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The evaluation of antimicrobial properties and phytoconstituent screening of *Brysocarpus coccineus* leaves grown in South-West Nigeria

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ABSTRACT

The preliminary phytochemical studies of Brysocarpus coccineus leaf extracts revealed the presence of saponins, tannins, steroids, reducing sugar, glycosides, flavonoids and anthraquinones. Hexane, ethylacetate and methanol successive extracts of Brysocarpus coccineus leaves effectively inhibited the growth of six test bacteria and six test fungi at different concentrations. The three extracts exhibited antibacterial properties on staphylococcus aureus and bacillus subtilis (gram positive), Escherichia coli, pseudomonas aeruginosa and klebsiellae pneumonae (gram negative), but no inhibition on salmonellae typhii at concentrations ranging from 25 to 200mg/ml. Meanwhile staphylococcus aureus and Escherichia coli were more sensitive to ethylacetate extract than hexane and methanol extracts. All the extracts exhibited intrinsic/higher antifungal properties on candida albicans, Aspergillus niger, Rhizopus stolon, penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum with activity comparable to that of the reference drug Tioconazole.

Keywords: *Bryocarpus coccineus*, antibacterial, antifungal, connaraceae, ethnomedicine.

INTRODUCTION

Bryocarpus coccineus schum and thonn. (Connaraceae) is a scandent or climbing scrub found in west and central Africa countries like Nigeria, Ghana, Cameroun, Ivory coast, Togo, Central African republic and Congo [1]. The plant is used in ethnomedicine for the treatment of veneral diseases, impotency, earache, jaundice, piles, sore of mouth and skin, tumour, wounds, stomatitis, swellings, rheumatism and as a urinary sedative [2,3,4]. The therapeutic properties of *Bryocarpus coccineus* as anti-inflammatory, antioxidant, analgesic, antidiarrhoeal and antipyretic have been established by various scientists [5,6,7,8,9]. The uterotonic, hepatoprotective, anxiolytic and sedative properties of various extracts of the plant have also been documented [10,11,12]. Dicoumarol, 4-hydroxy-coumarin, and flavonoids identified as quercetin, quercetin 3-O-α-arabinoside and quercetin 3-O-β-D-glucoside have been isolated from the leaves of

Brysocarpus coccineus [13,14]. In our efforts to study the biological activities of medicinal plants grown in Nigeria, we report on phytochemical and antimicrobial properties of *Brysocarpus coccineus* leaves grown in south-west Nigeria.

MATERIALS AND METHODS

Collection and authentication of the plant material

The plant material of *Brysocarpus coccineus* was collected from Ibadan, Oyo State of Nigeria, November 2009. Botanical identification and authentication was done by Mr. A.W. Ekundayo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen (FHI108801) was deposited.

Preparation of plant extracts

The whole plant of *Brysocarpus coccineus* was separated into leaves and stem, air-dried and weighed (stem, 1100g and leaves, 534g). The dried leaves was successively extracted in hexane, ethylacetate and methanol for 10 days respectively using cold extraction method. The resultant hexane (6g), ethylacetate (9g) and methanol (8g) extracts were obtained by evaporation and stored in the refrigerator for further use.

Phytochemical studies

The Preliminary phytochemical screening of the hexane, ethylacetate and methanol extracts of *B. coccineus* leaves was done using standard procedures [15,16,17,18,19].

Antimicrobial Assay

Microorganisms: Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive were used for the antibacterial assay. These were; *Salmonella typhii* (UCH 4801), *Escherica coli* (UCH 00260), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae* (UCH 2894) belongs to the gram-negative and *Bacillus subtilis* (UCH 74230) while *Staphylococcus aureus* (UCH 2473) belongs to the gram-positive. For the Antifungal assay, six fungi were also utilized. These were; *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum.* All the microorganisms used were clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays.

Antimicrobial agents: Gentamycin (10 µg/mL) and Tioconazole (0.7 mg/mL) were included as standard reference drugs in the study.

Antimicrobial activity determination

Agar diffusion-pour plate method (bacteria): An overnight culture of each organism was prepared by taken two wireloop of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24hr at 37^{o}C. From overnight culture, 0.1 mL of each organism was taken and put into the 9.9mL of sterile distilled water to obtained 10^{-2} inoculum concentration of the organism.

From the diluted organism (10^{-2}), 0.2mL was taken into the prepared sterile nutrient agar cooled to about 40-45^{o}C, then poured into sterile Petri dishes and allowed to solidify for about 45-60min. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of the test tubes for the experiment. For this work 8 wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24hr at 37^{o}C.

Agar diffusion-surface plate method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified properly. 0.2mL of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish cover to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8mm diameter. The graded concentrations of the extracts were put into the including the controls. All the plates were left on the bench for 2hr to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25^{o}C for 72hr [20,21,22].

