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Full Length Research Paper

The antimicrobial activities and phytochemical screening of ethanolic leaf extracts of *Hedranthera barteri* Hook and *Tabernaemontana pachysiphon* Stapf.

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The antimicrobial efficacy and phytochemical screening of ethanolic leaf extracts of *Hedranthera barteri* and *Tabernaemontana pachysiphon* against *Staphylococcus aureus*, *Escherichia coli* and *Fusarium phoseolida* were determined using the Agar diffusion well method. Sensitivity in terms of zones of inhibition, minimum inhibitory concentration (MIC), minimum fungicidal and bactericidal concentration (MBC) and phytochemical composition of the leaf extracts were also determined. Results obtained showed that the ethanolic extracts of the leaf of *H. barteri* was the most potent inhibiting all isolates with diameters of zones of inhibition ranging between 2 - 15 mm, than the ethanolic leaf extracts *T. pachysiphon* which did not inhibit the growth of *F. phoseolida*. The extracts from both plants inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs ranging between 25 - 100 mg/ml, while MFCs/MBCs gave a range of 6.25 - 100 mg/ml. Phytochemical screening of leaf extracts of both plants showed the presence of active principles such as alkaloids, saponins, resins, flavonoids, polyphenols and carbohydrates.

Key words: Antimicrobial, *Hedranthera barteri*, *Tabernaemontana pachysiphon,* phytochemical, bacterial isolates.

INTRODUCTION

Hedranthera barteri and *Tabernaemontana pachysiphon* (Family Apocynaceae) are shrubs, found in damp situations of the closed forest in Ghana, North and South Nigeria, West Cameroon and also in Congo Brazzaville. The fruits of *H. barteri* and *T. pachysiphon* have been used traditionally to prevent miscarriages, treatment of sores and ulcers respectively (Thomas, 1910; Green, 1994). In Nigeria, *H. barteri* fruits have been implicated in herbal remedies against gonorrhoea, as a vermifuge and the exudates from the leaf used to suppress painful tumor (Ainslie, 1937). The leaf decoction is drunk by Igbos of South- Eastern Nigeria in treating dizziness (Thomas et al., 1967) while the bark and seeds of *T. pachysiphon* have been reported to contain conophargngine and alkaloids, and has been implicated in reducing breast inflammation (Burkill, 1985). Chukwujekwu et al. (2005) and Onasanwo and Elegbe (2006) reported its use as anti-inflammatory, antimalarial, antibacterial and anti-nociceptive agents. This stimulated interest to further investigate these plants with a view of determing the antimicrobial properties and phytochemical composition of the leaf extracts of these plants.

MATERIALS AND METHODS

The fresh leaves of *H. barteri* and *T. pachysiphon* were collected from Enugu in South-Eastern Nigeria during the month of May 2008. The plants were identified by a plant taxonomist of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

Sample preparation and extraction

The leaves were dried at room temperature (28°C) until completely dry and grounded into fine power using a motor laboratory plant mill (Christy and Norris Ltd, Chemsford England).

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Table 1. Antimicrobial activity of leaf extracts of *H. barteri* and *T. pachysiphon*.

Isolate	Mean zone diameter of inhibition (mm)					Extract/plant
S. aureus	12	10	6	21	0	Ethanolic /*H. barteri*
S. aureus	15	9	7	17	0	Ethanolic /*T. pachysiphon*
E. coli	15	9	7	17	0	Ethanolic /*H. barteri*
E. coli	7	5	3	18	0	Ethanolic /*T. pachysiphon*
F. phoseolida	5	4	2	16	0	Ethanolic /*H. barteri*
Conc. of extracts (mg/ml)	200	150	100	+ve control	-ve control	

Negative = 30% dimethylsulphoxide (DMSO); Positive = 10 ug/ml ciprofloxacin for all extracts.

The powder of leaves (50 g) each was subjected to hot extraction in 250 ml of ethanol. The soxhlet extractions with ethanol (99%) as described in AOAC (1980) were adopted for the study. The samples were transfer into the thimble and inserted into the soxhlet extractor. The extracts (16 g) each obtained were stored in sterile bottles.

