



Antibacterial and antifungal activities of some extracts and fractions of *Mitracarpus scaber* Zucc. (Rubiaceae)

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Abstract

Objective: To evaluate *in vitro* the potential antibacterial and antifungal activities of two crude extracts (n-hexane and 80% MeOH) from *Mitracarpus scaber* (Zucc) (Rubiaceae) leaves and that of fractions from of the portion of the 80% MeOH extract aiming to locate active fractions. **Materials and methods:** The n-hexane and 80% MeOH extracts of *M. scaber* leaves were separately obtained by maceration followed by exhaustive percolation with corresponding solvent. They were dried *in vacuo* and their antibacterial and antifungal activities evaluated *in vitro* by the dilution method against 21 clinical isolates including 13 bacteria, 3 dermatophytes and 2 yeasts. **Results:** The two extracts exhibited a pronounced antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus faecalis* (minimal inhibitory concentration and minimal bactericidal concentration < 65 µg/ml) and antifungal activity against *Candida albicans*, *Candida tropicalis* and *Trichophyton rubrum* (minimal inhibitory concentration and minimal fungicidal concentration < 65 µg/ml). Only the n-hexane extract exhibited a pronounced antimycotic activity against *Aspergillus flavus*, *Microsporum canis* and *Trichophyton mentagrophytes* (minimal inhibitory concentration and minimal fungicidal concentration < 65 µg/ml). *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Aspergillus niger* were found to be resistant to the effect of these extracts (minimal inhibitory concentration and minimal bactericidal concentration or minimal fungicidal concentration > 500 µg/ml). Both activities found in the 80% MeOH extract were located in the diethylether fraction from its partition with minimal inhibitory concentration of 7.8 to 62.5 µg/ml and minimal bactericidal concentration of 15.7 to 62.5 µg/ml for the antibacterial activity and, minimal inhibitory concentration of 7.8 to 31.25 µg/ml and minimal fungicidal concentration of 15.7 to 62.5 µg/ml for the antifungal activity against all cited microorganisms above. It was however ineffective against *Proteus mirabilis* and *Pseudomonas aeruginosa* (minimal inhibitory concentration and minimal bactericidal concentration > 500 µg/ml). **Conclusion:** *Mitracarpus scaber* leaf extracts possess a wide antibacterial and antifungal spectra of activity that can justify and support its traditional use as a remedy for the treatment of fungal and bacterial skin diseases.

Keywords: *Mitracarpus scaber*, Rubiaceae leaves, antibacterial and antifungal activities, skin diseases, traditional medicine.

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1. Introduction

Mitracarpus scaber Zucc (synonyms : *Mitracarpus villosus* (Sw). DC., *M. verticillatus*, *M. verticilatum* Vakte, *Staurospernum verticilatum* Schum et Thonn.) is one of the medicinal plants commonly found in subtropical countries. The leaves are highly used for the treatment of skin diseases such as mycosis, scabies and eczema. The young leaves are squeezed and rubbed on the affected corporal parts two or three times per day.

Other medicinal indications of this plant include infantile toothache, antiparasitic and venereal infections, it is used as an antidote to arrow poison by applying the powdered leaves to the wound and drinking of an aqueous decoction, it is also used as an antiinflammatory agent [1, 2]. The same preparation is also employed for the treatment of other diseases such as jaundice, hepatitis, hepatitis disorders and syndromes.

In the Democratic Republic of Congo, this medicinal plant commonly called Kafua-nkusu in Kasai (Tshiluba), Kibelekele or Yembudi mbula in Bandundu (Kikongo) and Bana banzazi in Bas-Congo (Kikongo) is popularly used as a remedy for the treatment of various skin diseases with some success using the same mode of treatment as described above. But, no report was found in the literature on the evaluation of the antimicrobial or antifungal activity of crude extracts, that can justify and support its use in the Congolese traditional medicine.

