Antimicrobial activity of *Moringa oleifera* from different locations against some human pathogens

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**ABSTRACT**

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking for remedies and health approaches from side effects caused by synthetic chemicals. This has aggravated the search for antimicrobials from plants sources. In this study, aqueous extracts of *Moringa oleifera* samples from different areas of India were screened for antimicrobial activity against two yeast strains, three Gram positive and seven Gram negative bacteria by agar well diffusion assay. Optimal strategies for better antimicrobial activity was established by various methods. Comparison with standard antibiotics, minimum inhibitory concentration (MIC) and microbicidal properties for seeds coat, stem bark and pods’ husks (the best morphological parts of sample 1) were also determined. Most parts of the samples from Punjab region exhibited broad spectrum properties and were comparable with standard antibiotic. The MICs of seeds coat, stem bark and pods’ husks were organism specific and ranged from 0.5 – 1.25 mg/ml, 3.0 – 4.2 mg/ml and 4.0 – 5.6 mg/ml respectively. Both seeds' coat and pods’ husks extract exhibited microbicidal properties which were totally inhibited at 10 and 12 h respectively. Seeds' coat extract was the most effective against *E. coli* at 0 hours, while some pathogens, treated with stem bark extract, exhibited regrowth again after 24 h apparently. Antimicrobial activity of seeds' coat and pods’ husks is reported for the first time. The active constituents may be considered potential candidates for development of broad spectrum antimicrobial drugs.

**Key words:** Antimicrobial, minimum inhibitory concentration, *Moringa oleifera*, medicinal plants.

**INTRODUCTION**

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking for remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to eco-friendly and bio-friendly plants, which can prevent and cure different human diseases (Dubey et al., 2004). According to the World Health Organization reports, the use of traditional medicine in the first world countries is on the rise due to failure of conventional medicine that can cure chronic diseases, emergence of multi-drug resistant pathogens and parasites, adverse effects of chemical drugs, increasing cost and information of herbal medicine.

Antimicrobial potentiality of different medicinal plants is extensively studied all over the world (Ahmed et al., 1998; Arora and Kaur, 1999; Arora et al., 2009; Rojas et al., 2006). However, only a few studies have been carried out in a systematic manner. *Moringa oleifera* is medicinal species, belonging to monogeneric family Moringaceae (order Brassicales). It has 33 species of trees and shrubs
distributed in sub-Himalayan ranges of India, Sri Lanka, North Eastern and South Western Africa, Madagascar and Arabia (Francis and Liogier, 1991; The plant list, 2010). Today, it has become naturalized in many locations of the tropics and is widely cultivated in Africa, Ceylon, Thailand, Burma, Singapore, West Indies, Sri Lanka, India, Mexico, Malabar, Malaysia, and the Philippines (Fahy, 2005).

Almost all parts of this plant: root, bark, gum, leaf, pods, flowers, seeds, and seeds oil have been used for the various ailments in the indigenous medicine (Odebiyi and Sofowora, 1999). It has been known to be anti-helminthic activity, antimicrobial activity, detoxifier, immune booster, and anti-parasitic activity (Thilza et al, 2010). Antimicrobial activities of various M. oleifera morphological parts against some pathogenic microorganisms have been reported (Caceres et al, 1999; Doughari, 2007; Kekuda et al., 2010; Jamil et al., 2007; Nikkon et al., 2003). However, not so extensive work on its antimicrobial properties has been studied and more so, some of its morphological parts are unexplored. The objectives of the present study are to screen samples collected from different areas within India, which has antimicrobial activity and to establish the optimal extraction methods that can give maximal antimicrobial activity. In addition, minimal inhibitory concentration (MIC) was also determined.

MATERIALS AND METHODS

Procurement of plant samples and microbial cultures

Sample 1 seeds, fresh stem bark and dry pods' husks were obtained from Botanical garden of Guru Nanak Dev University, Amritsar Punjab. Later, the seeds were identified by the Forest Research Institute, Dehradun, India as M. oleifera Lamk and the specimen has been deposited in Guru Nanak Dev University, Amritsar –India’s Botanical Herbarium under accession number 6746 HERB. Sample 2 seeds were procured from Indo Exports, Tamilnadu - India and Sample 3 seeds were obtained from Indian Horticulture Research Institute, Bangalore - India. The chemicals and standard antibiotics used in this work were purchased from Hi-Media, Mumbai, India.

