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Advances in Applied Science Research, 2011, 2 (5): 624-631

Advances in Applied Science Research
Pelagia Research Library
ISSN: 0976-8610
CODEN (USA): AASRFC

Baleria opaca: Its phytochemical evaluation, antibacterial and antifungal properties

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ABSTRACT

The preliminary phytochemical studies of Baleria opaca (whole plant) extracts revealed the presence of saponins, tannins, glycosides, alkaloids and anthraquinones, but absence of reducing sugars and flavonoids. Hexane, ethylacetate and methanol successive extracts of Baleria opaca effectively inhibited the growth of six test bacteria and six test fungi at different concentrations. The Methanol extract exhibited higher antibacterial properties than both hexane and ethylacetate extracts on Staphylococcus aureus and Bacillus subtilis (gram positive), Escherichia coli, Pseudomonas aeruginosa, Salmonellae typhii and Klebsiellae pneumonae (gram negative). All the extracts also exhibited significant antifungal properties on Candida albicans, Aspergillus niger, Rhizopus stolon, penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum at concentrations between 25 and 200 mg/ml.

Keywords: *Baleria opaca*, antibacterial, antifungal, Acanthaceae, ethnomedicine.

INTRODUCTION

Medicinal plants have played essential and exceptional roles since early century in the treatment of all kinds of diseases in Africa and other parts of the world. Most Africans still depend on tradition medicine/ethnomedicine of these plants for their medical care because they are considered safer, cheaper, more effective and culturally reliable. Bacterial and fungal infections are prevalent in our society, especially in Africa and these manifests in various diseases like pneumonia, diarrhoea, typhoid, gonorrhoea, syphilis and other sexually transmitted diseases. The search for new, safer and cheaper drugs especially from plants, to cure these diseases is on the increase. *Barleria opaca* (also known as child's vegetable) belong to the family Acanthaceae (Acanthus family) which is a taxon of dicotyledonous flowering plant containing 221 genera and at least 4000 species [1]. Most of them are tropical herbs, shrubs, or twining vines; some are epiphytes. Only a few species are distributed in temperate regions. Their main centres of distribution are Indonesia and Malaysia, Africa, Brazil and Central America. The representatives

of the family can be found in nearly every habitat, including dense or open forests, in scrublands, on wet fields and valleys, at the sea coast and in marine areas, and in swamps and as an element of mangrove woods [2]. *B. opaca* is a scrambling shrub with hairy stems and sometimes rooting at the nodes. leaves opposite, simple; petiole up to 0.5cm long, blade elliptical, 7.5 by 3.5cm, tapering at both ends with scattered simple hairs at both surfaces. The plant occurs in Western and Central tropical Africa, from Cote d'Ivoire to Gabon. The leaves of *B. opaca* are used to treat children for piles by squatting in a warm decoction. In Nigeria the whole plant is used in treating jaundice, rheumatism and paralysis, and the leaf sap is applied against catarrh [3]. The pharmacological and phytochemistry of *B. opaca* have not been reported. In our efforts to study the biological activities of medicinal plants in Nigeria, we report on Preliminary phytochemistry, antibacterial and antifungal properties of *Baleria opaca*.

MATERIALS AND METHODS

Collection and authentication of the plant material.

The plant material of *Baleria opaca* was collected from Ibadan, Oyo State of Nigeria, October 2010. 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 (FHI108470) was deposited.

Preparation of plant extracts

The whole plant of *Baleria opaca* was air-dried, grounded and weighed (689g). The dried plant was successively extracted in hexane, ethylacetate and methanol for 10 days respectively using cold extraction method. The resultant hexane (5g), ethylacetate (5.5g) and methanol (7g) extracts were obtained by evaporation and stored in the refrigerator for further use.

Phytochemical studies

The Phytochemical screening of the hexane, ethylacetate and methanol extracts of *B. opaca* (whole plant) was done using standard procedures [4,5,6,7,8].

1) Saponins: Small quantity of each extract was boiled with 5 ml of distilled water, filtered and cooled.
a). Frothing: To the filtrate (2.5 ml) about 10 ml of distilled water was added and shaken vigorously for 2 minutes. Frothing observed indicates a positive test.
b). Emulsification: To the filtrate (2.5 ml) added 3 drops of olive oil and shaken vigorously for 2 minutes. An emulsified layer indicates a positive test.