Nevertheless, previous investigations on *Mitracarpus scaber* collected in other countries have shown that leaf or inflorescence extracts (water, acetone and ethanol) exhibited an antibacterial and antifungal activity against some limited bacteria, fungi, dermatophytes and yeasts [3,4] by a diffusion method. The minimum inhibitory concentrations of ethanolic and hydroalcoholic extracts of aerial parts

against some *Staphylococcus aureus* methicillin resistant strains and four *Candida* species including *C. albicans*, *C. guilliermondii*, *C. crusei* and *C. parapsilosis* have been reported [5].

A literature research has failed to reveal any investigation on the antibacterial and antifungal activities of the n-hexane and 80% MeOH extracts of *M. scaber* leaves. Therefore, the present study was designed to explain the rationale use of this medicinal plant growing in DR Congo by the *in vitro* assessment, the antibacterial and antifungal activities of these two leaf crude extracts and that of some fractions from the partition of a 80% MeOH extract with solvents of different polarities against some clinical isolates of bacteria, fungi, dermatophytes and yeasts constituting a test battery of 21 human pathogenic microorganisms.

2. Materials and methods

2.1 Plant material

Young leaves of *M. scaber* were collected in Kinshasa, capital of the Democratic Republic of Congo (DR Congo) in February 1989. The plant was identified by M. N. Nlandu of the Institut National d'Etudes et de Recherches en Agronomie of the University of Kinshasa where a voucher specimen has been deposited. The plant material part was dried and reduced to powder.

2.2. Preparation of extracts and fractionation

Two different 50 g of powdered leaves were separately macerated and exhaustively percolated with 80% MeOH. and n-hexane For each solvent, the resulting macerate and percolate were combined and evaporated *in vacuo* to yield corresponding semi-dried extracts of 23.76 g (47.52%) and 7.4 g (14.80%) denoted as extract A and B respectively. An amount of extract A (20 g) was dissolved in 200 ml of distilled water, filtered and exhaustively extracted with diethylether,

ethylacetate and n-BuOH. Each fraction was further treated as described above, to give corresponding dried extracts denoted as A1 (3.54 g, 17.7%), A2 (2.74g, 13.70%) and A3 (5.21 g, 26.05%) respectively. The residual aqueous phase was also evaporated to dryness and denoted as A4 (8.13 g, 40.65%).

2.3. Preparation of test samples

Ten milligrams of each dried extracts and fractions were separately dissolved in 10 ml MeOH to obtain corresponding stock solution of 1 mg/ml. These stock solutions were diluted two-fold with the same solvent to have a series of concentrations of the test samples ranging from 3.4 to 500 µg/ml. Penicilline Na and Ketozazole from the Laboratoire d'Analyse et de Contrôle des Médicaments et des Denrées Alimentaires (LACOMEDA), Faculty of Pharmacy, University of Kinshasa were used as antibacterial and antifungal reference antibiotics respectively. 10 mg of each were treated in the same way as extracts, to give corresponding and different test concentrations.

2.4. Sources of selected microorganisms

Selected microorganisms included bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium fortuitum*, *Proteus mirabilis*, *Proteus morgani*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexneri*, *Streptococcus faecalis*, *Streptococcus pyogenes*, the yeasts *Candida albicans* and *Candida tropicalis*, the fungi *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*, the dermatophytes *Microsporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. They were clinical isolates from different pathologic medium from patients diagnosed as having various infections at the Laboratory of Bacteriology, Cliniques Universitaires du Mont Amba, University of Kinshasa, DR Congo.

2.5. Antibacterial and antifungal testing

The antibacterial and antifungal activities of *M. scaber* extracts were evaluated in vitro by a dilution method using Muller-Hinton and Sabouraud Dextrose Agar (SDA) as medium respectively. Briefly, in test tubes containing 0.5 ml of each microorganism suspension, 0.5 ml of test sample prepared as described above was added. The mixture was stirred. Two sets of controls were used.

