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Antimicrobial activity and phytochemical screening of *Maerua pseudopetalosa* (Glig and Bened.) DeWolf

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Abstract

Chloroform, ethyl acetate and ethanol, which were used for the extraction of *Maerua. pseudopetalosa* parts, gave nine extracts with different colors, consistencies and extractabilities. Four bacterial strains (*Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi,* and three fungal species: *Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans*) were tested for antimicrobial activities of the extracts. The antibacterial activity was of the limited type (only 11%); while antifungal activity showed considerable variations depending on the type of the extract, the fungus and the duration of the treatment. Tests for secondary metabolites proved the presence of tannins, sterolsL and alkaloids. The detection of sterols, alkaloids, flavonoids and cardiac glycosides were proved by TLC separation in the crude extracts.

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Introduction

Maerua pseudopetalosa (Gilg and Bened.) De Wolf (Capparaceae) is a medicinal plant of great repute in South Central Sudan. It is commonly known locally as "Kordale" .The roots are used for cough and for treatment of tumors. The roots of *M. pseudopetalosa* showed activity against some bacterial strains (Henry,1948); however, there is insufficient information regarding the antimicrobial activities of other different parts of the plants . The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectrum of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (Sieradski, *et al.*, 1999).Hence, there is need to investigate the antimicrobial properties of plant extracts that have not been done.

The production of the secondary compounds is often low (less than 1% dry weight) and depends mainly on the physiological and developmental stages of plants (Nandani *et al.,* 2013). Even though this plant is of a wide spread use, yet there is little available literature on the scientific evaluation of their antimicrobial activity and secondary metabolites. Therefore this study is aimed to evaluate the phytochemical screening and antimicrobial activity of the plant *M. pseudopetalosa* extracts as a new potential sources of natural antimicrobial activities.

Materials and methods

Plant Materials

The plant under investigation (*M. pseudopetalosa*) was collected from the Aaly Al Neel state (Republic of South Sudan).The plant was authenticated at the Department of Botany by Prof. Hatil, H. Elkamali, Omdurman Islamic University.

Preparation of crude plant extracts

The plant material was air dried, ground into a coarse pawder form and150 grams from each plant part(Tuber,root and whole aerial parts) were extracted using solvents of increasing polarities (chloroform, ethyl acetate, and ethanol). The plant materials were soaked and shaken overnight on a shaker, then filtered, evaporated to dryness under reduced pressure in a rotatory evaporator and weighed. Three replicates were used for each sample.

Assay for Antibacterial Activity

The media used for antibacterial screening were nutrient agar and nutrient broth.

Dilution of the bacterial concentration was carried out by taking one ml of the bacterial suspension into 9 ml of normal saline in a sterile test tube. This suspension usually contains 10^8 Colony-Forming Units per ml (CFU/ml). A fresh stock suspension was prepared every two weeks intervals.

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted to assess the antibacterial activity of the extracts. Two ml of the standardized bacterial suspensions were mixed thoroughly with 250 ml of the nutrient agar. Aliquots of the inoculated agar (25ml) were distributed into sterile Petri dishes. The agar was left to set and 4 wells (7 mm in diameter) were made using a sterile cork borer (No 7). The crude plant extracts were dissolved in ethanol and three concentrations were made(50, 100 and 200 mg/ml). Then 0.1 ml of each concentration was dropped into each of the wells; leaving one well to be filled with 0.1 ml ethanol as a control.

The effect of the extracts and control on the bacterial growth were recorded after 18-24 hours by measuring the inhibition zone diameters for each treatment.

Preparation of the Culture Media of Fungi

The potato dextrose agar (PDA) medium was prepared by dissolving 39 grams and 500 mg chloramphenicol in one liter distilled water. Ten ml portions of the prepared medium were sterilized in 30 ml bottles. The fungal cultures were maintained in the PDA media as slopes and incubated for about seven days for *Aspergillus species* and two days for *Candida albicans*. The fungal growth was washed with 100 ml sterile normal saline and then the suspensions were kept at 4C° until use.

The Liquid Medium Method for Antifungal Activity

This method was carried out for the two Aspergillus fungi only; as was described by Mohammed (2007). CZAPEK DOX liquid medium was prepared by dissolving 33.4 grams of the powder in one liter of distilled water and were sterilized in large test tubes; 20ml in each tube. To the 20ml of the medium 0.1ml of the fungal suspension was added. Three concentrations of the plant extracts were used in order to test their effects on the complete growth of the two fungi

Activity Against non-Filamentous Fungi

The complete medium was prepared by dissolving 4grams yeast extract, 10grams malt extract and 4grams glucose in 1litre of distilled water, the pH was adjusted to 5.5. Hundred ml of the medium were taken in 500 ml Ehrlenmeyer flask and were inoculated with the corresponding test organism. This was followed by incubation in a rotary shaker (120 rpm) at room temperature for 24 h. The culture was centrifuged in sterile 10ml Falcon tubes. The supernatant was discarded and the residue was re-suspended in normal saline. The cells were vortexed in approx. 40 ml sterile saline, centrifuged and the supernatant was discarded. Finally the cells were re-suspended in sterile normal saline and the turbidity was adjusted to an O.D of 1.3 at 546nm.

