The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: A Tanzania medicinal plant

K. D. Mwambete

Muhimbili University of Health & Allied Sciences, School of Pharmacy, Department of Pharmaceutical Microbiology, P.O. Box 65013, Dar es Salaam, Tanzania. Tel: +255-22-2150302-6, Fax: +255-22-2151596. Email: kmwambete@muhas.ac.tz

**Abstract**

**Objective:** To evaluate the antimicrobial activity of *Momordica charantia* extracts on reference strains and microorganisms isolated from clinical specimens.

**Method:** Petroleum ether and methanolic crude extracts of fruits and leaves of the plant were evaluated for antimicrobial activity using the disk diffusion method on four reference microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*); and four clinical strains of *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Cryptococcus neoformans*.

**Result:** Antimicrobial activity was observed against all the tested microorganisms with exception to *P. mirabilis* and *C. neoformans*. Methanolic crude extracts exhibited relatively broader antimicrobial spectrum of activity than petroleum ether extracts with the as lower concentration as 0.075mg/μl. Methanolic fruit crude extract displayed the broadest antimicrobial spectrum by inhibiting majority (75%) of the tested microorganisms. Neither was there synergistic nor addition effect upon mixing leaf and fruit extracts of equal concentrations derived from the same solvent.

**Conclusion:** Extracts of *M. charantia* demonstrated antimicrobial activity on tested microorganisms except on *Proteus mirabilis* and *Cryptococcus neoformans*. Fruit extracts showed higher antimicrobial activity than leaf extract. Further studies are recommended that will involve various parts of the plant, select different fractions of extracts and purify the active antimicrobial components.

**Keywords:** Antimicrobial activity, *Momordica charantia*, petroleum ether and methanolic crude extracts

**Introduction**

In recent years, several diseases and microbial infections such as respiratory infections, bacterial meningitis, sexually transmitted as well as hospital acquired infections, particularly those caused by the members of the family Enterobacteriaceae have shown considerable resistance to a number of antimicrobial agents, such as penicillin, ampicillin, and fluoroquinolones among many others. There is an increasing trend in the emergence of resistance to antimicrobial agents, not only due to the poor quality drugs, patient non-compliance, and irrational use of antimicrobial agents, but also to spontaneous mutations within the microbial populations. Therefore, drastic measures should be adopted to control the use of antimicrobial agents, to understand the genetic mechanisms of bacterial resistance, and to continue studies to develop new drugs. Ultimately, this may greatly contribute to provision of more appropriate and efficient antimicrobial agents to the patient.

Plants have been an integral part of human civilization. Medicinal plants have also been relied upon by over 80% of the world population for their basic health care needs. Among these plants, *Momordica charantia* (Family Cucurbitaceae) are used in the Amazon, Brazil and parts of Asia, among its many uses, for treatment of skin infections. But also the plant is grown in some parts of East Africa, Tanzania inclusive, where it is locally known as *Zukini* and used as appetizer among other utilities. The fruits and leaves contain alkaloids, glycoside, saponin-like substances, resin, an aromatic volatile oil and mucilage. Reports also show that the plant has anti-tumor and anti-HIV activities. A leaf tea is used for diabetes, to expel intestinal gas, promote menstruation, and as anti-viral agent against measles and hepatitis viruses.

Most recent researches on the plant show that it has ability to inhibit the enzyme guanylate cyclase that is thought to be associated with psoriasis, leukemia and tumor pathogenesis. However, a few previous studies have had evaluated the antimicrobial activity, and very variable results had been reported. But none of those studies was conducted in Tanzania or East Africa. Therefore, this study intends to evaluate the antibacterial and antifungal activities of the plant which is found in Dar es Salaam, Tanzania.

**Materials and methods**

**Collection of plant materials**

Leaves and fruits of the plant were collected at Msasani area an outskirt of Dar es Salaam City. Herbarium specimen was made and sent for authentication at the University of Dar es Salaam, Department of Botany, where a voucher specimen (No. UD/MUCHS 14/04) was deposited. Fresh plant materials (fruits and leaves)
were washed under running tap water, sun dried, then homogenized to fine powder and finally stored in air tight bottles until further use.