One control was microorganism control and consisted of the culture medium with no plant extract or reference antibiotic; extract or reference cultured in medium without microorganisms was considered as a second control for sterility. All tubes were plugged with sterile cotton and incubated at 37°C for 24 h. The MIC (minimum inhibitory concentration) was regarded as the lowest concentration of samples in medium that did not permit any visible growth of microorganisms when compared with drug-free broths inoculated with lack of the microorganism suspensions.

To determine the MBC (minimal bactericidal concentration) or MFC (minimal fungicidal concentration), all tubes used in the MIC study which did not show any turbidity or growth of the microorganisms were subcultured onto the surface of a freshly prepared medium (Muller-Hinton for bacteria and Sabouraud Dextrose Agar for yeasts, fungi and dermatophytes). They were incubated at 37°C for 24 h for bacteria and 7 days for yeasts, fungi and dermatophytes). The MBC or MFC was defined as the lowest concentration that did not permit any visible microorganism colony growth on the medium after the period of incubation fixed in the present study.

3. Results and discussion

Two crude extracts (80% MeOH and n-hexane) and four fractions from the portion of the 80%

MeOH of *M. scaber* leaves were investigated for their potential antibacterial and antifungal activities *in vitro*. Results from the antibacterial testing using a dilution method are presented in Table 2. They show that both extracts had a similar antibacterial spectra. Considering their MIC and MBC values, it was clearly observed that the n-hexane extract (extract B) is in general more active than the 80% MeOH one (extract A).

The values of different inhibitory or bactericidal concentrations ranged from 7.5 to 62.5 µg/ml for extract B while that of extract A varied from 31.25 to 125 µg/ml. These effects were particularly observed against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus faecalis* implicated in various skin infections [6].

Other sensitive bacteria included *Bacillus cereus*, *Mycobacterium fortuitum*, *Shigella flexneri*, *Bacillus subtilis* at different degrees. The two extracts were however inactive against *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Proteus morganii* and *Pseudomonas aeruginosa* growth (MIC and MBC > 500 µg/ml).

Our results are only qualitatively in good agreement with those reported [5] using the same testing method concerning the antibacterial activity of polar extracts of this medicinal plant against *S. aureus*, the only antibacterial tested by these authors. The fractionation of extract A with solvents of different polarities resulted in the obtaining of 4 fractions tested in the same conditions. Results presented in Table 1 indicate that fraction A1 (Et₂O) possessed a wide antibacterial spectra than fractions A2 (EtOAc), A3 (n-BuOH) and A4 (residual aqueous phase).

It exhibited a pronounced antibacterial activity against *B. cereus*, *B. subtilis*, *M. fortuitum*, *S. flexneri*, *S. aureus*, *S. epidermidis*, *S. faecalis*, and *S. pyogenes* (MIC and MBC < 65 µg/ml)

than its parent extract. It was however moderately active without a significant bactericidal effect against *K. pneumoniae* and *P. morganii*, and did not inhibit the growth of *P. mirabilis* and *P. aeruginosa* at the higher test concentration of 500 µg/ml (Table 1).

Fraction A2 showed a pronounced activity against *B. cereus*, *S. aureus* and *S. epidermidis* (MIC and MIC < 65 µg/ml, but higher than that of A1, A2 also exhibited a good bacteriostatic (MIC < 65 µg/ml) and a moderate bactericidal (125 ≤ MBC ≤ 500 µg/ml) effect against *S. flexneri* and *S. faecalis*.

Its activity against *P. morganii* and *S. pyogenes* was moderate without a bactericidal action. Fraction A3 was moderately active against *B. cereus*, *B. subtilis*, *S. flexneri* and *S. epidermidis*. A2 and A3 fractions were however inactive against the remaining selected bacteria when tested at the higher test concentration of 500 µg/ml. A4 were found to be inactive against all selected bacteria (Table 2).

Although the same fractions were previously obtained from an EtOH extract of *M. scaber* leaves, their MIC and MBC reported [5] are in the same order than those reported here concerning their effects against *S. aureus* and *S. epidermidis* growth.