Two ml of the prepared suspension were used to inoculate 200 ml of melted agar in 500 ml Erlenmeyer flasks. The contents of the flasks were mixed thoroughly and 10 ml of each of the inoculated media were poured aseptically in Petri-dishes. Filter paper disks (6 mm) were loaded each with 10 µl of each of the extracts, left to dry and placed into the agar plates. The plates were incubated for 24 h at 27 C° or 37 C° depending on the test organism (Fiedler, 2004). The diameters of the growth inhibition zones were then measured and recorded.

Chemical Tests

Preliminary screening for the major classes of secondary metabolites was carried out using techniques described by Harborne (1998).

Tannins

Two ml of ethanol extract were placed in a test tube and 2-3 drops of 5% ferric chloride solution were added. Formation of a blue or a green color indicates the presence of tannins.

Saponins

Five ml of the ethanol extract were taken in a test tube and 5 ml of water were added and the tube was corked and shaken vigorously. Formation of persistent foam, which remains stable for at least one hour, indicates the possible presence of saponins.

Sterols and triterpenes

One gram of the powdered plant material was macerated with 20 ml of petroleum ether for six hours, filtered and the ether was evaporated to dryness. The residue was dissolved in 2 ml acetic anhydride, transferred to a test tube and concentrated sulphuric acid was poured, cautiously, along the side of the test tube. Possible presence of sterols or triterpenes is indicated by the immediate appearance of a violet color in case of the presence of triterpenes, which changes to green on standing in case of sterols.

Flavonoid glycosides

Detection of flavonoid glycosides was carried by using the following procedure:

Six grams of the pawdered material were extracted with 100 ml methanol in a Soxhlet apparatus for three hours. The methanol extract was evaporated to dryness using rotatory evaporator. The residue was dissolved in 50 ml of distilled water and filtered. The aqueous extract was tested for the possible presence of glycosides as follows:, 5 drops of 2% ferric chloride solution in methanol were added to 2 ml of the extract in a test tube. Formation of a green color indicates the presence of flavonoid compounds.

Alkaloids

Ten grams of the powdered plant material were macerated with 10% acetic acid in 50 ml 80% aqueous ethanoland were allowed to stand for 24 hours.They were, then, filtered and concentrated using rotatory evaporator. Small portions of the

concentrated extract were placed in separate test tubes to which 2-3 drops of the alkaloidal reagent (Dragendorff) were added. The remaining portion of the extract was made alkaline with strong ammonia solution, allowed to stand for one hour, and filtered. The residue was air-dried and extracted with chloroform.

The chloroform extract was evaporated to dryness and the residue was dissolved in a few ml of methanol. The methanol solution was acidified with diluted hydrochloric acid and tested for the presence of alkaloids. The test was recorded positive when turbidity or precipitation was obtained.

Chromatographic Separation

TLC plates were prepared by using silica gel F254 type G 60 as a stationary phase according to Harborne (1973). A number of developing systems were prepared and many trials were carried out in order to attain the most suitable solvent systems that give good separation.

Results and discussion

Antibacterial Activity

The antibacterial effects exhibited by the three solvent extracts varied with the extracted parts and the bacterial strains used (Table 1).

Table 1. Mean inhibition zone diameter (mm) caused by the three concentrations (50, 100, 200 mg/ml) of different extracts against the tested bacterial strains.

Plan t part	Used extract	Bacterial strain											
		S.aureus			S.typhi			B.cereus			E.coli		
		Cons.mg/ml											
		50	100	200	50	100	200	50	100	200	50	100	200
Tuber	Chloroform	2 0	-	-	-	-	-	-	-	-	-	-	-
	Ethanol		-	-	-	-	-	-	-	-	-	-	-
	Ethyl acetate	15	17	17	-	-	-	-	-	-	-	-	-
Root	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
	Ethyl acetate	-	12	19	-	-	-	-	14	20	-	-	17
W.A	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	19	21	20	-	19	-	-	-	-	18	19	20
	Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-

S.aureus = Staphylococcus aureus; *S.typhi = Salmonella typhi* ; *B.cereus =Pacillus cereus* ; *E.coli ; Escherichia coli* ; - = no inhibition; W.A = Whole aerial parts.

