Antibacterial Activity of Crude and Fractions of
Momordica foetida Leaf Extracts

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ABSTRACT
The leaves of Momordica foetida were screened for their phytochemical composition and antimicrobial potential. Phytochemical screening of the crude extract showed the presence of various secondary metabolites which included alkaloids, flavonoids, terpenoids, steroids, saponins and tannins. *In vitro* antimicrobial activities of the crude extract and five solvent fractions were screened against 28 bacterial strains using traditional methods. Among the five fractions, ethyl acetate exhibited the highest broad spectrum antibacterial activity against the tested bacteria and the decreased in the order of ethyl acetate > butanol > chloroform > aqueous > hexane fraction at a concentration of 5.0 mg mL−1. The zone of inhibition exhibited by the EtOAc and BuOH fractions ranged from 8 to 17 mm while the CHCl₃ fraction was between 7.5 and 15 mm. The hexane and aqueous fractions showed a zone of inhibition that ranged from 7 to 14 mm. The minimum inhibitory concentration (MIC) exhibited by EtOAc, CHCl₃ and HEX fractions against the bacterial strains ranged between 0.156 and 2.5 mg mL⁻¹ while that of BuOH and aqueous fractions ranged between 0.3125 and 5.0 mg mL⁻¹. The antibacterial activities of the plant were comparable with standard antibiotics such as neomycin, ampicillin and tetracycline at the same concentration.

Keywords: antimicrobial, Cucurbitaceae, isolates, medicine, phytochemical

INTRODUCTION

Bioactive plants are commonly used by traditional healers or in Folklore medicines for the relief of pain or cure of some alignments. This act of medication has made some plant families popular (Heinrich 2000; Kuete et al. 2006). The acceptance of traditional medicine as an alternative form of health care has assisted scientists in elevating these claims, by studying the chemical composition and biological potential (Bisignano et al. 1996; Hammer et al. 1999). This had led to the advent of new drugs on the market.

In the last decade, the pharmaceutical industry has been faced with an influx of drug-resistance pathogens in treating infectious diseases (Hugo and Russel 1984). The increase in the number of antibiotic-resistant bacteria and the side effects of some known drugs has aroused the interest of scientists in tapping bioactive plants to control and cure some of the global infections (Hostettmann and Hamburger 1991).

South African is a country with great cultural diversity and biodiversity, with many people still using a wide variety of plants in medicine and other necessities of life (van Wyk and Gericke 2000). These plants, often referred to as traditional vegetables, account for 10% of the world’s higher plants.

![Momordica foetida](image)

*Fig. 1* Momordica foetida at flowering stage (left) and fruiting stage (right).

Momordica foetida Schumach. & Thonn. is widespread in tropical Africa and in South Africa. *M. foetida* occurs in forest edges and clearings, margins of swamps. *M. foetida* are dioecious, perennial herbs, trailing or climbing plants with simple or bifid tendrils. The stem is up to 4.5 m long, with dark green flecks when young, woody when old, rooting at the nodes. The leaves are alternate, simple while stipules are absent. Flowers are unisexual, regular, white or pale yellow to orange-yellow (*Fig. 1*). The fruit is a long-stalked, ellipsoid berry up to 7 × 5 cm and orange when ripe (*Fig. 1*). Seeds are oblong, flattened, about 1 cm long and brown (Burkill 1985; Jackson 1990).

Traditional medicinal uses *M. foetida* leaves and root in treating coughing, stomachache, intestinal disorders, headache and earache. The plant is also used for skin problems caused by smallpox and boils (Burkill 1985). Pharmacologically, it has shown to be a potentially strong antinicotinic and antimuscarinic (Waako 2005). Leaf extracts showed antitrichomonas activity against *Trichomonas vaginalis* (Burkill 1985). Previous phytochemical studies resulted in the isolation of cucurbitane triterpenoids from leaf extracts (Mulolland et al. 1997), alkaloids and glycosides from the complete plant (Olanipi 1975; Olanipi and Marquis 1975) and the identification of sitosterol glycoside, 5,25-stigmasteradien-3β-yl-glucoside and 1β-hydroxyfriedel-6-en-3-one from the leaves of *M. foetida* (Olanipi 1980). Even though this valued plant also grows in South Africa, to the best of our knowledge, there is no adequate information on the antibacterial activities and medicinal potential of the South African species. In this study, we report the *in vitro* antibacterial potential of the leaf extracts (crude and various fractions).