On the other hand, results presented in Table 2 show that both the 80% MeOH and n-hexane extracts exhibited an antifungal activity at different extents. Extract A showed a pronounced activity against *Candida albicans*, *Candida tropicalis* and *Trichophyton rubrum* (MIC and MFC < 65 µg/ml). It also showed a good fungistatic effect against *Microsporum canis* (MIC = 62.5 µg/ml) with a moderate fungicidal effect (MFC = 250 µg/ml), its fungistatic effect against *Aspergillus fumigatus* was moderate (125 ≤ MIC ≤ 250 µg/ml) without a fungicidal property (Table 2). It also exhibited

Table 1.
Antibacterial activity of extracts and fractions from *Mitracarpus scaber* leaves (MIC and MBC in µg/ml)

Microorganisms/ext.and fract.	A		B		A1		A2		A3		A4	
	MIC	MBC										
<i>Bacillus cereus</i>	62.5	125	31.25	31.25	15.7	31.25	31.25	62.5	125	250	> 500	> 500
<i>Bacillus subtilis</i>	250	> 500	31.25	125	62.5	62.5	250	> 500	125	250	> 500	> 500
<i>Escherichia coli</i>	> 500	> 500	> 500	> 500	125	250	> 500	> 500	> 500	> 500	> 500	> 500
<i>Klebsiella pneumonia</i>	> 500	> 500	> 500	> 500	250	> 500	> 500	> 500	> 500	> 500	> 500	> 500
<i>Mycobaterium fortuitum</i>	31.25	125	62.5	62.5	15.7	31.25	> 500	> 500	> 500	> 500	> 500	> 500
<i>Proteus mirabilis</i>	125	> 500	250	> 500	>500	>500	> 500	> 500	> 500	> 500	> 500	> 500
<i>Proteus morgani</i>	> 500	> 500	> 500	> 500	125	> 500	125	> 500	> 500	> 500	> 500	> 500
<i>Pseudomonas aeruginosa</i>	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
<i>Shigella flexneri</i>	62.5	62.5	31.25	62.5	7.8	31.25	62.5	125	62.5	125	> 500	> 500
<i>Staphylococcus aureus</i>	31.25	62.5	7.8	31.25	7.8	15.7	15.7	32.25	> 500	> 500	> 500	> 500
<i>Staphylococcus epidermidis</i>	62.5	62.5	15.7	31.25	15.7	15.7	15.7	62.5	250	> 500	> 500	> 500
<i>Streptococcus faecalis</i>	125	250	62.5	125	125	62.5	62.5	125	> 500	> 500	> 500	> 500
<i>Streptococcus pyogenes</i>	62.5	250	62.5	125	62.5	62.5	250	> 500	> 500	> 500	> 500	> 500

MIC: minimal inhibitory concentration, MBC: minimal bactericidal concentration, MFC: minimal fungicidal concentration, ext.: extracts, fract.: fractions, A : 80% MeOH extract, B : n-hexane extract, A, A1, A2, A3 and A4 : diethylether, EtOAc, n-BuOH and aqueous fractions from the partition of the 80% MeOH extract respectively,

a moderate antimycotic activity against *Trichophyton mentagrophytes* ($125 \leq \text{MIC}$ and $\text{MFC} \leq 250 \mu\text{g/ml}$) and was however inactive against *Aspergillus niger* (MIC and $\text{MFC} > 500 \mu\text{g/ml}$).

In contrary, extract B showed a pronounced antimycotic activity against *Aspergillus flavus*, *C. albicans*, *C. tropicalis*, *M. canis* and *Trichophyton rubrum* (MIC and $\text{MFC} < 65 \mu\text{g/ml}$). It also showed a good fungistatic effect and a moderate fungicidal action against *T. mentagrophytes*, a moderate antimycotic effect against *A. fumigatus* (Table 3).