Test micro-organisms

The reference bacterial and yeast strains used in this study were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh - India. These included; Gram positive bacteria: Enterococcus faecalis (MTCC 439), Staphylococcus aureus (MTCC 740) and Staphylococcus epidermidis (MTCC 435); Gram negative bacteria: Escherichia coli (MTCC 119), Klebsiella pneumoniae 1 (MTCC 109), K. pneumoniae 2 (MTCC 530), P. aeruginosa 2 (MTCC 741), Salmonella typhimurium 1 (MTCC 98), S. typhimurium 2 (MTCC 1251) and Shigella flexneri (MTCC 1457) and Yeast strains: Candida tropicalis (MTCC 230) and Candida albicans (MTCC 227). The cultures were maintained on nutrient agar slants except Enterococcus faecalis, Candida tropicalis and Candida albicans were maintained on trypticate soya agar (TSA), Sabouraud agar and yeast malt agar respectively. All these were sub-cultured regularly and preserved on solid media at 4°C as well as in 10% glycerol suspensions at -20°C.

Inoculum preparation

The inoculums were prepared as follows; a loopful of 3 to 4 isolated colonies was inoculated into 5 ml of suitable broth, incubated at 37 and 25°C for bacterial and yeast strains respectively. These actively growing bacterial suspensions were then adjusted with their respective suitable broth so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate (BaCl₂. 2H₂O) with 99.5 ml of 1% (v/v) sulphuric acid (H₂SO₄). This turbidity is equivalent to approximately 1 × 10⁶ CFU/ml.

Preparation of plant extracts

Extracts of different plant parts of the three samples were prepared as follows:

Sterilization

The stem bark (B₁) was cut into small pieces and shade dried as recommended by Chirinos et al. (2007). The seeds were shelled to obtain the seeds coat (SC) and seed kernels (S) separately and some were used without shelling as Unshelled seeds (US). The dry pods' husks (PH₁) were cut into small pieces. These plant parts were surface sterilized separately by soaking them in 1% mercuric chloride (HgCl₂) for 5 min and rinsing them 3 to 4 times using sterile distilled water and lastly drying at 40°C. The small pieces of dried plants were ground separately into powder form using an electric grinder.

Preparation of aqueous extracts

The aqueous extracts were prepared by weighing respective amount of powdered material of various parts and suspending the desired quantity in sterile distilled water. The suspensions were kept in a hot water bath initially at 40°C and later at their respective optimum temperatures as established) for 20 min for extraction. Each extracted material was filtered through muslin cloth and the filtrate subjected to antibacterial testing by agar
well diffusion assay.

Screening for antimicrobial activity of M. oleifera samples by agar well diffusion method

The aim of this experiment was to compare the antimicrobial effectiveness of the plant samples against a wide range of human pathogens. The various parts of the three M. oleifera samples were subjected to antimicrobial activity against 3 Gram positive, 7 Gram negative bacteria and 2 yeast strains at different concentrations ranging from 1-20% by well diffusion method (Doughari et al., 2007). Different agar plates of suitable media were prepared and inoculated with 0.1ml of respective test organisms by spread plate method. Wells measuring 8 mm in diameter were cut out under aseptic conditions using a stainless steel borer. Each well was filled with 0.1 ml extract of the different concentrations and refrigerated for 2 h to allow proper diffusion before further incubation in an upright position at respective temperatures and time (37°C for 24 h in case of bacteria and 25°C for 24 - 48 h in case of yeast). A negative control (sterile distilled water) was used in each case. A clear zone of inhibition, which is more than 12 mm, was considered to be sensitive.

Optimization studies

Based on the screening results obtained, unshelled seeds, seeds' coat, stem bark and pods' husks of sample 1 were selected for further experimentation.

Effect of grinding by pestle mortar and electric grinder

This was to determine whether grinding strategy had any significant effect on antimicrobial potential of the plant parts. The surface sterilized plant materials were ground using pestle mortar and electric grinder and antimicrobial activity of their respective extracts was determined as described earlier. The experiment was repeated thrice and the mean inhibition zone recorded and analysed by t-test.