2) Alkaloids: Small quantity of each extract was stirred with 5 mL of 1% hydrochloric acid for five minutes on a water bath and then filtered. Of the filtrate of each extract was divided into two portions. Mayer's reagent was added to one portion; occurrence of creamy white precipitate was taken as positive. To the second portion few drops of Dragendorff's reagent was added and appearance of orange red precipitate was regarded as positive for the presence of alkaloids.

3) Glycosides (Keller-killiani Test): Small quantity of each extract was diluted in 5 ml of distilled water. Add 2 ml of glacial acetic acid containing one drop of ferric chloride solution (3.5%) to each. This was underlay with 1 ml of concentrated sulfuric acid. A radish brown ring is formed at the interface and upper layer turns bluish green on standing indicates the presence of a deoxy sugar characteristic of cardiac glycosides.
b) Method-2: Small quantity of each extract moistened with 5 ml in distilled water and filtered.

Few drops of chloroform were added to each (to enhance enzymatic activity). A sodium picrate–saturated filter paper strip was hanged at the neck of the flask with the help of the cork and warmed the flask. The filter paper strip turned brick-red or maroon is indicated the presence of cyanogenetic glycosides.

4) Tannins:

a) Ferric Chloride Test: Small quantity of each extract was boiled in 10 ml of water in a test tube and then filtered while hot and a few drops of 0.1% ferric chloride solution were added to the filtrate. A brownish green or a blue-black coloration indicates as a positive test.

***b)* Lead Acetate Test:** Small quantity of each extract was taken in a test tube and diluted with 5 ml of distilled water. Add few drops of a 1% solution of lead acetate to each. A yellow or red precipitate indicates a positive test.

5) Flavonoids: Three methods were used to determine the presence of flavonoids in the extracts.

a) Method-1: Dilute ammonia solution (5 ml) was added to aqueous filtrate of each extract followed by addition of concentrated H_2SO_4 acid (1 ml). A yellow colouration that disappears on standing indicates the presence of flavonoids.

b) Method-2: Few drops of 1% aluminium solution were added to aqueous filtrate of the each extract. A yellow coloration indicates the presence of flavonoids.

c) Method -3: A small portion of the each extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

6)Steroids:

a)Liebermann-burchard's Test: Small amount of each extract was dissolved in 1 ml of chloroform. Add 2 ml of acetic anhydride and 1 ml of concentrated H_2SO_4 acid to each portion. A greenish color is produced which turns blue on standing indicates the presence of steroids.

b) Salkowski's Test: Small amount of the extract was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added to a lower layer. A reddish-brown colour at the interphase indicates the presence of deoxysugar characteristics of cardenolides. A violet ring may form just above the ring and gradually spread throughout the layer.

7) Reducing sugars (Fehling's Test): A small portion of each of the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for 2 minutes. An orange-red precipitate on boiling with Fehling's solution indicates the presence of reducing sugars.

8) Anthraquinones: A small portion of each extract was boiled with 10 ml of sulfuric acid, traces of ferric chloride solution was added and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was taken into another test tube and 1 ml of dilute ammonia was added to each portion. Rose-pink color in the aqueous layer indicates the presence of anthraquinones.

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 0026), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae* (UCH 2894) belongs to the gram-negative while *Bacillus subtilis* (UCH 7423) and

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°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°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 *B. opaca* 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°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 *B. opaca* 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°C for 72hr [9,10,11].

RESULTS AND DISCUSSION

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

There was presence of saponins and alkaloids in ethylacetate and methanol extracts of *Baleria opaca* but absent in hexane extract of the plant, while steroids was present only in methanol extract of *B. opaca*. However, both reducing sugars and flavonoids were absent in all the extracts.