Although these two crude extracts have a similar antifungal spectra, the antimycotic activity of the n-hexane extract (extract B) is in general, higher than that of the 80% MeOH extract (extract A). As observed for the antibacterial activity of fractions from the partition of the extract A, fraction A1 was found to be the most active against all selected fungi, dermatophytes and yeasts with MIC and MFC ranging from 7.8 to $62.5 \mu\text{g/ml}$ according to their susceptibility.

The most sensitive microorganisms were *C. albicans*, *M. canis* and *T. mentagrophytes* with MIC and MFC ranging from 7.8 to $15.7 \mu\text{g/ml}$, followed by *A. fumigatus*, *A. flavus*, *C. tropicalis* and *T. rubrum* with MIC and MFC comprised between 15 and $32 \mu\text{g/ml}$ whereas *A. niger* (MIC and $\text{MFC} = 31.25 \mu\text{g/ml}$) was the most resistant. Fraction A2 exhibited only a pronounced antimycotic activity against *C. tropicalis* and *Microsporum canis* (MIC and $\text{MFC} < 65 \mu\text{g/ml}$).

It exhibited also a good fungistatic effect against *A. flavus*, *T. mentagrophytes* and *C. albicans*, ($\text{MIC} = 62.5 \mu\text{g/ml}$) and a moderate fungicidal effect ($125 < \text{MFC} \leq 250 \mu\text{g/ml}$). Its antimycotic effect against *T. rubrum* growth was moderate, this fraction was however ineffective against *A. niger* growth (MIC and $\text{MFC} > 500 \mu\text{g/ml}$).

Fractions A3 and A4 were devoid of any appreciable antimycotic effect against the growth of all selected fungi, dermatophytes and yeasts when tested at the highest test concentration of $500 \mu\text{g/ml}$.

Our results qualitatively corroborate with those reported [5] for the effect of polar extracts and fractions from the partition of the first one against only *C. albicans*. The reference products used in our present investigation have shown a higher antibacterial and antifungal activity (MIC , MBC or $\text{MFC} < 1 \mu\text{g/ml}$) than that exhibited by extracts and fractions from *M. scaber* leaves.

Other previous investigations on *M. scaber* have also confirmed the antibacterial and antifungal activities of ethanolic and aqueous leaf and inflorescence extracts by the diffusion method (amount of tested extracts : 5 to 30 mg) [3, 4, 7].

Although the n-BuOH fraction (A2) had been previously shown to be inactive against *S. aureus* and *C. albicans*, the only selected and tested microorganisms [5] has also confirmed in the present work, [8] reported the isolation and structure elucidation of active constituents from this inactive fraction.

These active principles included gallic acid and 3,4,5-trimethoxy-benzoic acid inhibiting *S. aureus* growth with $\text{MIC} = 3.90$ and $0.97 \mu\text{g/ml}$ respectively; 4-methoxyacetophenone and 3,4,3'-trimethoxyacetophenone had been shown to be effectively active against *C. albicans* growth ($\text{MIC} = 1.95 \mu\text{g/ml}$). Other isolated compounds such as kaempferol 3-O-rutinoside, rutin and psoralen have been reported to exhibit a low antibacterial and antimycotic activity ($\text{MIC} = 125 - 500 \mu\text{g/ml}$).

This finding has some significance in this sense that an inactive extract or fraction may contain constituents which, when isolated and tested alone might show some activity that was not

Table 2.
Antifungal activity of extracts and fractions from *Mitracarpus scaber* leaves (MIC and MFC in µg/ml)