Preparation of crude extract

The method of Akujobi et al. (2004) was adopted. The crude extract was diluted with 30% Dimethylsulpoxide (DMSO) to obtain 200, 150, 100, and 50 mg/ml concentrations. These were stored at 15°C until required.

Test microorganisms

The test organisms *Staphylococcus aureus* (NCTC 8532) and *Escherichia coli* (NCTC 09001) were grown on Nutrient agar while *Fusarium phoseolida* (NCTC 8863) was grown on potato dextrose agar for 24 h. These test organisms were obtained from Pharmaceutical Microbiology Department, University of Benin, Nigeria. These organisms (*S. aureus* (NCTC 8532) and *E. coli* (NCTC 09001) were subcultured and the pure cultures re-subcultured on nutrient agar and *F. phoseolida* (NCTC 8863) on potato dextrose agar slants and stored until required for the study.

Antimicrobial assay

Semi-solid nutrient agar and potato dextrose agar plates were seeded with (0.1 ml) containing 2×10^6 cells/ml of the standard inoculum of the test organisms. The plates were swirled to allow the inoculum to spread evenly on the surface of the agar, and the excess discarded in a disinfectant jar. The plates were kept on the bench for about 20 min to set, and dried in the incubator for 30 min at 37°C. With the aid of the sterile standard cork borer (5.0 mm), 6 wells were bored at equal distance around the plates. The bottoms of the wells were sealed with one drop of the sterile nutrient agar to prevent diffusion of the extracts under the agar. The 5th and 6th wells served as positive and negative controls. The negative control well was filled with dimethylsulphoxide (DMSO). Ciprofloxacin was used as the positive control. 0.2 ml of each prepared concentration of the extracts was aseptically introduced into wells 1 - 4. The plates were allowed on the bench for 40 min for pre-diffusion and then incubated at 37°C overnight. The resulting zones of inhibitions were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the organisms in questions at that particular concentration (Abayomi, 1982).

Minimum inhibitory concentration (MIC)

The MIC of the potent extracts was determined according to the macro-broth dilution technique (Baron and Finegold, 1990). Standardized suspension of the test organisms were inoculated into a series of sterile tubes of nutrient broth and potato dextrose broth containing two fold dilutions of leaf extracts and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Minimum fungicidal and bactericidal concentration

Minimum fungicidal and bactericidal concentrations were determined by first selecting tubes that showed no growth during MIC determination, a loopful from each tube was sub-cultured onto extract free agar plates and incubated for further 24 h at 37°C. The least concentration, in the MIC test at which no growth in the subculture was observed, was noted as the minimum fungicidal and bactericidal concentration

Preliminary phytochemical analysis of plants extract

This was carried out according to the methods described by Trease and Evans (1989).

Statistical analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA) (Sanders, 1990).

RESULTS

The antimicrobial and phytochemical properties of the leaf extracts of *H. barteri* and *T. pachysiphon* on the test isolates were revealed. The antimicrobial activities of ethanolic leaf extract of *H. barteri* and *T. pachysiphon* gave different mean diameter of zones of inhibition on the organisms tested (Table 1). The ethanolic leaf extract of *H. barteri* inhibited the growth of all the isolates giving mean diameter of inhibitions ranging between 6 – 12 mm for *S. aureus*, 7 - 15 mm for *E. coli* and 2 – 5 mm for *F. phoseolida*, while *T. pachysiphon* ethanolic leaf extracts gave the mean zone diameter of inhibition ranging from 9 – 15 mm for *S. aureus*, 3 – 7 mm for *E. coli* but did not inhibit the growth of *F. phoseolida*. All the data obtained

Table 2. Minimum Inhibitory concentration (MIC) of leaf extract of *H. barteri* and *T. pachysiphon.*

Isolate	Concentration of extracts (mg/ml)					Extract/plant	MIC
	100	50	25	6.25	3.13		
E. coli	-	-	-	+	+	Ethanolic/*H. barteri*	25
E. coli	-	-	+	+	+	Ethanolic/*T. pachysiphon*	50
S. aureus	-	-	+	+	+	Ethanolic/*H. barteri*	50
S. aureus	-	+	+	+	+	Ethanolic/*T. pachysiphon*	100
F. phoseolida	-	+	+	+	+	Ethanolic/*H. barteri*	100

- , No growth; +, Growth.