Table 2. Antifungal screening of *M. pseudopetalosa* of chloroformic extracts at different concentrations.

concentrations→		Control		50 mg/ml		100 mg/ml		200mg/ml	
Parts↓	Days after treatment ↓	A.fl.	A.fu.	A.fl.	A.fu.	A.fl.	A.fu.	A.fl.	A.fu.
Tuber	2	+++	++	++	+	+	++	++	++
	5	++++	+++	+++	++	++	++++	+++	+++
	8	++++	++++	++++	+++	++	++++	+++	+++
Root	2	+++	+	+++	+	++	++	+	+
	5	++++	+++	++++	+++	+++	++	+++	+++
	8	++++	++++	++++	++++	++++	++	++++	+++
W.A	2	+++	++	++	++	++	++	+	++
	5	++++	+++	+++	++++	+++	++	+++	++++
	8	++++	++++	++++	++++	++++	++++	++++	++++

≡No growth A.fl. ≡*Aspergillus flavus*

\+ ≡ Weak growth A.fu. ≡*Aspergillus fumigatus*

++ ≡ Moderate growth

+++ ≡ Good growth

++++ ≡ Vigorous growth

Control ≡ Ethanol.

The tuber chloroform extract showed one activity incidence against *S. aureus* at a concentration of 100 mg/ml. However , the ethanol extract of the whole aerial parts reflected inhibitory effects against all bacterial strains except for *B.cereus,* while *E.coli* and *S.aureus* responded in a positive manner (inhibition of growth) to all concentrations used, in addition to that *S,typhi* exhibited inhibitory effects.

Table 3. Antifungal screening of *M. pseudopetalosa* ethanolic extracts at different concentrations.

Concentration→		Control		50mg/ml		100 mg/ml		200mg/ml	
Part↓	Days after treatment↓	A.fl.	A.fu..	A.fl.	A.fu.	A.fl.	A.fu.	A.fl.	A.fu.
Tuber	2	++++	+	+++	+	++	++	+	++
	5	++++	+++	+++	+++	++	+++	+	+++
	8	++++	+++	+++	+++	+++	++	+	+++
Root	2	++++	+	+++	+	+++	++	++	++
	5	++++	++++	++	++++	+++	++++	++	++
	8	++++	++++	+++	++++	++++	++++	+++	+++
W.A	2	+++	+	++	+	+	++	+	++
	5	+++	+++	+++	+++	++	+++	+	+++
	8	++++	++++	++++	++++	+++	+++	++	++++

Control ≡ Ethanol A.fl. ≡ *Aspergillus flavus*

\- ≡ No growth A.fu. ≡*Aspergillus flavus*

\+ ≡Weak growth

++ ≡ Moderate growth

+++ ≡ Good growth

++++ ≡ Vigorous growth.

Table 4. Antifungal screening of *M. pseudopetalosa* different parts ethyl acetate extracts tested against *A. flavus* and *A. fumigatus* at different concentrations.

Concentration→		Control		50mg/ml		100 mg/ml		200 mg/ml	
Parts↓	Days after ↓treatment	A.fl.	A.fu.	A.fl.	A.fu.	A.fl.	A.fu.	A.fl.	A.fu.
Tuber	2	++	+	++	+	++	+	++	+
	5	+++	++	+++	+++	++	+++	++++	+++
	8	++++	++++	++++	++++	+++	++++	++++	++++
Root	2	++	+	+	+	+	+	+	+
	5	+++	++	++	+++	++	+++	+	++
	8	++++	++++	+++	++++	++	++++	++	+++
W.A	2	++	+	++	+	++	++	++	++
	5	+++	+++	+++	+++	+++	++	++++	+++
	8	++++	+++	+++	++++	++++	++++	++++	++++

\- ≡No growth A.fl. ≡ *Aspergillus flavus*

\+ ≡ Weak growth A.fu. ≡ *Aspergillus fumigatus*

++ ≡ Moderate growth

+++ ≡ Good growth

++++ ≡ vigorous growth

Control ≡Ethanol.

The results clearly indicated that the plant had a limited antibacterial effect which is in agreement with what was reported by Al Magboul *et al.* (1987) who examined extracts of other members of the same family. Henry (1948) tested solutions of tetra-amine iodide of *M. pseudopetalosa* tubers against *S. aureus* and *S.typhi,* he also obtained negative results in each case.

Antifungal Activity

At day 8,the date set for the termination of the experiment which gives the most relative results for deciding whether or not an inhibition effect had occurred, it was observed that the chloroform extract was the most effective at a concentration of 100 mg/ml relative to its ability to induce similar effects on both *A. fumigatus and A. flavus* for both tubers and roots (Table 2). The ethanol extract of the tuber and whole aerial parts exhibited delayed growth of *A. flavus* at a concentration of 200 mg/ml (Table 3).