Effect of filtration method on antimicrobial efficacy of extracts

To ascertain whether there was any significant effect of filtration on antimicrobial efficacy of the plant parts, their aqueous extracts were prepared as described earlier and filtered by different means viz; cheese cloth, Whatman filter paper No.1 and centrifugation at 8000 rpm for 15 min. The filtrates obtained were subjected to antimicrobial testing by agar well diffusion method. The experiment was repeated thrice.

Effect of storage on the efficiency of extracts

To assess the shelf life of the extracts, the experiment was designed to check the stability of the extracts stored at 4 °C and 28°C for 30 days. Five percent aqueous extracts of the 4 selected plant parts were prepared in enough quantity and stored at temperatures stated above. The antimicrobial testing against test organisms was conducted after every 5 days within the experimentation period. The results were recorded the size of zone of inhibition and analysed by t-test at 5% confidence level.

Effect of extraction temperature

The experiment was performed to determine the optimum extraction temperature for the four selected plant parts. The surface sterilized samples were ground by electric grinder and 5% of each powder was suspended in sterile distilled water and incubated at different temperatures (30 – 100°C) for 20 min. The controls were prepared without incubation. The suspensions were then filtered using Whatman filter paper No. 1. The filtrates were subjected to antimicrobial testing by agar well diffusion assay.

Thermostability of the extracts

This experiment was to determine the thermostability of the bioactive components of the selected parts of sample 1. Three sets of the aqueous extracts of each plant part were prepared according to their respective optimum extraction temperatures that is, (50°C: unshelled seeds; 70°C: seeds coat; and 80°C: stem bark and pods’ husks) for 20 min. One set was kept as controls and the other two sets for each sample were subjected to heat treatment at their respective optimal temperature (50,70, and 80°C) and 100°C separately for 1 h. Antimicrobial assay of these extracts was performed and their results were compared with their respective controls.

Up to this point, the seeds’ coat, the stem bark and pods’ husks were showing better results as compared to the unshelled seeds and therefore, they were used in the remaining experiments.

A comparison of antimicrobial activity of Moringa oleifera sample 1 and standard antibiotics

This experiment was designed to compare the efficiency of the seeds’ coat, stem bark and pods’ husks of sample 1 with organism specific standard antibiotics. The 5% of the three aqueous extracts including seeds’ coat stem bark and pods’ husks were concentrated by lyophilization and re-suspended in 2 ml sterile distilled water. Several discs were prepared by impregnating each disc with 20 µl of the
lyophilized crude extract and were dried under aseptic conditions. Antimicrobial activity of the concentrated aqueous extracts of sample 1 and standard antibiotics was determined by disc diffusion method (Doughari et al., 2007; Bauer et al., 1966).

Minimum inhibitory concentration of seeds coat, stem bark and pods’ husks

This study was planned to determine the MIC of the extracts selected as discussed earlier by agar dilution method (Parekh and Chanda, 2006; Mahajan, 1992). An aqueous extract stock solution of 5% (50 mg/ml) was prepared and various concentrations ranging from 0.025 – 0.125% (v/v) an equivalent of 0.25- 1.25 mg/ml in case of seeds coat extract, 0.3 – 0.42% (v/v) an equivalent of 3- 4.2 mg/ml in case of stem bark extract and 0.4-0.56% an equivalent of 4 - 4.2 mg/ml in case of pods’ husk were dispensed into suitably cooled (to about 40ºC) agar medium. The suspensions were plated and inoculated with 0.1 ml of a 4h activated test organisms by streaking with a sterile tooth pick. The plates were incubated at 37ºC for 24 h in case of bacterial strains and 25ºC for 24 h in case of yeast strain. The lowest concentrations exhibiting complete inhibition of the microbial growth was taken as the MIC. The experiment was performed in duplicates with positive and negative controls and was repeated three times.

