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Antimicrobial Activity of Extracts from *Felicia muricata* Thunb

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Abstract: The antimicrobial activities of the acetone, methanol and water extracts from the leaves, stems and roots of this herb were investigated against 10 bacterial and 5 fungal species using the dilution method on solid agar medium. The acetone extracts from the leaf and root were active against Gram-positive bacteria with Minimum Inhibitory Concentration (MIC) ranging between 1.0 and 7.0 mg mL⁻¹, whereas the acetone stem extract was able to inhibit all the bacterial strains at 0.5-7.0 mg mL⁻¹. The methanol extracts of the 3 plant parts showed activity against all the bacterial isolates with MIC values ranging between 0.1 and 10.0 mg mL⁻¹. Again all the extracts exhibited appreciable activity against all the fungi species investigated. The methanol extract of the root showed 100% inhibition against *Aspergillus niger*, *A. flavus* and *Penicillium notatum* at 5 mg mL⁻¹ while it was 88.61% inhibition in *Mucor hiemalis*. The ability of the extracts of *F. muricata* to inhibit the growth of several bacteria and fungi is an indication of its broad-spectrum antimicrobial potential which may be employed in the management of bacterial and fungal infections.

Key words: Antimicrobial activity, *Felicia muricata*, fungicidal activity, infections, minimum inhibitory concentration, Gram-positive bacteria

INTRODUCTION

Significant number of people in developing countries have limited access to modern drugs for the treatment of common diseases, thus, medicinal plants are their only therapeutic resource (Waller, 1993). The use of traditional herbal remedies as alternative medicine by the indigenous population of South Africa plays a major role in their culture and also features significantly in the primary health care system. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important as potential sources of novel antibiotic (Meurer-Grimes *et al.*, 1996; Rabe and Van Staden, 1997).

Felicia muricata Thunb. (Asteraceae), locally known as Ubosisi in the Eastern Cape of South Africa, is a small drought resistant perennial herb growing up to 20 cm in height. The leaves are simple and are arranged in alternate fashion. Its name was derived from muricate (rough, with sharp tubercles or protuberances). The species is regarded as an indicator of desertification, becoming increasingly invasive in grassland regions (Jordaan and Kruger, 1993). *F. muricata* is used by the traditional healers in the management of headaches, stomach catarrh, pains and inflammation (Hutchings, 1989; Hutchings and Van Staden, 1994; McGaw *et al.*, 1997). Information gathered during our preliminary investigation on the local

uses of the species also revealed its medicinal importance for the treatment of stomach ache and cancer. Extracts from the plant have been reported to show 80-90% inhibitory activity against cyclooxygenase, an important enzyme in the prostaglandin biosynthesis pathway (McGaw *et al.*, 1997; Okoli and Akah, 2004) and this may be responsible for the anti-inflammatory activity of this plant. Despite the reported medicinal uses of this species, especially for stomach ache and inflammation, the antimicrobial activity of this herb has not been reported in literature. This study investigated the antimicrobial activity of *F. muricata* by screening its extracts against 10 selected bacterial and 5 fungal strains, with the aim to validate the use of this species in the management of pains and inflammation that could have resulted from microbial infections. According to Mathekga and Meyer (1998), *in vitro* antimicrobial screening could provide the preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations.

MATERIALS AND METHODS

Plant material: Plants used for this study were collected in August 2007 from several populations of *F. muricata* growing within premises of Alice campus of the University of Fort Hare (33°11.10'S and 7° 10.60'E; altitude 695 m). The mean annual rainfall of this area is about

700 mm and temperature range of 13 to 25°C. The species was authenticated by Mr. Tony Dold, Selmar Schonland Herbarium, Rhodes University, South Africa. A voucher specimen (AshafaMed.2007/1) was prepared and deposited in the Griffen Herbarium of the University of Fort Hare.