Table 3. Minimum fungicidal/bactericidal concentration of leaf extract of *H. barteri* and *T. pachysiphon.*

Isolate	Concentration of extracts (mg/ml)					Extract/plant	MFC/MBC
	100	50	25	6.25	3.13		
E. coli	-	-	-	-	+	Ethanolic/*H. barteri*	6.25
E. coli	-	+	+	+	+	Ethanolic/*T. pachysiphon*	100
S. aureus	-	-	+	+	+	Ethanolic/*H. barteri*	50
S. aureus	-	-	+	+	+	Ethanolic/*T. pachysiphon*	50
F. phoseolida	-	+	+	+	+	Ethanolic/*H. barteri*	100

-, No growth; +, Growth.

Table 4. Phytochemical analysis of leaf extract of *H. barteri* and *T. pachysiphon.*

Extract compound	*H. barteri*	*T. pachysiphon*
Alkaloids	+	+
Saponin	+	+
Resins	+	+
Tannins	-	+
Flavonoids	+	+
Anthraquinone	-	-
Cardiac glycoside	-	-
Carbohydrates	+	+
Polyphenol	+	+

+, Present; -, Absent.

showed no significant difference ($p > 0.05$). The negative control showed no inhibition on any of the bacterial isolates while the positive control inhibited all the bacterial isolates (Table 1).

The ethanolic leaf extracts of *H. barteri* MIC results appeared more potent than *T. pachysiphon* with MIC at 25, 50 and 100 mg/ml against *E. coli*, *S. aureus* and *F. phoseolida* respectively, while *T. pachysiphon* gave MIC values of gave 50 and 100 mg/ml against *S. aureus* and *E. coli* (Table 2). However, the ethanolic leaf extracts of the plants gave MBC of 6.25, 50 and 100 mg/ml for *E. coli*, *S. aureus* and *F. phoseolida* respectively for *H. barteri*, while leaf extracts of *T. pachysiphon g*ave MBC 50 and 100 mg/ml for *S. aureus* and *E. coli* (Table 3).

The phytochemical screening showed that the different leaf extracts of *H. barteri* and *T. pachysiphon* contain alkaloids, tannins, resins, saponins, glycosides, flavonoids and carbohydrates at different concentrations. However, some phytochemicals like tannins (*H. barteri)*, cardiac glycoside and carbohydrates (*H. barteri* and *T. pachysiphon)* were absent in these plant extracts (Table 4).

DISCUSSION

The results obtained in the study showed the anti-microbial efficacy of ethanolic leaf extract of *H. barteri* and *T. pachysiphon* on test isolates. The inhibitory activities agree with previous study for its use as anti-inflammatory and antibacterial (Chukwujekwu et al., 2005). The results obtained is also similar to the findings of Obi and Onuoha (2002), who documented alcohol as the best solvent for the extraction of plant active ingredients of medical importance.

In the study, the ethanolic leaf extracts of *H. barteri* inhibited all the organisms tested. The diffusion of those phytochemical within the agar matrix may explain the wider zone of inhibition (Cowan, 1999). The ethanolic leaf extracts of *T. pachysiphon* were non-inhibitory to *F. phoseolida*. The minimum inhibitory concentrations

observed from the ethanol leaf extracts of the two plants are quite high between a range of 25 - 100 mg/ml or *H. barteri*, and 50 - 100 mg/ml for *T. pachysiphon*. The results obtained for the minimum fungicidal and bactericidal concentration (MFCs/MBCs) gave a range of 6.25 - 100 mg/ml for *H. barteri* and a range of 50 - 100 mg/ml for *T. pachysiphon.* The ethanolic leaf extracts varied considerably from the results obtained for the minimum inhibitory concentrations. These variations in results implies that the MFCs/MBCs results obtained from plates cultures after plating on various dilutions of extracts are more reliable (cidal) compared to MICs results (static) obtained visually using turbidity as an index.

Conclusion

The study has shown that the seed extracts of *H. barteri* and *T. pachysiphon* have active ingredients/ phyto-chemicals which are able to inhibit pathogenic isolates. The observed antimicrobial effects of these two medicinal plants on the organisms tested, though *in-vitro* appear interesting and promising and may be effective as a potential sources of novel antimicrobial drugs.

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