Table l: Phytochemical constituents of the hexane, ethylacetate and methanol extracts of *Baleria opaca* (whole plant)

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

- *Absent* ++ *Present*

Table ll: Antibacterial properties of the hexane, ethylacetate and methanol extracts of *Baleria opaca* (Whole plant)

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	-	-	-	10	-	-
	25	10	-	10	14	10	-
	50	12	10	12	16	12	10
	100	14	14	14	18	14	12
	200	16	18	18	20	16	14
	Hexane	-	-	-	-	-	-
	Gentamycin	38	36	34	34	36	38
Ethylacetate	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	-	10	-	-	10
	50	10	10	12	10	12	12
	100	12	12	14	12	14	14
	200	14	14	16	14	16	18
	Ethylacetate	-	-	-	-	-	-
	Gentamycin	38	36	36	34	36	34
Methanol	6.25	-	-	-	10	-	-
	12.5	10	10	-	14	10	10
	25	12	12	10	16	12	12
	50	14	14	14	18	14	14
	100	16	18	16	20	16	18
	200	20	22	20	24	18	20
	Methanol	-	-	-	-	-	-
	Gentamycin	37	33	36	34	32	34

Table lll: Antifungal properties of the hexane, ethylacetate and methanol extracts of *Baleria opaca* (Whole plant)

Extracts	Extract conc/Ref./ Control (mg/ml)	Diameter of well = 8 mm					
		Diameter of zone of inhibition of fungi(mm)					
		C.a	A.n	Rhiz	Pen	T.r	E.f
Hexane	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	10	-	-	-	-	-
	50	12	10	10	-	-	10
	100	14	12	12	10	10	12
	200	16	16	14	12	12	14
	Hexane	-	-	-	-	-	-
	Tioconazole	26	24	20	24	26	26
Ethylacetate	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	10	-	10	-	-	10
	50	12	-	12	10	-	12
	100	14	10	14	12	10	14
	200	18	12	16	14	12	16
	Ethylacetate	-	-	-	-	-	-
	Tioconazole	26	26	24	24	24	26
Methanol	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	10	-	-	10	-
	50	-	12	-	10	12	-
	100	10	14	10	12	14	10
	200	12	16	12	14	18	12
	Methanol	-	-	-	-	-	-
	Tioconazole	26	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 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). methanol extract showed higher inhibition on the six test organisms than the Hexane and ethylacetate extracts at different concentrations.

All the bacteria strains were sensitive to the three extracts at concentrations ranging from 12.5 to 200 mg/mL using the agar broth cup diffusion procedure. Hexane and methanol extracts exhibited higher inhibition against the growth of *staphylococcus aureus, escherichia coli, bacillus subtilis, pseudomonas aeruginosa and klebsiellae pneumonae* than ethylacetate extract of the plant. Meanwhile methanol extract of *B. opaca* showed higher antibacterial properties on the six test organisms than both the hexane and ethylacetate extracts.

The result of the antifungal activities of the hexane, ethylacetate and methanol extracts of *B. opaca* at concentrations between 6.25 and 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. Candida albicans* showed higher sensitivity on hexane and ethylacetate extracts than methanol extract, while methanol extract revealed higher antifungal activities on *Aspergillus niger and Tricophyton rubrum* than both hexane and ethylacetate extracts of *B. opaca*. Further, ethylacetate showed higher inhibition on *Rhizopus stolon* and *epidermophyton floccosum* than *Aspergillus niger, Penicillum notatum* and *Tricophyton rubrum,* while hexane extract showed higher inhibition on *Candida albicans* and *Aspergillus niger* than other test fungi at concentrations between 25 and 200mg/mL. However, the sensitivity of the test bacteria and fungi to all the extracts were concentration dependent, activity being higher at higher concentration of the extracts.

CONCLUSION

The antibacterial and antifungal properties of *Baleria opaca* extracts suggest the use of the plant for the treatment of infectious diseases caused by fungi and bacteria. The need for development of new antibacterial and antifungal drugs, and more importantly from natural sources cannot be overemphasized. This plant provides a good opportunity for drug development in this area. The continuation of study on the plant is on to isolates, identify, characterize and elucidate the structure of bioactive compounds responsible for the observed pharmacological activities.

Acknowledgements
H.A.A is grateful to the Department of Chemistry, University of Ilorin, Nigeria for the vital assistance rendered during this research.

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