Microbicidal activity of seeds’ coat, stem bark and pods’ husks by viable cell count method

The experiment was performed to compare the time taken by the three extracts to completely kill the pathogens. Stock solutions of 5% aqueous extracts of the plant parts under study were prepared as already described earlier. The microbicidal activity of the extracts was determined by viable cell count method (Toda et al., 1989). Five ml of grown inoculum at 4 h was serially diluted to 10⁻³ CFU/ml with suitable double strength broth medium. Each diluted inoculum was mixed separately with 5⁸ MIC of the extracts under study and incubated at respective temperatures as mentioned earlier. At different time intervals viz.0, 1, 2, 3, 4...24 h, 0.1 ml of the mixed suspension was spread on suitable agar plates in duplicate and incubated for 24 – 48 h at respective suitable temperature. The mean number of colonies were determined and compared with that of control in which the plant extract was replaced with sterilized distilled water.

Data analysis

Most of the experiments were performed in triplicate and repeated thrice.

RESULTS AND DISCUSSION

Screening for antimicrobial activity of aqueous extracts of Moringa oleifera samples

The preliminary screening for antimicrobial activity of various concentrations of aqueous extracts against 10 bacterial and two yeast pathogens indicated a sharp increase in zone of inhibition with increase in concentration of extracts from 1% to 5% by different microorganisms. Beyond 5% concentration, a marginal increase was observed in all M. oleifera samples. This may be attributed to the viscous nature of the extract at higher concentrations thus giving not so uniform results. Therefore, the subsequent results are reported based on 5% concentration. The seeds’ coat, stem bark and pods’ husks of sample 1 exhibited considerable inhibitory activity against a wide range of microorganisms (Figure 1) which was better in case of stem bark than the earlier reports using aqueous and organic extracts of M. oleifera Lam.(Anith et al., 2011; Chetia and Gogoi, 2011; Sarin et al., 2010). The shelled seeds of the sample 2 exhibited better activity than those of sample 1 and sample 3 and this concurs with previous studies regarding antimicrobial properties of the seeds of M. oleifera which were found to have inhibitory activity against a number of pathogens (Anwar and Rashid, 2007; Jamil et al., 2007; Kebreab, 2005; Lockett et al., 2000). However, seeds of all the samples showed no activity against some of the selected pathogens of Gram negative bacteria and C. tropicalis (Table 1). Though previous studies reported the use of M. oleifera seeds for water purification, (Anwar and Rashid, 2007; Broin et al., 2002; Kalogo et al., 2000; Kawo, 2007; Muyibi and Evison, 1995) the seeds under study exhibited resistance to some of the waterborne pathogens. Though unshelled seeds of sample 1 tested against a wide range of test organisms, the level of effectiveness as compared with those of the other samples was significantly indifferent at 5% confidence level. K. pneumoniae 1 was the most sensitive while K. pneumoniae 2 demonstrated resistance to all plant parts. In yeast strains, C. albicans was sensitive while C. tropicalis exhibited resistance. The seeds’ coat of the sample 1 exhibited the highest antimicrobial activity than any of the reported plant parts of M. oleifera (Caceres et al., 1999; Renitta et al., 2009). Apparently, this is the first report on antimicrobial properties for the seeds’ coat and pods’ husks of this plant species.

Optimization studies

Based on the results of the screening experiments conducted above, sample 1 was selected for further analysis.
Figure 1. Antimicrobial activity of 5% of various plant parts from the three samples collected from different areas against the test organisms. (n=3)

Effect of grinding by pestle mortar and electric grinder

To determine whether the activity of the extracts under study was affected by electric grinding as reported earlier (Kaur and Arora, 2009) six bacterial strains and one yeast pathogen were tested for susceptibility against extracts of samples prepared by grinding in pestle mortar and electric grinder. The results indicated a slight variation in zone of inhibition size between the two methods in all the extracts except significant loss of activity of pestle and mortar prepared US extract against S. flexneri and seeds coat, stem bark and pods’ husks against K. pneumonia 1. The loss of activity for extracts ground by pestle and mortar may be attributed to the sample particle size as reported by (Eloff, 1998b) regarding leaching out of higher quantities of compounds in case of fine particles as compared to courser ones. However, the difference between the two grinding methods was found to be statistically insignificant (p<0.05). Therefore electric grinder was used throughout the remaining work. Seeds coat exhibited the highest inhibition in all test organisms followed by the stem bark and pods’ husks which showed a marginal variation and lastly unshelled seeds.