Extract preparation: The separated leaves, stems and roots of the plant samples were carefully washed under running tap water, air dried at room temperature (30°C) and pulverized before extraction. Powdered plant material (40 g each) was separately extracted in acetone, methanol and water for 48 h at 30°C, on an orbital shaker (Stuart Scientific Orbital Shaker, UK). Acetone and methanol were of high analytical grade. The extracts were filtered through Whatman No. 1 filter paper and the filtrate was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000-efficient, Heidolph, Germany). The water extract was freeze-dried using Savant Refrigerated Vapor Trap, (RVT4104, USA). The freeze-dried extract was stored at 4°C before bioassay. The different extracts were re-dissolved in their respective solvents to give 50 mg mL⁻¹ stock solution (Taylor *et al.*, 1996). This was diluted to the required concentrations of 0.1, 0.5, 1.0, 5.0, 7.0 and 10 mg mL⁻¹ for the bioassay analysis.

Antibacterial assay: Five Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus kristinae*, *Streptococcus faecalis*) and five Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsella pneumoniae*, *Serratia marcescens*) bacteria species used in this study were all laboratory isolates. They were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. The organisms were maintained on nutrient agar plates and were revived for bioassay by subculturing in fresh nutrient broth (Biolab, Johannesburg, South Africa) for 24 h before being used.

Nutrient agar (Biolab, Johannesburg, South Africa) was prepared by autoclaving and allowed to cool to 55°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 0.1, 0.5, 1.0, 5.0, 7.0 and 10 mg mL⁻¹ were poured into Petri dishes, swirled gently until the agar began to set and left over night for solvent evaporation (Afolayan and Meyer, 1997). Agar plates containing 1% acetone, methanol and water served as controls (Dulger and Ugurlu, 2005). Organisms were streaked in radial pattern on the agar plates (Meyer and Afolayan, 1995). The inoculum size of

each test strain was standardized at 5 × 10⁵ cfu mL⁻¹ using McFarland Nephelometer standard according to the National Committee for Clinical Laboratory Standards. The plates were incubated under aerobic conditions at 37°C and examined after 24 and 48 h. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of an extract was required for it to be declared active (Sindambiwe *et al.*, 1999; Mathekga *et al.*, 2000). Chloramphenicol and streptomycin (standard antibiotics) were used as positive controls in the experiment.

Antifungal assay: Antimycotic activity of *F. muricata* was investigated using five fungal species (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum*, *Mucor hiemalis* and *Candida albicans*). All fungal cultures were maintained on Potato Dextrose Agar (PDA) (Biolab, Johannesburg, South Africa) and were recovered for testing by subculturing on PDA for 3 days at 25°C prior to bioassay. PDA plates were prepared by autoclaving before the addition of the extracts. Each extract was vortexed with the molten agar at 45°C to final concentrations of 0.1, 0.5, 1.0, 5.0, 7.0 and 10.0 mg mL⁻¹ and poured into Petri dishes. Blank plates containing only PDA or PDA with the respective solvent served as controls. The prepared plates containing the extracts were inoculated with plugs (5 mm in diameter) obtained from the actively growing portions of the mother fungal plates and incubated at 25°C for 5 days. The diameter of fungal growth was measured and expressed as percentage growth inhibition of three replicates (Lewu *et al.*, 2006; Koduru *et al.*, 2006). Due to the nature of *Candida albicans*, the organism was streaked radially like the bacteria.

Statistical analysis: Significant differences within the means of treatments and controls were measured and calculated using the LSD statistical test (Steel and Torrie, 1960). LC₅₀ (the concentration at which 50% of growth was obtained) was calculated by extrapolation.

RESULTS AND DISCUSSION

Antibacterial activity: The Minimum Inhibitory Concentration (MIC) values of the reference drugs, acetone, methanol and water extracts from the leaves, stems and roots of *F. muricata* against the tested organisms are shown in Table 1. The acetone extract of the leaves was active only against Gram-positive bacteria with MIC ranging between 1.0-5.0 mg mL⁻¹,

Table 1: Antibacterial activity of the extracts from the leaves, stems and roots of *Felicia muricata*

