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Antibacterial and antioxidant activity of three compounds isolated from *Mitracarpus scaber*

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ABSTRACT

The antibacterial and the free radical scavenging activities of the alcoholic extract, total alkaloid extract and three compounds isolated from *Mitracarpus scaber* were evaluated using the p-iodonitrotetrazolium method and the DPPH method. The extracts and the three compounds identified as methoxy-4-acetophenon, 3,4,5-trimethoxybenzoic acid, azaanthraquinone were tested *in vitro* against five micro organisms. Azaanthraquinone showed the most interesting activity with minimum inhibitory concentrations values of 7.5 µg/ml, 19 µg/ml, 38 µg/ml and 150 µg/ml on *Dermatophilus congolensis*, *Staphylococcus aureus*, *Enteroccocus feacalis, Escherichia coli* and *Pseudomonas aeruginosa,* respectively. On the other hand, the three tested compounds showed mild scavenging activity in the DPPH test, with IC_{50} values between 12 µg/ml and 45.74 µg/ml.

Keywords: *Mitracarpus scaber*, azaanthraquinone, antimicrobial activity, antioxidant activity.

INTRODUCTION

Resistance to antimicrobial agents is a major global public health problem. In the recent years incidence of multi-drug resistance in Gram positive (*Staphylococcus aureus, Streptococcus pneumoniae*), Gram negative (*Escherichia coli, Shigella spp., Haemophilus influenzae*) and other bacteria like *Mycobacterium tuberculosis,* has been reported from all over the world (Mulligen et al., 1993; Sajduda et al., 1998; Sanches et al., 1998). Many strains of *Streptococcus pneumonia*, *Streptococcus pyogenes* and *Staphylococcus spp.*, the organisms that cause respiratory and cutaneous infections, as well as *Pseudomonas spp.* and members of the *Enterobacteriaceae*, causing diarrhoea, urinary tract infections, and sepsis, are now resistant (Harold, 1992).

Likewise, the treatment of dermatophilosis, an enzootic and recurrent skin infection of bovine in tropical and subtropical countries caused by the gram positive bacterium *Dermatophilus congolensis* (Ogwu et al., 1981), still remains a matter of great concern. Similarly, oxidative stress and its adverse effects on human health has become a subject of considerable interest. Many efforts have been made to discover new antimicrobial and antioxidant compounds from various kinds of sources such as micro-organisms, animals, and plants. Systematic screening of folk remedies is another strategy in the discovery of novel effective compounds (Sanogo et al., 1996; Eloff et al., 2005). Recent studies have shown that the traditional use of an ointment containing alcoholic extracts of *Mitracarpus scaber* had a high efficiency against bovine dermatophilosis and cured tested animals without recurrence (Ali et al., 2003).

The purpose of this study was to evaluate the antibacterial and antioxidant activities of a *Mitracarpus scaber* ethanolic or methanolic extract and three isolated compounds (Bisignano et al., 2000) from this extract on strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enteroccocus feacalis* and *Dermatophilus congolensis*.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts of *Mitracarpus scaber* Zucc. ex Schult. & Schult. f. (Rubiaceae) were collected in the area of Cotonou, Abomey-Calavi and were identified and authenticated by the National Herbarium of the University of Abomey - Calavi (Benin) where a voucher specimen was deposited (n°: AA.6272/HNB). The plant was first dried at room temperature for 5 days during which it was turned over every day. Then, the plant was dried in an oven at 50 °C for 48 hours and subsequently reduced to coarse powder using a grinder and stored at room temperature. Part of this powder (500 g) was macerated in 4 L of ethanol 95° (Merck) for 72 hours by constant shaking. This extract was filtered and concentrated (yield = 15.9%). 5g of this extract was dissolved in 100 ml of 1N H_2SO_4 (final pH of the solution 2.5) and extracted three times by 100 ml of n-hexane. The acidic solution was basified (1N NaOH) to pH 9.9 and extracted three times with 100 ml of CH_2Cl_2. The organic phase (total alkaloid extract) was concentrated (2.9 % yield).

Antibacterial activity

In the first part of this work, the following test organisms were used to determine the minimal inhibitory concentration (MIC) of the plant extract and the isolated pure compounds: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enteroccocus feacalis* ATCC 29212. These species are the major cause of nosocomial infections in hospitals (Sacho and Schoub, 1993) and these strains are widely used in screening tests and references.

To determine the MIC of compounds isolated from *Mitracarpus scaber* (Bisignano et al., 2000) against each of these organisms, the microplate dilution method using tetrazolium violet to indicate growth of the bacteria was used (Eloff, 1998). Compounds were reconstituted to 20 mg/ml with acetone. 100 µl of the extract solution obtained were serial diluted in 96-well microplates. One colonie of each organism was introduced into 5 ml Luria Bertani (LB) broth and incubated for 1 hour. 100 µl of the resulting culture (10^6–10^7 CFU/ml) were added to each well. The plate was sealed and incubated at 37 °C for 18 hours. 40 µl of a 0.2 mg/ml solution of *p*-iodonitrotetrazolium violet (p-INT) dissolved in water were added to the microplate wells and incubated at 37 °C for 1-2 hours. The MIC value, which is the lowest concentration of plant extract, and compounds, at which bacterial growth was inhibited was then determined.

In the second part of our work, a gram-positive bacterium, *Dermatophilus congolensis* ATCC 14637 DSMZ (Deutsche Sammlung Van Mikoorganismer und Zellkulturen Gmbtl) was grown overnight on Tryptone Soya broth. The MIC of extract and compounds was determined using the agar dilution method (National Committee for Clinical Laboratory Standards, 1990). Inoculates of 10^3-10^4 CFU were spotted on

Muller-Hilton agar supplemented with the extract, compounds and antibiotic at concentrations ranging from 1000 to 2 µg/ml and 64 to 0.5 µg/ml, respectively, for the extract and for the antibiotic and compounds. The plates were incubated for 4 days at 37 °C. Tests were performed at least in duplicate. Gentamicin, tetracyclin and ampicillin were used as positive controls.