RESULTS AND DISCUSSION

The preliminary phytochemical studies of the hexane, ethylacetate and methanol extracts indicated the presence of steroids, glycosids, flavonoids and anthraquinones (Table 1).

There was presence of saponins in ethylacetate and methanol extracts of *Brysocarpus coccineus* leaves but absent in hexane extracts of the plants, while tannins, was present only in ethylacetate extracts of *B.coccineus* leaves.

Table 1: Phytochemical constituents of the hexane, ethylacetate and methanol extracts of Brysocarpus coccineus leaves

Secondary metabolites	Extracts (whole plant)		
	Hexane	Ethylacetate	Methanol
Alkaloids	–	–	–
Saponins	–	++	++
Tannins	–	++	–
Reducing sugars	++	–	–
Steroids	++	++	++
Glycosides	++	++	++
Flavonoids	++	++	++
Anthraquinones	++	++	++

- Absent *++ Present*

The bacteria used for the antibacterial assay (Table 2) of the hexane, ethylacetate and methanol extracts were clinical strains of *staphylococcus aureus* and *bacillus subtilis* (gram positive). *Escherichia coli, pseudomonas, aeruginosa, klebsiellae pneumonae and salmonella typhii* (gram negative). Hexane and ethylacetate extracts showed higher inhibition on the six test organisms than the methanol extract.

Table 2: Antibacterial activities of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* Leaves

Extracts	Extract conc/Ref./ Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		S.a	E.coli	B.sub	Ps.a	Kleb	Sal.
Hexane	6.25	–	–	–	–	–	–
	12.5	–	–	–	–	–	–
	25	10	–	–	–	–	–
	50	12	10	10	–	–	–
	100	14	13	12	10	–	–
	200	16	17	14	12	12	–
	Hexane	–	–	–	–	–	–
	Gentamycin	37	33	36	34	33	33
Ethylacetate	6.25	–	–	–	–	–	–
	12.5	10	–	–	–	–	–
	25	12	11	–	–	–	–
	50	14	14	–	–	–	–
	100	16	17	10	–	10	–
	200	19	20	12	10	12	–
	Ethylacetate	–	–	–	–	–	–
	Gentamycin	37	33	36	34	34	33
Methanol	6.25	–	–	–	–	–	–
	12.5	–	–	–	–	–	–
	25	–	–	–	–	–	–
	50	10	–	–	10	–	–
	100	12	10	10	14	10	–
	200	15	14	14	16	12	–
	Methanol	–	–	–	–	–	–
	Gentamycin	37	33	36	34	32	34

S.a *Staphylococcus aureus*
E.coli *Escherichia coli*
B.sub *Bacillus subtilis*
Ps.a *Pseudomonas aeruginosa*
Kleb *Klebsiellae pneumonae*
Sal *Salmonellae typhii*
C.a *Candidas albicans*
A.n *Aspergillus niger*
Rhiz *Rhizopus stolon*
Pen *Penicillum notatum*
T.r *Tricophyton rubrum*
E.f. *Epidermophyton floccosum*

All the bacteria strains were sensitive to the three extracts of concentrations ranging from 12.5 to 200mg/ml using the agar broth cup diffusion procedure, except *salmonella typhii* which exhibit no sensitivity from the extracts. Further, the sensitivity of the test bacteria to all the extracts were

concentration dependent, activity being higher at higher concentration of the extracts. Meanwhile, hexane and ethylacetate extracts exhibited higher inhibition against the growth of *staphylococcus aureus and escherichia coli* than methanol extract while methanol extract of *B.coccineus* showed higher inhibition against the growth of *pseudomonas aeruginosa* than both the hexane and ethylacetate extracts.

Table 3: Anfungal activities of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* Leaves

Extracts	Extract conc/Ref./ Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		C.a	A.n	Rhiz	Pen	T.r	E.f
Hexane	6.25	–	–	–	–	–	–
	12.5	10	–	10	–	–	–
	25	12	10	13	10	10	10
	50	14	13	17	12	13	13
	100	16	17	20	15	16	16
	200	23	20	23	17	18	19
	Hexane	–	–	–	–	–	–
	Tioconazole	25	22	25	23	24	23
Ethylacetate	6.25	–	–	–	–	–	–
	12.5	–	–	–	–	10	10
	25	10	–	12	10	12	11
	50	12	11	14	12	14	13
	100	17	15	15	15	17	16
	200	19	19	17	18	19	19
	Ethylacetate	–	–	–	–	–	–
	Tioconazole	25	23	25	23	24	23
Methanol	6.25	–	–	–	–	–	–
	12.5	–	–	–	–	10	10
	25	10	–	10	10	11	11
	50	13	11	14	11	14	14
	100	15	15	16	14	17	16
	200	19	19	19	17	20	19
	Methanol	–	–	–	–	–	–
	Tioconazole	25	24	25	22	22	24

S.a *Staphylococcus aureus*
E.coli *Escherichia coli*
B.sub *Bacillus subtilis*
Ps.a *Pseudomonas aeruginosa*
Kleb *Klebsiellae pneumonae*
Sal *Salmonellae typhii*
C.a *Candidas albicans*
A.n *Aspergillus niger*
Rhiz *Rhizopus stolon*
Pen *Penicillum notatum*
T.r *Tricophyton rubrum*
E.f. *Epidermophyton floccosum*

The result of the antifungal activities of the hexane, ethylacetate and methanol extracts at concentrations ranging from 6.25 to 200mg/ml is presented in Table 3.

