candida albicans* were clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University, Zaria. Twenty four hour broth cultures of these organisms were diluted in normal saline as this: *Staph. aureus* and *Candida albicans* were diluted to 1:1000, *E. coli* and *S. typhi* were diluted to 1:5000. Agar diffusion method was used for the susceptibility test. Sterile nutrient agar plates were flooded with the various dilutions of the test suspension of the organisms. The suspension of the organisms was then drained with sterile Pasteur pipettes. After draining, wells were bored into the inoculated plates with number 4 cork borer. The wells were filled with the ethyl acetate and *n*-butanol fractions (200mg/ml). The extracts were allowed to diffuse with the agar medium for 1 hour after which they were incubated for 24 hours at 37°C for bacteria and 25°C for 5-7 days for *C. albicans*. After incubation, diameter of zones of inhibition were measured to the nearest millimeter.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):- Broth dilution method of Ericsson and Sherris, (1971) was used. Two fold serial dilution of the ethyl acetate fraction (28mg/ml) and the butanolic fraction (32mg/ml) were made in nutrient broth suspensions (0.2ml) of 1:1000 dilutions of *Staph. aureus, C. albicans* and 1:5000 dilutions of *E. coli* and *S. typhi* were added to the tubes. Two controls: negative and positive controls were also set up. The positive control contained the nutrients broth and test organism to show the viability of the organisms and the growth properties of the medium, while the negative control contained only the nutrient broth to show the sterility of the medium. All the tubes were incubated at 37°C for 24 hours. After inoculation, sub cultures were made from each tube in the sterile nutrient agar plates as the mixtures were coloured. The plates were then

incubated for 24 hours at 37°C to determine the MIC. The least concentration of the fraction that showed no growth after 24 hours were considered as the MIC.

To determine the MBC, the plates were further incubated for 24 hours after which the least concentration that still showed no growth was considered the MBC and minimum fungicidal concentration (MFC).

Results

The results of the phytochemical screening revealed the presence of flavonoids, tannins, and sugar in the ethanolic extract, ethyl acetate and *n*-butanol fractions, while saponins was present in only the ethanolic extract and *n*-butanol fraction (Table 1). The ethyl acetate fraction was very active on the test organisms producing zones of inhibition ranging from 22.5 to 35mm (Table 2). The activity was most on *C. albicans* against which the fraction produced a zone of inhibition of 35mm and least on *S. typhi* with a zone of inhibition of 22.5mm. The butanolic fraction also had antimicrobial activity against all the test organisms but activity was most on *Staph. aureus* and least on *C. albicans* with zones of inhibition of 23.00mm and 15.2mm respectively (Table 2). The ethyl acetate fraction showed higher activity than *n*-butanolic fraction against each of the test organisms as the zone of inhibition produced by the former are higher than the ones produced by the latter in each case. Table 3 shows the MIC and the MBC of the two fractions against the test organisms. The ethyl acetate fraction showed lower MIC and MBC values against each of the test organisms. Ethyl acetate fraction showed the least MIC values of 0.44mg/ml against *Staph. aureus*, while the butanolic fraction had the least MIC value of 1.75mg/ml against *Staph. aureus* and *S. typhi*.

Table 1: Results of phytochemical screening

Class of constituents	Ethanolic extract	Ethyl acetate extract	*n*-butanol extract
Alkaloids	-	-	-
Flavonoids	+	+	+
Tannins	+	+	+
Steroids/ triterpenes	-	-	-
Saponins	+	-	+
Sugars	+	+	+

Table 2: Zone of inhibition for ethyl acetate and *n*-butanol soluble portion

Test Organism	Zone of inhibition (mm)	
	Ethyl acetate	*n*-butanol
E. coli	28.0	17.00
S. aureus	32.0	23.00
S. typhi	22.5	21.00
C. albicans	35.0	15.2

Table 3: Minimum Inhibitory and Minimum Bacterial Concentration (MIC & MBC) (mg/ml) of ethyl acetate and butanolic fractions