Bacteria	Gram+/-	MIC (mg mL ⁻¹)									Chl (µg mL ⁻¹)	Strep. (µg mL ⁻¹)
		Acetone			Methanol			Water				
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root		
<i>Staphylococcus aureus</i>	+	5.0	5.0	5.0	5.0	5.0	5.0	na*	na	na	<2	<2
<i>Staphylococcus epidermidis</i>	+	1.0	0.5	5.0	5.0	10.0	5.0	na	na	na	<2	<2
<i>Bacillus cereus</i>	+	1.0	0.5	5.0	5.0	10.0	5.0	na	na	na	<2	<2
<i>Micrococcus kristinae</i>	+	1.0	1.0	7.0	5.0	0.1	5.0	na	5.0	na	<0.5	<2
<i>Streptococcus faecalis</i>	+	na	7.0	na	10.0	7.0	10.0	na	na	na	<2	<4
<i>Escherichia coli</i>	-	na	7.0	na	10.0	7.0	10.0	na	na	na	<2	<2
<i>Pseudomonas aeruginosa</i>	-	na	7.0	10.0	5.0	1.0	1.0	na	na	na	<10	<4
<i>Shigella flexneri</i>	-	na	7.0	na	10.0	10.0	10.0	na	na	na	<2	<2
<i>Klebsella pneumoniae</i>	-	na	7.0	na	10.0	10.0	10.0	na	na	na	<2	<2
<i>Serratia marcescens</i>	-	na	7.0	na	10.0	10.0	7.0	na	na	na	<2	<2

+: Positive, -: Negative, MIC: Minimum Inhibitory Concentration, Chl: Chloramphenicol, Strep.: Streptomycin, *Not active at 10 mg mL⁻¹, which was the highest concentration tested

whereas the methanol extract of the leaves suppressed the growth of all tested organisms with inhibition range of 5.0-10.0 mg mL⁻¹. Both the acetone and methanol extracts of the stem showed activity against all the bacteria strains with MIC ranging between 0.1 - 7.0 mg mL⁻¹. The acetone extract of the roots was active only against Gram-positive bacteria apart from *P. eruginosa* that was inhibited at 10.0 mg mL⁻¹ which was the highest concentration tested, whereas the methanol extract of the roots inhibited all the bacteria tested at inhibition range of 1.0 to 10.0 mg mL⁻¹. Water extracts from all the plant parts were not active against any of the organisms except *M. kristinae* that was inhibited at 5 mg mL⁻¹ by extract from the stem. Generally, the methanol extracts were more active than other extracts. This may be associated with the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007).

The acetone and methanol extracts were active against *Bacillus cereus*, a respiratory pathogen commonly associated with colds and flu (Viljoen *et al.*, 2004) at MIC values of 0.5-5.0 mg mL⁻¹. The inhibitory property of extracts of *F. muricata* against pathogenic bacterial strains can introduce the plant as a candidate for drug development for the treatment of ailments caused by these pathogens. The non-activity of the water extract against most bacterial strains investigated is in accordance with the reported cases of earlier workers who showed that aqueous extracts of plants generally showed little or no antibacterial activities (Madamonbe and Afolayan, 2003; Koduru *et al.*, 2006; Aliero *et al.*, 2006). Traditionally, however, plant extracts are prepared with water as infusions, decoctions and poultices; therefore it seems unlikely that the traditional healer is able to extract those compounds which are responsible for activity in the acetone and methanol extracts. Gram-negative bacteria

have been reported to be more resistant to plant extracts than the Gram-positive strains (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Afolayan, 2003). The ability of the stem extracts to inhibit all Gram-negative bacteria strains tested was an indication of the plants broad spectrum of the antimicrobial property.

Antifungal activity: The majority of the extracts showed broad antimycotic activity against the tested organisms at 0.5 mg mL⁻¹ or lower (Table 2). Only the acetone extract of the leaves and the methanol extract of the roots showed inhibition (100%) against *C. albicans* at 10 mg mL⁻¹, which was the highest concentration tested in this study. Table 2 does not include the column for *C. albicans*. Extracts from the roots were inhibitor to the fungi species than the leaf and stem extracts. Apart from the water extract of the leaves which did not show any activity against any of the fungi, all the extracts were fungicidal (100% inhibition) on *P. notatum* and *M. hiemalis*. The susceptibility of *A. flavus* to the extracts of *F. muricata* is noteworthy, as the fungus has recently been implicated in cases of immuno-compromised patients that frequently develop opportunistic and superficial mycosis (Portillo *et al.*, 2001). In general, the methanol extract had the highest activity against both bacteria and fungi strains. This was followed by the acetone extract and the least activity was observed in the water extract. The ability of the extracts of this plant to inhibit the growth of several bacteria and fungi species is an indication of the broad spectrum antimicrobial potential of *F. muricata*, which makes the species a candidate for bioprospecting for antibiotic drugs. Work is therefore in progress on the isolation, purification and structural elucidation of the bioactive compounds in this plant.