Antioxidant activity

Free radical scavenging activity was determined by means of the method previously described by Schmeda-Hirschmann et al. (2003) in which DPPH was used. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark at 4 °C between the measurements. 0.75 mL of a methanolic extract solution at different concentrations (1, 10 and 100 µg/mL), was placed in a test tube, and 1.5 mL of a DPPH methanolic solution (20 mg/L) was added. All determinations were performed in triplicate. The samples were incubated for 20 min in the dark at 30 °C and the decrease in absorbance at 517 nm was measured against a control prepared with methanol and a sample blank, using a spectrophotometer (Genova). The radical scavenging activities were calculated according to the formula below:

$$\text{RSA (\%)} = \frac{Ab - As}{Ab} \times 100$$

where, RSA = radical scavenging activity,
Ab = Absorbance of DPPH radical solution (t=0 min),
As = Absorbance of reaction mixture (t≠0 min) - Absorbance of sample plus methanol.

The RSA percentages were plotted against the logarithmic values of concentrations of test samples and, by extrapolation, EC_{50} value (which is defined as the concentration of sample that causes 50% loss of the DPPH activity) of each sample was determined.
Each assay was run in triplicate. Quercetol was used as a positive reference compound in this assay.

RESULTS AND DISCUSSION

The antibacterial activities of the alcoholic extract, total alkaloids extract and the three compounds previously isolated from *M. scaber* are reported in Table 1. There were differences in the inhibition of growth among the compounds against different strains of micro-organisms. The results obtained showed that the alcoholic and alkaloid extracts of *Mitracarpus scaber* as well as azaanthraquinone (AAQ) possess *in vitro* antimicrobial activity against *Dermatophilus congolensis* with IC_{50} values between 1 and 0.0075 mg/ml. The most interesting activity was obtained with AAQ, with an IC_{50} value of 7.5 µg/mL. This makes this compound a potent agent with potential clinical application. 4-Methoxyacetophenon and 3,4,5-trimethoxybenzoic acid did no show inhibitory effects at the levels tested against *D. congolensis*. *S. aureus* was the most sensitive of all the four test organisms, with a MIC value of 0.02 mg/ml followed by *E. faecalis* (0.04 mg/ml) and *E. coli* (0.15 mg/ml) (Table 1). 4-methoxyacetophenon was mildly active against *E. coli* (2.5 mg/ml). 3,4,5-trimethoxybenzoic acid also showed moderate activity against *E. faecalis* (1.25 mg/ml) and the three other organisms.

The results of our investigations confirmed the antimicrobial properties of *M. scaber* reported in previous studies (Irobi and Daramola, 1993; 1994; Sanogo et al., 1996). 2-aza-antraquinone (AAQ) identified in a sample of this specie from Nigeria (Okunade et al., 1999) possessed many properties: antiviral, antimicrobial on several germs (Okunade et al., 1999), antiprotozoal on *Trypanosoma congolense* and on chloroquine resistant *Plasmodium falciparum* (Solis et al., 1995). Moreover, other authors (Moulis et al., 1992) have reported the antifungal activity of pentalongin, a naphthoquinoid pigment isolated from the fresh aerial parts of *M. scaber*.

In addition, 3,4,5-trimethoxybenzoic acid, azaanthraquinone and 4-méthoxyacetophenonemoderate showed mild antioxidant activity in the DPPH assay, with an IC_{50} values of 45.74 µg/ml , 39.72 µg/ml and 11.65µg/ml respectively (Table 2).

Table 1: MIC values in mg/ml of alcoholic extract, total alkaloid extract, 4-méthoxyacetophenon, 3,4,5-trimethoxybenzoic acid and AAQ.

Extract/Compound	**Minimum inhibitory concentration (mg/ml)**				
	Dermatophilus Congolensis **ATCC 14637**	***Pseudomonas aeruginosa*** **ATCC 27853.**	***Enterococcus Faecalis*** **ATCC 29212**	***Staphylococcus Aureus*** **ATCC 25923**	***Escherichia coli*** **ATCC 25922**
Alcoholic extract	1	-	-	-	-
Total alkaloid extract	0.750	-	-	-	-
4-methoxyacetophenon	-	10	>10	>10	2.5
3,4,5-trimethoxybenzoic acid	-	5	1.25	5	5
Azaanthraquinone	0.0075	>10	0.038	0.019	0.15
Ampicillin	-	0.156	0.156	0.078	0.156
Gentamicin					0.0125
Tétracyclin	0.001				

nd: not determined

Table 2: Free radical scavenging activity, IC_{50} values (µg/ml).

Compound	**Free radical scavenging activity**	
	IC_{50} (µg/mL)	**R2**
3,4,5-trimethoxybenzoic acid	45.74	0.97
Azaanthraquinone	39.72	0.98
4-méthoxyacetophenone	11.65	0.91

Mitracarpus scaber is used to treat skin diseases in traditional medicine. The presence of phenolic compounds in this plant can justify its use for the treatment of skin infections caused by *Staphylococcus aureus* and *Candida albicans*.

Conclusion

The most active compound, azaanthraquinone, was isolated from *M. scaber* in our previous work. Further work should be directed towards testing the cytotoxicity against myoblastic rat L6 cell, expanding the assay to other micro-organisms and possible structure-activity study pre-clinical development. The high concentration of this compound in leaves also makes the use of leaf extracts a viable possibility. These results partly validate the ethnobotanical use of *M. scaber* as antimicrobial.

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