**Extraction procedures**

Sun dried plant materials were subjected to exhaustive extraction by cold maceration, with 2.5L of either petroleum ether or methanol at room temperature for 72 hours (23). The extracts were filtered through Whatman® No.1 filter paper (England) and concentrated to dryness using a Rotavapor® (Buchi, Essen); and then freeze dried in order to obtain dry powder. Only the methanolic extracts yielded powder. Table 1 summarizes the extraction yields per each plant part. Varying concentrations of the plant (leaf and fruit) extracts (0.075, 0.10, 0.20, 0.40, 0.60, and 1.00mg/μl) were prepared with petroleum ether and methanol as solvents/diluents. Two separate mixtures of 1.00mg/μl each composed of leaf and fruit crude extracts that were extracted with identical solvent were also prepared. All these preparations were employed in the subsequent antimicrobial activity testing. Paper discs (5mm) were punched from the Whatman® qualitative filter paper (England) that was then impregnated with 20μl of the extract.

**Test microorganisms and control antibiotics**

Four clinical strains were used in the study: *Klebsiella pneumoniae* (TK43/04), *Proteus vulgaris* (FN25/04), *Salmonella typhi* (LP06/04) and *Cryptococcus neoformans* (MD81/04). Another four reference strains of constituted by *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 90028) and *Staphylococcus aureus* (ATCC25923) were also tested. Gentamycin (500 mg) was dissolved in 1000ml distilled water for bacteria and Clotrimazole (1500 mg) for fungi was dissolved 1000 ml distilled water were used as positive controls.

**Antimicrobial activity testing**

A loopful of each assayed strain was inoculated into 15 ml of Nutrient broth (Oxoid) in the universal bottle and incubated at 37°C for 24 hours to activate the strains. The inoculum’s density was estimated visually to match the turbidity of McFarland 0.5 standard (24). One milliliter of microbial suspension was pipetted onto the surface of solidified agar plate, and gently rotated by hand so as to cover the entire surface with the microbial suspension. The plates were allowed to dry for at least 15 minutes prior introduction of the shortly prepared extract-impregnated paper disks onto the agar by means of sterile forceps. The control antibiotic disks were also incorporated at the centre; subsequently the agar-plates were incubated overnight at 37°C. Pure solvents (petroleum ether and methanol) were used as negative controls and the control activity was deducted from the test. Diameters of zones of inhibition (ZI) were measured and recorded in millimeters, which were interpreted as susceptible (S), intermediate (I) or resistant (R) in accordance with the NCCLS protocol (25).

**Statistical analysis**

All the above assays were conducted in duplicate and repeated twice for consistency of results and statistical purpose. Statistical analysis (descriptive and comparison of means) was performed using Independent Samples T-Test (p<0.05) for antimicrobial data (Table 2) to compare means of ZIs among the plant extracts, solvents used and the positive controls (gentamycin and clotrimazole).

**Results**

**Antimicrobial activity of methanolic crude extracts**

The findings of this study show that methanolic crude extracts exhibited a considerably broader antimicrobial activity compared to petroleum ether crude extracts (Table 2). The maximum ZI was produced by methanolic crude extract against *Klebsiella pneumoniae*. Within the methanolic extracts, fruit extract was more active with a broader antimicrobial spectrum than leaf extract by inhibiting 6 out of 8 tested microorganisms (Table 2; Figure 1). The effective antimicrobial concentrations for methanolic crude extracts of *M. charantia* ranged from 0.075-1.0mg/μl. Furthermore, methanolic leaf extract (MLE) manifested antimicrobial activity solely against *S. aureus* (Table 2, Figure 1).