Effect of filtration on antimicrobial activity of sample 1 plant parts

Three methods of filtration were used in this study to determine the efficacy of the filtrates obtained against the test organisms. The results indicated sample specific efficiency. Centrifuged extracts showed higher activity followed by extracts which were vacuum filtered by Whatman filter paper and lastly muslin cloth with the seeds’ coat giving higher activity in all the three cases. Variation in activity of these filtrates could be attributed to quality of the filtrates with the centrifuged ones expected to be clear of suspended particles which may interfere with the quantity of the volumes titrated resulting to lesser activity. In case of US, the efficacy was organism as well as method of filtration specific. An increase of activity against some pathogens and a reduction in others was noted between centrifuged and muslin cloth extracts. However, t-test analysis between the methods used indicated insignificant difference (p<0.05) hence, Whatman filtration was taken as the method of choice in the rest of the study. Among the bacterial pathogens tested, K. pneumoniae 2 was resistant to all the sample extracts and similar results were noted in case of C. tropicalis.

Effect of storage on the efficiency of extracts

All the extracts deteriorated at ambient temperature and the extent was sample specific. The study revealed that the aqueous extracts of all the samples were stable at ambient temperature (28°C±2°C) up to the 20th day of storage except bark which deteriorated within the first 5 days. The activity of bark extracts at ambient temperature, deteriorated in all the attempts perhaps due to inactivation of active constituents by the sample flora since no preservative was added to the extracts prior to storage and therefore no further experimentation was carried out on this. In the remaining period in case of the other extracts,
The optimum extraction temperatures were determined which varied for the four extracts in concurrence with previous reports that the extraction temperature for plant extracts is plant part specific (Chirinos et al., 2007). Seeds coat showed maximum activity at 70ºC with the highest average inhibition zone of 22.2 mm, followed by the stem bark and pods’ husks at 80ºC recording the highest average inhibition zone of 19.8 and 18.2 mm respectively while US showed its highest activity at 50ºC with average inhibition zone of 19.8 and 18.2 mm respectively. Though, SC when tested separately gave the highest activity, reduction of activity might be interfering with the active components. The maximum activities at higher temperatures by both samples as compared to the controls could be due to the fact that higher temperatures promotes higher analyte solubility by increasing both solubility and mass transfer rate as well as decreasing the viscosity and surface tension of the solvent thus enabling the solvent to reach the sample matrices, and consequently improving the extraction rate (Castro et al., 1998).

**Thermostability**

Samples treated with high extraction temperatures was an indication that the active constituents might be thermostable in nature. The experiment was carried out to ascertain this. Initial extraction was done by the usual protocol as mentioned earlier; incubation was done at their respective optimal temperatures and further given heat treatment at the same temperatures and another set at 100ºC temperatures.
each for one hour to check the thermostability of the active compounds. The samples were compared with their respective controls extracted at the initial stage. As previously reported by Chirinos et al. (2007) longer extraction time runs the risk of thermal degradation for most of the phyto–constituents, the tested samples exhibited nearly similar results. All the samples exhibited loss of activity to some extent at both temperature treatments (respective optimal temperatures and 100°C) as compared to their respective controls. Unshelled seeds extract was the most sensitive with 7.3 and 82.5%, seeds' coat showed relative stability with 1.38 and 19.8% lose of activity while stem bark and pods' husks showed a marginal variation of 7.1, 34.0 and 4.9%, 36.8 respectively. Lots of activity was noted (Figure 3). These findings may serve as a wakeup call for the traditional healer which is in their routine practice boil for longer period, thus putting in question the potency credibility of the resulting products.

**A comparison of antimicrobial activity of sample 1 extracts and standard antibiotics**

The various parts of plant, exhibited broad spectrum
properties against a wide range of pathogenic organisms, comparable with standard antibiotics (Table 2). The seeds’ coat portrayed higher potency to both some Gram negative and Gram positive pathogens as compared to some of the antibiotics tested (Alam et al., 2009). These results indicate that the active constituents are plant part specific. These findings suggest that all the samples were potent candidates for drug development.