Table 2: Antifungal activity of extracts from the leaves, stems and roots of *Felicia muricata*

Concentration (mg mL ⁻¹)	Growth inhibition (%)											
	Leaf				Stem				Root			
	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>	<i>M. hiemalis</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>	<i>M. hiemalis</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>	<i>M. hiemalis</i>
Acetone												
10	88.10 ^c	88.33 ^d	100.00 ^c	100.00 ^d	69.17 ^a	72.87 ^a	100.00 ^a	100.00 ^d	87.78 ^a	90.46 ^c	86.67 ^a	100.00 ^a
5	81.94 ^c	83.06 ^c	100.00 ^c	100.00 ^d	55.28 ^d	68.61 ^d	86.94 ^d	100.00 ^d	81.11 ^d	86.11 ^a	80.28 ^d	88.89 ^d
1	76.67 ^b	57.22 ^b	76.67 ^b	75.28 ^c	42.50 ^f	56.39 ^c	78.30 ^c	100.00 ^d	73.33 ^c	74.72 ^d	70.83 ^c	77.78 ^c
0.5	72.50 ^b	0.00 ^a	0.00 ^a	56.39 ^b	37.78 ^b	46.39 ^b	73.06 ^b	73.61 ^c	60.83 ^b	71.11 ^c	61.11 ^b	63.61 ^b
0.1	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	59.44 ^b	0.00 ^a	62.78 ^b	0.00 ^a	0.00 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.38	0.94	0.83	0.45	3.35	0.68	0.37	0.08	0.43	0.08	0.43	0.41
Methanol												
10	84.72 ^d	90.93 ^c	100.00 ^c	100.00 ^c	81.67 ^a	90.56 ^d	82.22 ^d	100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^d	100.00 ^d
5	83.19 ^d	69.71 ^b	78.33 ^b	68.06 ^d	79.72 ^a	90.56 ^d	75.00 ^c	100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^d	88.61 ^d
1	73.61 ^c	0.00 ^a	0.00 ^a	45.83 ^c	65.28 ^d	80.83 ^c	61.11 ^b	80.28 ^d	95.56 ^d	91.67 ^d	79.44 ^c	79.44 ^d
0.5	70.83 ^c	0.00 ^a	0.00 ^a	36.67 ^b	58.61 ^c	61.67 ^b	0.00 ^a	72.50 ^c	86.94 ^c	87.50 ^c	67.22 ^b	66.39 ^c
0.1	65.20 ^b	0.00 ^a	0.00 ^a	0.00 ^a	45.83 ^b	0.00 ^a	0.00 ^a	67.78 ^b	80.28 ^b	75.83 ^b	0.00 ^a	63.33 ^b
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.08	3.87	3.55	1.75	0.23	0.42	0.91	0.07	0.06	0.07	0.40	0.08
Water												
10	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	75.09 ^a	77.69 ^d	82.69 ^d	66.39 ^a	91.48 ^a	95.19 ^a	100.00 ^a	82.69 ^a
5	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	65.83 ^d	75.83 ^d	74.44 ^a	63.06 ^d	91.11 ^e	91.67 ^d	100.00 ^a	60.28 ^d
1	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	53.33 ^c	63.33 ^c	62.22 ^d	43.33 ^c	79.72 ^d	89.17 ^d	67.50 ^d	53.61 ^c
0.5	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	43.61 ^b	56.94 ^b	51.67 ^c	32.22 ^b	75.83 ^c	74.17 ^b	61.39 ^c	40.56 ^b
0.1	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	40.83 ^b	0.00 ^a	71.11 ^b	0.00 ^a	57.78 ^b	0.00 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀					0.83	0.45	0.44	2.35	0.07	0.37	0.09	0.86

Values are means of percentage growth inhibition of three replicates. Values within a column followed by the same superscript are not significantly different at $p < 0.05$. LC₅₀ values in mg mL⁻¹ were calculated by extrapolation

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