Table 5. Biochemical tests for the secondary metabolites present in the different parts of *M. pseudopetalosa*.

Parts	Flavonoid	Tanins	Terpenes	Sterols	Alkaloids	Saponins
Root	-	+	-	-	++	-
Tuber	-	+	-	+++	+	-
W.A	-	+	-	-	-	-

- ≡ Absent + ≡ Weak presence++ ≡ Moderate presence

+++ ≡ High presence W.A ≡ Whole aerial part.

Table 6. TLC separation of *M. pseudopetalosa* ethyl acetate extracts obtained by using a solvent system of hexane:ethyl acetate(8:2) and sprayed with vanillin-sulphuric acid.

Plant part	Under UV (365nm.)	With reagent	Rf. Values					
Tuber	Blue and bright blue	Dark and pale blue	0.13	0.4	0.8	0.93	0.99	-
Root	Blue and bright blue	Dark and pale blue	0.17	0.88	0.99	-	-	-
Whole Aerial Parts	Blue, dark blue	Dark and pale blue	0.5	0.97	-	-	-	-

- ≡ Absent.

Whereas the ethyl acetate root extract reflected a moderate growth delay against *A. flavus* at the concentrations of 100 and 200 mg/ ml (Table 4).

In the case of *C. albicans* no clear effect of growth inhibition was recorded for the different concentrations of plant parts extracted with the different solvents.

Table 7. TLC separation of *M. pseudopetalosa* chloroform extracts obtained by using a solvent system of chloroform: methanol: water (7:5:1) and sprayed with anisaldehyde-sulphuric acid.

Plant parts	Under UV	Spray reagent	Rf. Values					
Tuber	Blue, pale and dark blue, red and dark red	Brown and pale brown	0.1	0.2	0.3	0.73	0.97	-
Root	Blue, pale and dark blue, red and dark red	Brown and pale brown	.09	0.1	0.21	0.55	0.99	-
Whole Aerial Parts	Blue, pale and dark blue, red and dark red	Brown and pale brown	0.05	0.1	0.3	0.97	-	-

- ≡ Absent.

Chemical Tests

The results indicated a weak presence of tannins in all the tested plant parts; which is in agreement with what was reported for the seeds of *Buccholzia coricea*; another member of the family Cappariaceae (Amaechi, 2009).

Sterols were detected only in the tuber parts, while alkaloids were in a weak and moderate presence in the tuber and root parts respectively which is

contrary to what was reported by Henry (1948) who did not detect alkaloids in the tuber parts of the same plant. The presence of the two secondary metabolites was reported in another member of the family, namely *Cleome rutidosperma* by Ojiako and Igwes (2007).

Table 8. TLC separation of *M. pseudopetalosa* ethanol extracts obtained by using a solvent system of hexane : ethyl acetate (7:3) and sprayed with vanillin-sulphuric acids.

Plant part	Under UV.	With reagent	Rf. values				
Tuber	Fluorescent, pale red	Greenish blue, dark brown and dark blue	0.1	0.37	0.70	0.79	0.97	-
Root	Fluorescent, pale red	Dark brown, brown and blue	.08	0.18	0.71	.098	-	-
Whole Aerial Parts	Fluorescent, pale black	Dark brown, brown and blue	0.08	0.31	0.69	0.77	0.84	0.90

- ≡ Absent.

On the other hand test for flavonoids, terpenes and saponins revealed negative results in all plant parts tested (Table 5).

Thin Layer Chromatography (TLC)

TLC separation of the different crude extracts of *M. pseudopetalosa* was carried out in order to try to identify the different constituents of the extracts with respect to their Rf. values and colors of spots. The data obtained were compared with those from available literature, with similar experimental conditions. After many trials, the best solvent systems and detection spray reagents were chosen which are: A solvent system which consisted of hexane: ethyl acetate in a ratio of 8:2was used. (Table 6) show the results obtained by chromatographic separation of the ethyl acetate extracts. Such colors which were showed for the spots of the different extracts indicated the presence of sterols.

The second solvent system which consisted of chloroform : methanol : water in a ratio of 7:5:1 was applied to the chloroform extract. Positive results were obtained using both UV (365 nm.) and anisaldehyde-sulphuric acid as a spray reagent. (Table 7) These colors indicated the presence of cardiac glycosides (Wagner *et al.*,1996).

The last solvent system which consisted of hexane: ethyl acetate in a 7:3 ratio was a modification of that used by Upasani *et al.* (2003) for the detection of alkaloids and flavonoids. The chromatographic separation gave positive results with the ethanol extract. The results of the Rf. values and chromatography plates (Table 8) indicated the presence of alkaloids and flavonoids.

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