Six clinical strains of human pathogenic fungi were used in the study. *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum* and *epidermophyton floccosum.* The six test fungi were sensitive to hexane, ethylacetate and methanol extracts. Further, hexane, ethylacetate and methanol extracts exhibited higher antifungal properties on *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum* and *epidermophyton floccosum* with activity comparable to that of the reference drug tioconazole trosyd against the six test organisms at concentrations between 25 and 200mg/ml.

CONCLUSION

All tested extracts effectively inhibited the growth of bacteria and fungi, except *salmonella typhii.* The obtained results further confirm the use of the plant in traditional medicine for the treatment of veneral diseases, impotency, earache, sore mouth and skin, stomatitis, wounds, rheumatism etc [2,3,4].

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REFERENCES

[1] Dalziel JM, *The useful plants of West Tropical Africa,* A crown agent for oversea publication London, **1955,** pp 568-570
[2] Burkill HM, *Useful plants of west tropical Africa,* Ed.2, Vol 1, families A-D, Royal Botanic Gardens, kew, **1985.**
[3] Adjanohoun EJ, Ahyi AMK, Ake Assi L, Akpagana K, Chibon P, El-Hadji A, Eyme J, Garba M, Gassita JN, Gbeassor M, Goudote E, Guinko S, Houdote KK, Houngnon P, Keita A, Keoula A, Klugoocloo WP, Lo I, Siameri KM, Tafferne KK, *Contribution auxetudes ethnobotaniques et floristiques an togo,* Agence de cooperation culturelle et Techinique. Paris, **1986.**
[4] Irvine FR, *Woody plants of Ghana,* An oxford university press publication, London, **1961,** pp 568-570
[5] Akindele AJ, Adeyemi OO, *Fitoterapia,* **2007**[a], 78, 25-28.
[6] Oke JM, Hamburger MO, *African journal of Biomedical Research,* **2002,** 5(1-2), 77-79
[7] Akindele AJ, Adeyemi OO, *Nigerian journal of health and biomedical sciences,* **2006**[a], 5(1), 43-46.
[8] Akindele AJ, Adeyemi OO, *Journal of Ethnopharmacology,* **2006**[b]**,**108, 20-25
[9] Amos S, Binda L, Kunle OF, Wambebe C, Gamaniel K, *Pharmaceutical Biology,* **2002,** 40, 33-38
[10] Akindele AJ, Adeyemi OO, *International Journal of Pharmacology,* **2007**[b], 3(4), 357-361
[11] Akindele AJ, Ezenwanebe KO, Anunobi CC, Adeyemi OO, *Journal of Ethnopharmacology,* **2010,** 129, 46-52.
[12] Akindele AJ, Adeyemi OO, *International journal of Applied Research in Natural plant,* **2010,** 3(1), 28-36
[13] Vickery M, Vickery B, *Toxicological Letter,* **1980,** 5, 115-118
[14] Ahamadu AA, Hassan HS, Abubakar MU, Akpulu IN, *Africa journal of Traditional, Complimentary and Alternative Medicines,* **2007,** 4(3), 257-260

[15] Trease GE, Evans WC, *Drugs of Biological origin*, In: Pharmacognosy 12th ed, United Kingdom, BalliereTindall, **1983,** pp 305-540
[16] Trease GE, Evans WC, Pharmacognosy. Brailliar Tiridel Can, 13th ed. Macmillan Publishers, **1989**.
[17] Harborne JP, *Phytochemical Methods*, 2nd ed, Oxford University Press, London, **1991,** pp 51.
[18] Harborne JP, *Phytochemical Methods*, A guide to modern technique of plants analysis, 2nd ed, Chapman and Hall, London, **1998**.
[19] Edeoga HO, Okwu DE, Mbaebie BO, *African Journal of Biotechnology*, **2005,** 4, 685-688.
[20] Kavanagh F, *Analytical Microbiolology*, Academic press, New York, **1972**.
[21] Ziwadyk P, *Enteriobacteriaceae in Zinsser Microbiology*, 20th ed, Georg Thiene verlag, Stuttgart, **1972,** pp 544-546.
[22] Van den Berge AD, Vlietinck AJ, *Methods in Plants Biochemistry*, Vol.6, Assays for bioactivity. Hostettmann K. Ed, Academic press, London, **1991,** 47-69.