MATERIALS AND METHODS

Plant material

The study was conducted in 2007. Fresh leaves of *M. foetida* were
collected from the University of Zululand and from their natural habitat around the University campus in March, 2007. The plant was identified by the herbarium unit of the Botany Department, University of Zululand, KwaZulu Natal, South Africa and deposited therein for reference purposes [Odeleye O.M. 1(ZULU)].

Preparation of extracts

The leaves were oven dried at 40°C for 18-24 h and electrically milled. Exactly 700 g of the powdered plant material was cold-extracted using 90% ethanol for 2 days with occasional shake and repeated 3 times days. The extracts were combined after TLC analysis to ensure complete extraction. The mixture was then filtered through Whatman filter paper No. 1 and the filtrate was concentrated to dryness using a rotary evaporator. This gave a yield of 123.44 g.

Fractionation of the ethanol crude extract

The ethanol extract (118.0 g) was dissolved in a mixture of methanol:water (2:3, v/v) at room temperature and partitioned successively with hexane, chloroform, ethyl acetate and butanol. The remaining 5g ethanolic extract was reserved for crude antibacterial test. The following yields were obtained: hexane (17.58 g), chloroform (2.6 g), ethyl acetate (6.29 g) and butanol (11.66 g) and aqueous (75.76 g). The fractions were concentrated under vacuum.

Phytochemical screening of the plant extract

A small portion of the dry extract was used for phytochemical screening using standard procedures described by Sofowora (1982), Trease and Evans (1996) and Harborne (1998) for the determination of some secondary metabolites.

Bacterial isolates used in the study

Bacterial strains used in this study consisted of reference strains obtained from the University of Fort Hare, namely Escherichia coli (ATCC 8739), E. coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 19852), Staphylococcus aureus (ATCC 6538), S. faecalis (ATCC 29212), Bacillus cereus (ATCC 10702), B. pumilus (ATCC 14884), Pseudomonas aeruginosa (ATCC 7700), Enterobacter cloacae (ATCC 13047), Klebsiella pneumoniae (ATCC 10031), K. pneumonia (ATCC 4352), Proteus vulgaris (ATCC 6830), P. vulgaris (CSIR 0030), Serratia marcescens (ATCC 9986), Acinetobacter calcoaceticus (Ac1), A. calcoaceticus (Ac2). Also environmental strains of K. pneumonia, Bacillus subtilis, Shigella flexneri, Salmonella sp., Staphylococcus epidermidis, P. aeruginosa, P. vulgaris, Enterococcus faecalis, E. coli, S. aureus, Microccoccus kristinae and M. luteus were used in this study.

Assay for antibacterial activity

Antibacterial activity of the extract and fractions were evaluated using the agar disc diffusion method described by Bauer et al. (1966). Bacteria were maintained on Mueller-Hinton agar (MHA) at 4°C. Molten MHA was inoculated with a broth culture of the respective bacterial strains and poured into sterile 90 mm Petri dishes supplied Merck Laboratory supplies (South Africa). The extract and fractions were dissolved in methanol to a final concentration of 5 mg mL⁻¹ and sterile Whatman No. 1 (6 mm) discs (one sheet per dish) were impregnated in each sample to be tested at a concentration of 5 mg mL⁻¹. The plates were incubated at 37°C for 24 h and the zones of inhibition were measured at the end of the incubation period. The standard antibiotics for reference drugs used were neomycin, ampicillin and tetracycline at 5 mg mL⁻¹.

Minimum Inhibitory Concentration

The Minimum Inhibition Concentration (MIC) of the plant extract and fractions were determined using a 96-well microplate dilution method described by Elloff (1998). Stock solution of each fraction and extract was dissolved in methanol. The final plate concentrations were 5.0, 2.5, 1.25, 0.625, 0.313, 0.157, 0.078 and 0.039 mg mL⁻¹ for the extract, fractions and standard antibiotics used as positive control. Bacteria were grown for 18 h in Mueller-Hinton nutrient broth and cultures of 105 Colony Forming Units (cfu) mL⁻¹ were used. The microplates were prepared using serial dilutions (Elloff 1998) and incubated for 24-48 h at 37°C. As an indicator of bacterial growth, 40 μl of 0.2 mg mL⁻¹ p-nitrotetrazolium (INT) solution was added to each well and incubated at 37°C for 30-120 min. The colourless tetrazolium salt is reduced to a red colored product by biological activity of the organisms, thereby making the inhibition of bacterial growth clearly visible. MIC values were recorded as the lowest concentration resulting in complete inhibition of bacterial growth. Each treatment was replicated thrice.