**Antimicrobial activity of petroleum ether crude extracts**

Petroleum ether crude extracts were equally effective against *K. pneumoniae*, *S. aureus*, *C. albicans* and *S. typhi* (p<0.05), with efficacious concentrations ranging from 0.2 -0.6 mg/μl. But petroleum ether extracts were inactive against the rest of the tested microorganisms (Table 2). Also petroleum leaf extract (PLE) showed no antimicrobial activity against *C. albicans*, *E. coli* and *P. aeruginosa*.
Table 1: Percentage yields of leaf and fruit crude extracts of *Momordica charantia*.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Wt. of starting material (g)</th>
<th>Weight of dry extract (g)</th>
<th>Yield (%) (n= 3; mean ± SED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic leaf extract</td>
<td>35</td>
<td>2.51</td>
<td>7.17±1.3</td>
</tr>
<tr>
<td>Petroleum ether leaf extract</td>
<td>35</td>
<td>1.26</td>
<td>3.6±1.1</td>
</tr>
<tr>
<td>Methanolic fruit extract</td>
<td>50</td>
<td>1.96</td>
<td>3.92±1.2</td>
</tr>
<tr>
<td>Petroleum ether fruit extract</td>
<td>50</td>
<td>0.83</td>
<td>1.66±0.9</td>
</tr>
</tbody>
</table>

Figure 1: Antimicrobial activity of *M. charantia* on tested microorganisms expressed as means of zones of inhibition (mm). Error bars show 95% of confidence interval and n = 28 for each point.

Antimicrobial activity of mixtures of leaf and fruit crude extracts

Upon mixing the two extracts (leaf and fruit) of equal concentrations and volumes that were extracted with the same solvent, the antimicrobial activity was sharply decreased (Figure 2). The mixture of methanolic leaf and fruit extracts was only active on *E. coli* and *P. aeruginosa* exhibiting smaller ZI of 7 and 9 mm respectively. While the mixture of petroleum ether leaf and fruit extracts showed mild antibacterial activity on *E. coli* (data not shown).

Figure 2: Concentration-dependent antimicrobial activity and effect of mixture of leaf and fruit extracts on test microorganisms (n = 128 for each point).

The overall comparative evaluation on antimicrobial activity of the assayed extracts displayed the microbial inhibitory efficacy in this order: methanolic fruit extract (MFE) > petroleum ether leaf extract (PLE) > methanolic leaf extract (MLE) > petroleum ether fruit extract (PFE) with means of 4.22, 2.33, 1.57 and 1.52 mm of ZI respectively. The antimicrobial efficacy was also demonstrated to be different between groups (p=0.0001; df=3, F = 15.9, n= 896). A positive correlation between concentrations...
had also demonstrated that M. charantia is very rich in triterpenes depend on interactions between their lipid constituents with the net surface charge of microbial components. It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity.

The mixture of methanolic leaf and fruit extracts were observed to be weakly active on E. coli with only 6mm of ZI. This probably is due to dilution effect or chemical antagonism of the various constituents on each other, resulting in inactive or less active products. Nonetheless, S. aureus was sensitive to three out of four assayed extracts. This justifies the traditional use of this plant in treatment of skin infections. Previous studies had also demonstrated that M. charantia is very rich in triterpenes, proteins, and steroids. Those of major interest include momordin, alpha- beta-momorcharin, cucurbitacin B1 and oleanolic acid.

It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity. The fact that two microorganisms viz. Proteus and Cryptococcus were not susceptible to any of the assayed extracts, this needs to be further investigated. It would not be unusual for S. aureus and P. aeruginosa if they were resistant to the assayed extracts because of their multidrug resistance-characteristics. Moreover, infections caused by these microorganisms are the most difficulty to treat with conventional antibiotics. However, the observed resistance of Proteus and Cryptococcus might be attributable to the presence of more active enzymes in these microbes, which deactivate the active antimicrobial agents, or low affinity of the active agent on the target molecules may be the reason behind

**Antimicrobial activity of standard antibiotics**

Comparison of ZI of the tested microorganisms for extracts, positive control (antibiotics) and solvents were considered significantly different at p<0.05. Salmonella typhi was strongly inhibited by gentamycin, while Klebsiella pneumoniae and E. coli were equally susceptible to the antibiotic. No significant difference was observed (p<0.05) with regard to antifungal activity of clotrimazole against C. neoformans and C. albicans (Table 2).