**Table 2. Comparison of standard antibiotics and aqueous extracts of sample 1.**

<table>
<thead>
<tr>
<th>Org</th>
<th>SC*</th>
<th>B*</th>
<th>P*</th>
<th>T</th>
<th>M</th>
<th>AMX</th>
<th>PN</th>
<th>K</th>
<th>S</th>
<th>C-T</th>
<th>APN</th>
<th>IMP</th>
<th>PP/T</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>31±0.6</td>
<td>24.3±0.9</td>
<td>20.3±0.9</td>
<td>21.0±0.6</td>
<td>-</td>
<td>35.5±0.5</td>
<td>27.5±0.5</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>SE</td>
<td>31.7±0.3</td>
<td>26±1.2</td>
<td>21±0.6</td>
<td>16.7±0.3</td>
<td>38.7±0.3</td>
<td>32±1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28±0.6</td>
<td>18±0.6</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>22±1.2</td>
<td>17.3±0.3</td>
<td>18.7±0.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28±0.6</td>
<td>18±0.6</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>ST1</td>
<td>23±0.6</td>
<td>20±0.6</td>
<td>20.7±0.9</td>
<td>-</td>
<td>-</td>
<td>35.5±1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21.7±0.9</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>25±0.6</td>
<td>22±1.2</td>
<td>29±0.6</td>
<td>13.3±0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31±0.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KP1</td>
<td>44.3±1.2</td>
<td>35±0.6</td>
<td>29±0.6</td>
<td>-</td>
<td>-</td>
<td>40±2</td>
<td>12.5±0.5</td>
<td>-</td>
<td>-</td>
<td>42±0.6</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>32.3±0.9</td>
<td>30±0.6</td>
<td>30±0.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29±0.6</td>
<td>-</td>
<td>28±0.6</td>
<td>18±0.6</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>ST2</td>
<td>28±0.6</td>
<td>22.3±0.3</td>
<td>26.3±0.9</td>
<td>-</td>
<td>-</td>
<td>33.5±0.5</td>
<td>18.5±0.5</td>
<td>-</td>
<td>-</td>
<td>10±0.6</td>
<td>43.3±0.7</td>
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<td>-</td>
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<tr>
<td>EF</td>
<td>21±0.6</td>
<td>16.3±0.3</td>
<td>19±0.6</td>
<td>-</td>
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<td>11.5±0.5</td>
<td>13±0.6</td>
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<tr>
<td>CA</td>
<td>26±0.6</td>
<td>20.3±0</td>
<td>17.7±1.2</td>
<td>28.7±0.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25±0.6</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \ast \) (N=3); \( \ast \) Not tested; \( \ast \) 5% of crude aqueous extract SC-15mg – Seeds Coat; B-15mg- Bark; T-30ug- Tetracycline ; M-5ug- Methicillin; AMX-30ug- Amoxycilin; PN-10ug- Penicillin K-30ug- Kenamycin; S-10ug- Streptomycin; C-T25ug- Co-Trimaxazole; APN-10ug- Ampicillin; IMP-10ug- Imipenem; PP/T-10ug- Piperacillin/Tazobactam and CH-30ug- Chloromphenical.