Test Organism	Ethyl acetate Fraction	Butanolic Fraction
E. coli	1.75 (3.5)	7.0 (14.0)
S. aureus	0.44 (0.88)	1.75 (3.5)
S. typhi	1.75 (3.5)	1.75 (3.5)
C. albicans	0.88 (1.75)	7.0 (14.0)

Values in parenthesis represent MBC

Discussion

The two fractions have good anti-microbial activity. The ethyl acetate fraction with lower MIC and MBC values has more activity than the butanolic fraction against all the tests organisms. The activity of the two fractions is more on the gram positive bacteria than on the gram negative ones as the zones of inhibition produced by these fractions are higher for *Staph. aureus* than the ones for *E. coli* or *S. typhi* (Table 2). The differences in susceptibility of the Gram-positive and Gram-negative bacteria to the two fractions might be related to the differences in the cell envelope structure of the two groups of bacteria species (Collee, 1978). While the structure is very simple in Gram-positive bacteria, it is complex in Gram-negative bacteria. The complex nature of the Gram-negative cell envelope has been found to retard or prevent the passage of antimicrobial agent through the envelope (Jawetz *et al.*, 1978; Davies, 1994). The antimicrobial activity of the extract can be explained by the presence of flavonoids and tannins in the fractions, these secondary metabolites are well known antimicrobial agents (Cowan, 1999; Mendoza *et al.*, 1997; Tereshuk *et al.*, 1997; and Waage and Hedin, 1985).

References

Akerele, O. (1988). Conservation of Medicinal Plant. A Cambridge University Press Publication, London18-20.

Amos, S.; Binda, L.; Kunle, O.F; Wambebe, C. and Gamaniel, K. (2002). Uterotonic properties of the ethanol extract of *Byrsocarpus coccineus. Pharm. Biol.* 40(1) 33-38.

Collee, J.G. (1978). Morphology and Nature of Bacteria, In Mackie and McKartney Medical Microbiology. 13th Edn. Eds. J.P. Duguod, *et al.* A Swan Publication, London. pp10-30.

Cowan, M.M. (1999). Plants products as antimicrobial agents. Clinical Microbiology Review 12. 560-565.

Dalziel, J.M. (1955). The Useful Plants of West Tropical Africa. A Crown Agents for Oversea Publication. London. pp568-570.

Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistant genes. Science 264, 375-382.

Ericsson H.A and Sherris, J.C. (1971). Antibiotics Sensitivity Testing Report of an International Collaborative Study. Acta. Pathological Microbiology 217B Supplement 1-90.

Irvine, F.R. (1961). Woody Plants of Ghana, An Oxford University Press Publication, London. pp 568-570.

Jawetz, O.E.; Melnick J.I. and Adelberg, E.A. (1978). Review of Medical Microbiology: 13th Edition Lange Publications, California pp

Mendoza, L; Wilkens, M. and Urzua, A. (1997). Antimicrobial study of the resinous exudates of diterpenoids and Flavonoids from some Chilean Pseudognaphalium (Asteralae). *J. Ethnopharmacol.* 58(2) 85-88.

Okunji, C.O and Iwu, M.M. (1988) Control of Schistosomiasis using some Nigerian medicinal plants as molluscicides. *Int.Journ. of crude drug.Res.* 26(4) 246-252.

Schultes, R.E. (1986). Ethnopharmacology Conservation, A Key To Progress In Medicine. Opera Botanica 92. 217-224.

Silva, G.L., Lee, I.S, and Kinghorn, A.D. (1998). In Natural Products Isolation Edited by Richard J. Cannel, A Humana Press Publication, New Jersey. pp 356-360.

Sofowora, A. (1993). Traditional Medicinal Plant in Africa. A Spectrum Publication, Ibadan. pp 102, 150-154.

Tereschuk, M.L; Riera, M.V; Castro, G.R and Abdala, I.R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagates minuta. J. Ethnopharmacol.* 50(1) 27-34.

Vickery,M and Vickery,B(1980) Coumarins and related compounds from members of connaraceae . *Toxicol letters* 5, 115-118

Waage, S.K. and Hedin, P.A. (1985). Quercertin 3-O-galactosyl (1→6)-glucoside with antibacterial activity. *Phytochemistry* 24 (2) 243-245.