Statistics

All experiments were performed in triplicate. The mean and standard deviation of at least three experiments were determined. Significant differences within the means of the treatment and the controls were calculated using one way analysis of variance (ANOVA) followed by LSD statistical test at 5% probability using Primer of Biostatistics program by Stanton A. Glantz.

RESULTS AND DISCUSSION

Table 1 reveals the secondary metabolites present in the crude extracts. The presence of terpenoid, steroids, tannins, flavonoids and cardiac glycosides were pronounced. These classes of compounds were reported earlier with antimicrobial activity (Hostettman and Nakaniishi 1979). These compounds may be responsible for the antibacterial activity of the leaf extract and fractions of M. foetida. Tannins, for example, have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler 1981). Herbs that contain tannins as their main constituents are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda 2003), which is one of the traditional uses of M. foetida plant. This result thus supports the use of M. foetida as a herbal remedy. One of the groups of secondary metabolites produced by plants is alkaloids and their amazing effect on humans has led to the development of powerful pain killers (Raffauf 1996). The presence of alkaloids supports the report of Ola-niyi and Marquis (1974) who isolated alkaloids and glycosides from M. foetida. Quinlan et al. (2002) and Neumann et al. (2004) showed that steroidal and steroid extracts exhibit antibacterial and antiviral activity, respectively. Thus, the presence of these compounds in M. foetida corroborates the antibacterial activities observed.

Many bacteria are implicated in opportunistic infections.
butanol and aqueous fractions of the leaf extracts displayed good antibacterial activity against tested bacterial and the fractions showed varying degrees of antibacterial activities (Table 2). The most sensitive bacteria to the five fractions were *P. aeruginosa*, *B. cereus*, *S. flexineri*, and *K. pneumonia*. The ethyl acetate and butanol fractions were the most...
active fractions but ethyl acetate was very active with zones of inhibition ranging between 8 and 17 mm. Hexane and aqueous fractions indicated low activity with hexane showing least activity with zones of inhibition ranging between 7.5 and 14 mm. The MIC of the fractions and crude extract of *M. foetida* leaves ranged from 5.0 to 0.156 mg mL$^{-1}$ (Table 3). The MIC of the most active fraction (ethyl acetate) ranged from 2.5 to 0.156 mg mL$^{-1}$.

**PERSPECTIVES**

*M. foetida* is a plant that has shown tremendous potential as a source of novel chemotherapeutic agents. The plant is extensively utilized in traditional medicinal practices in West, Central and South Africa. It is therefore one of the prime medicinal plants of Africa that can provide relief to the millions of poor people on the continent if its potential is adequately explored. This investigation justifies its ethnomedical use, having displayed activities with the human pathogenic bacteria. This study therefore, supports the medicinal value of the plant. Since *M. foetida* is used in the preparation of decoctions, different formulations could be prepared for clinical trials. This study serves as a base for the subsequent detection of specific compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Further studies on the isolation and structural elucidation of the active components and toxicological studies of the most active fraction are in progress.

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**REFERENCES**


Burkill HM (1985) *The Useful Plants of West Tropical Africa, Families A–D* (Vol 1, 2nd Edn), Royal Botanic Gardens, Kew, Richmond, UK, 960 pp

Dharmananda S (2003) Gallnut and the uses of Tannins in Chinese Medicine, A paper delivered at the Institute for Traditional Medicine, Portland, Oregon, 15 pp

Ellan (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* 64, 711-713


Jackson WPJ (1990) *Origins and Meanings of Names of South African Plant Genera*, Ecology laboratory, Botany Department, University of Cape Town, 71 pp


Olaniyi AA (1980) 1 β-Hydroxyfriedel-6(7)-ene-3-one-3-one from *Momordica foetida*. *Journal of Natural Products* 40, 303-305


Quinlan MB, Quinlan RJ, Nelan JM (2002) Ethiophysiology and herbal treatments of intestinal worms in Dominica, West Indies. *Journal of Ethnopharmacology* 80, 75-83