**Minimum inhibitory concentration of aqueous extracts of seeds’ coat, stem bark and pods husks**

The pathogens used in this study were those which exhibited considerable sensitivity in preceding experiments. The MIC of sample 1 for all the extracts was strain specific. The concentrations used ranged from 0.25-1.25 mg/ml (w/v) in case of seeds’ coat, 3-4.2mg/ml (w/v) for the bark and 4.0-5.6mg/ml for pods’ husks. Most of the organisms were inhibited at MIC of 0.5 mg/ml (w/v) for seeds’ coat and no similar work has been reported earlier on this part of the plant under study except that reported on Detarium microcarpum seeds coat which showed MIC values higher than the present work (Ebi and A fiero, 2011). Stem bark and pods’ husks exhibited much higher MIC values than those of seeds’ coat and their lowest values noted were 3.0mg/ml and 4.0mg/ml respectively (Table 3). However, the concentration for stem bark was much lower as compared to previous report on organic extract (Sarin et al., 2010) of the same plant part. The MIC values as exhibited by all extracts lies within the range of active crude extracts (<8mg/ml) as defined by Fabry et al. (1998). K. pneumoniae was the most sensitive organism to all the extracts and showed MIC values of 0.25, 3 and 4 mg/ml (w/v) respectively and this correlates to the antimicrobial assay studies performed earlier which revealed these organism to be the most sensitive. Though C. albicans exhibited relative sensitivity in
Table 3. Minimum inhibitory concentrations of sample 1 extracts.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Seeds’ coat (SC)</th>
<th>Bark (B)</th>
<th>Pods’ husks (Ph)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.5</td>
<td>3.0</td>
<td>4.5</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0.5</td>
<td>3.4</td>
<td>4</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>0.5</td>
<td>3.2</td>
<td>5</td>
</tr>
<tr>
<td><em>Salmonella typhimurium 1</em></td>
<td>0.5</td>
<td>3.0</td>
<td>5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.25</td>
<td>3.0</td>
<td>4.2</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia 1</em></td>
<td>0.25</td>
<td>3.0</td>
<td>4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.5</td>
<td>3.0</td>
<td>4</td>
</tr>
<tr>
<td><em>Salmonella typhimurium 2</em></td>
<td>0.5</td>
<td>3.4</td>
<td>5.2</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1.0</td>
<td>4.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Figure 4. Viable cell count studies of aqueous extract to sample 1 seeds’ coat against different organisms.

In antimicrobial assays, it was the least sensitive to seeds’ coat, stem bark and pods husks with MIC of 1.25, 4.2 and 5.6 mg/ml (w/v) respectively. Despite this, sample 1 proved to be more effective against *C. albicans* which was reported to be resistant to most plants extracts used earlier (Buwa and Staden, 2006; Heisey and Gorham, 1992; Ncube et al., 2011). Candidiasis is a common opportunistic infection among HIV/AIDS patients and is one of the major causes of death in developing countries (Reichart, 2003). Therefore, *M. oleifera* being readily available in most places of the third world countries could be of great significance in abating such opportunistic infections.

**Microbicidal activity of seeds’ coat, stem bark and pods’ husk**

The microbicidal activity was based on the MIC values of the three extracts obtained earlier. The study was conducted using 5x MIC values of respective organisms for all the three extracts which ranged from 2.5 – 5 mg/ml (w/v) for seeds’ coat, 15 – 21 mg/ml (w/v) for stem bark and 20 – 28 mg/ml for pods husks. *C. albicans* was the least sensitive in all the extracts while *E. coli* was found to be the most sensitive to seeds’ coat and demonstrated 100% killing while most pathogens were killed in 6 h except *C. albicans* and *K. pneumoniae 1* which took 10 hours for complete killing (Figure 4). In case of stem bark, *K. pneumoniae 1* was the most sensitive which exhibited viability of 0.6% at 0 hours whereas reduction in growth for *E. coli, P. aeruginosa, S. typhimurium 1* and *S. typhimurium 2* was noted as from 2 h. *K. pneumoniae 1* and *P. aeruginosa* showed 100% killing in 8 hours with stem bark while the rest of the organisms took 12 hours for complete killing except *S. epidermidis, S. flexneri* and *S. typhimurium 2* which exhibited re-growth after 24 h of incubation (Figure 5) an indication that the active compound (s) may have both cidal...
and static properties. For pods’ husks, initial killing was noted at 4 h of incubation with some of the organisms persisting to 12 h (Figure 6). There was no re-growth of any pathogen after 24 h of incubation in case of seeds’ coat and pods’ husks indicating the cidal nature of the bioactive compounds. To the best of our knowledge, no literature on similar work has been reported on these plant parts of *M. oleifera* plant. However, work done on stem bark aqueous extract of *Holarrhena antidysenterica (kurchi)* against some enteric pathogens was based on higher concentrations as compared to present studies (Ballah et al., 2001).

**Conclusion**

Therefore the active constituents of both the samples could be a source of potential candidates in the search for active constituents that could lead to development of drugs or drug leads of broad antimicrobial spectrum. However, it should be noted that research on antimicrobials at *in vitro* level may not always culminate with the same effects when administered into human body. It regards *in vivo* studies and elucidation of active compounds to ascertain their mode of action. The study also revealed that efficiency of
the active constituents depends on the method of extraction and this could be an important message to traditional herbalists who prefer boiling herbs for long time thus inactivating thermo sensitive active molecules.

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