Microorganisms/ext.and fract.	A		B		A1		A2		A3		A4	
	MIC	MFC										
<i>Aspergillus flavus</i>	62.5	125	62.5	62.5	15.7	31.25	62.5	125	> 500	> 500	> 500	> 500
<i>Aspergillus fumigatus</i>	250	> 500	125	250	15.7	31.25	125	> 500	> 500	> 500	> 500	> 500
<i>Asergillus niger</i>	> 500	> 500	> 500	> 500	31.25	31.25	> 500	> 500	> 500	> 500	> 500	> 500
<i>Candida albicans</i>	62.5	62.5	15.62	31.25	7.8	7.8	62.5	250	250	> 500	> 500	> 500
<i>Candida tropicalis</i>	31.25	62.5	31.25	31.25	15.7	31.25	31.25	62.5	125	250	> 500	> 500
<i>Microsporium canis</i>	62.5	250	62.5	62.5	7.8	15.7	31.25	62.5	> 500	> 500	> 500	> 500
<i>Trchophyton mentagrophytes</i>	125	500	31.25	62.5	7.8	15.7	62.5	125	> 500	> 500	> 500	> 500
<i>Trichophyton rubrum</i>	31.25	62.5	31.25	62.5	15.7	31.25	125	250	> 500	> 500	> 500	> 500

MIC: minimal inhibitory concentration, MBC: minimal bactericidal concentration, MFC: minimal fungicidal concentration, ext.: extracts, fract.: fractions, A : 80% MeOH extract, B : n-hexane extract, A, A1, A2, A3 and A4 : diethylether, EtOAc, n-BuOH and aqueous fractions from the partition of the 80% MeOH extract respectively,

observed with the parent extract or fraction. The existence of antagonism between different constituents of these kind of extracts or fractions, which is in general favourable for the loss or to the decreasing of a biological activity could not be excluded.

Furthermore, an investigation conducted [9] on an ethanolic extract of the leaves had resulted in the isolation and structure elucidation of isoquinoline-5,10-dione, psychorubrin, 3-O-ethylpsychorubrin, tectoquinone as an artifact and stigmasterol. With the exception of the last two compounds, the remaining isolated constituents have been shown to be active against *S. aureus*, the only tested microorganism with MIC ranging from 3.125 to 12.5 µg/ml.

The most active compound was psychorubrin (MIC=3.125 µg/ml) followed by 3-O-ethylpsychorubrin (MIC = 6.25 µg/ml while benz (g) isoquinoline-5,10-dione showed an activity 10 times less than that reported [10] against *S. aureus*.

Our preliminary phytochemical screening of the 80% MeOH extract of *M. scaber* leaves has revealed the presence of flavonoids, coumarins, steroids, terpenes, tannins and anthraquinones. The presence of alkaloids was doubtful although it has been reported [10] that the isolation of an azaanthraquinone alkaloid, a benz[g] isoquinoline-5,10-dione with a pronounced antibacterial activity against *S. aureus* and *B. subtilis* (MIC = 1.5 µg/ml), and *Mycobacterium intracellulare* (MIC = 6.25 µg/ml). It exhibited also an interesting antifungal activity against *C. albicans* and *Cryptococcus neoformans* (MIC = 6.25 µg/ml).

The same compound was also isolated [9] from an ethanolic extract of *M. scaber* leaves. This compound isolated from *Psychotria*

camponutans (Rubiaceae) was also reported to exhibit an antimalarial activity [11]. Results from the phytochemical screening of the n-hexane indicated the presence of steroids and terpenes as the major constituents. The extract also gave a positive test with Neu's reagent (1% diphenylboric ethanolamine complex in MeOH) suggesting the presence of lipophilic flavonoids.

Moreover, previous studies on other medicinal plants have reported the antibacterial and/or the antifungal activity of phenolic and phenylpropanoic compounds, coumarins, methylated and prenylated flavonoids [12-15] terpenic compounds [16-21] and anthraquinone [9].

Even tannins have also been reported to exhibit some levels of antibacterial activity [22, 23]. Both activities of *M. scaber* leaf extracts reported here could be related to the presence of these kind of phytochemical groups also present in this medicinal plant as revealed by phytochemical screening.

Further phytochemical investigations are in progress to isolate and identify active principles mainly in the diethylether fraction from the portion of the 80% MeOH extract. The results reported here may justify and support the use of *M. scaber* leaves as a remedy for the treatment of some skin diseases particularly the corporal mycosis in the Congolese traditional medicine.

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