**Table 2: Antimicrobial activities of M. charantia methanolic and petroleum ether leaf and fruit extracts on test microorganisms expressed as zone of inhibition in millimeters.**

<table>
<thead>
<tr>
<th>Conc. (mg/μl)</th>
<th>C. albicans</th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
<th>E. coli</th>
<th>S. typhi</th>
<th>P. aeruginosa</th>
</tr>
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<tbody>
<tr>
<td>0.2</td>
<td>- - - -</td>
<td>13 - 8 11</td>
<td>- - - - - -</td>
<td>8 - -</td>
<td>- - -</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>0.4</td>
<td>- - 10 8</td>
<td>16 7 8 11</td>
<td>- - 13 -</td>
<td>8 - 7</td>
<td>- 15 9  -</td>
<td>- - 10 -</td>
</tr>
<tr>
<td>0.6</td>
<td>- - 11 13</td>
<td>16 9 11 11</td>
<td>- 12 18 -</td>
<td>- 8 -</td>
<td>17 10 -</td>
<td>- 10 -</td>
</tr>
<tr>
<td>1.00</td>
<td>- - 11 13</td>
<td>16 9 11 13</td>
<td>- 13 18 -</td>
<td>- 8 -</td>
<td>17 10 6</td>
<td>- 12 -</td>
</tr>
<tr>
<td>Positive control</td>
<td>18 23 25 25 27</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Petroleum ether extracts of both leaves and fruits showed milder antimicrobial activity compared to the methanolic extracts, which certainly indicates that methanolic extracts contain higher concentration of active antimicrobial agents. But also this could be attributable to the polarity nature of active antimicrobial agents. These may include alkaloids, glycosides, volatile oils or tannins, which are all found in more abundant amount in fruits of M. Charantia. Previous studies had also demonstrated that M. charantia is very rich in triterpenes, proteins, and steroids. Those of major interest include momordin, alpha- beta-momorcharin, cucurbitacin B1 and oleanolic acid.

It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity. The fact that two microorganisms viz. Proteus and Cryptococcus were not susceptible to any of the assayed extracts, this needs to be further investigated. It would not be unusual for S. aureus and P. aeruginosa if they were resistant to the assayed extracts because of their multidrug resistance-characteristics. Moreover, infections caused by these microorganisms are the most difficulty to treat with conventional antibiotics. However, the observed resistance of Proteus and Cryptococcus might be attributable to the presence of more active enzymes in these microbes, which deactivate the active antimicrobial agents, or low affinity of the active agent on the target molecules may be the reason behind
the observed findings. In line to our findings, other previous studies have demonstrated both in vitro and in vivo antibacterial activities against *E. coli*, *Staphylococcus, Pseudomonas, Salmonella*, *Streptobacillus* and *Streptococcus*. Additionally, an extract of the entire plant was also shown to possess antiprotozoal activity against *Entamoeba histolytica*. In another study, a fruit extract has exhibited activity against the stomach ulcer-causing bacteria *Helicobacter pylori*.

Recently, researchers have found that *M. charantia* contains several proteins that can inhibit HIV in vitro. These proteins, known collectively as ribosome-inactivating proteins (RIPs) are alpha-momarchorin, beta-momarchorin and MAP-30 (Momordica Anti-HIV Protein). As research is still in progress, it is unclear which active ingredients in have clinical usefulness. However, some HIV-positive individuals report no benefits from the treatment of HIV/AIDS. From the above results it can be concluded that methanolic and petroleum ether crude extracts of leaves and fruits of *Momordica charantia* have adequate antimicrobial activity. Fruit crude extracts possess relatively higher antimicrobial activity compared to leaf crude extract. Nevertheless, mixtures of the fruit and leaf extracts seem to have neither a synergistic nor additive antimicrobial activity on the tested microorganisms. This study represents the preliminary report on antimicrobial activity of the crude extracts of *M. charantia* against both the clinical isolates and reference bacterial strains that are implicated in opportunistic as well as nosocomial infections. Further studies are recommended that will involve various parts of the plant from distinct areas, select different fractions of crude extracts and purify the most active antimicrobial components. Toxicity studies should also be done to determine their safety.

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