Antimicrobial Effect of Mangifera Indica Woodash on Bacterial Isolates From Human Skin

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Accepted 16th November, 2014.

There is a concerted and continued search for interesting and novel biologically active compounds found in nature that could be used against pathogenic microorganisms. Ethanol extracts of Mangifera indica woodash were investigated for in-vitro antibacterial activity against isolated skin bacteria, namely Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Streptococcus spp. The agar well diffusion method was used to assay for the antibacterial properties of the woodash extracts. Data obtained from the susceptibility tests showed that the woodash extracts of Mangifera indica at different concentrations inhibited the growth of all test organisms showing varying zones of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol woodash extract were determined against these microorganisms using tube dilution. All test woodash extract of Mangifera indica exhibited a maximum MBC value of 7.0 mg/mL and MIC value of 13.0 mg/mL.

Keywords: Mangifera indica woodash, Inhibitory effect, plant nanomaterials, ethanol extracts.

INTRODUCTION

There is a concerted and continued search for interesting and novel biologically active compounds found in nature that could be used against pathogenic microorganisms. Current research has documented the therapeutic values of natural compounds in a bid to validate claims of their biological activity (Abraham et al., 1996). Because of its constant exposure to and contact with the environment, the skin is particularly apt to contain transient microorganisms. Nevertheless, there is a constant and well defined resident flora modified in different anatomic areas, secretions, habitual wearing of clothing or proximity to mucus membrane (Maisbach et al., 1981). The predominant microorganisms on the skin are aerobic and anaerobic diphtheroids (e.g. Corynebacterium, Propionibacterium), non-hemolytic aerobic and anaerobic Staphylococci (Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus species), Woodash is composed of many major and minor elements which are extracted from plants from the soil during growth. As a product of the combustion of organic matter, plant woodash has a lot of constituents with chemical, physical and biological applications (Abrahams et al., 1996). Data from research studies show the utility of natural products as sources of novel structures, but not necessarily the final drug entity (Newman et al., 2007). The objective of this research project is to investigate the effect of the broad spectrum antimicrobrial activity of woodash obtained from combusted woody parts of Mango (Mangifera indica) on cultures of skin bacterial colonies. Mangifera indica (mango) has been an important herb in indigenous medical systems for 400 years. Reports have shown the antibacterial activity of M. indica. In an in-vitro agar-diffusion technique, M. indica extract showed activity on seven bacterial species; Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Staphylococcus citrens, Escherichia coli, Salmonella agona, Klebsiella pneumonia, one yeast (Sacchchromyces cerevisiae) and four fungi (Thermoasans autantiacus, Trichoderma raesei, Aspergillus flavus and Aspergillus fumigatus. The antimicrobial activities of methanolic extracts of M. indica has shown to exhibit antimicrobial activities at a concentration of 20mg/mL (). Ethano-medical studies indicates that 15 different bioactive compounds extracted from different parts of the i.e. seeds, fruits, resins, exudates, sap are possessing antimicrobial properties ().
MATERIALS AND METHODS

Stem, bark and root parts of M.indica were obtained from the Forest Reserve of the Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The plants were identified by a Plant taxonomist in the Department of Plant Science and Biotechnology, This was to authenticate the botanical identity of the plants by an authority as to validate findings of this research.

The woody parts of Mangifera indica were burnt completely aerobiocly after sun-drying to reduce its moisture content. The resulting charcoal was further subjected to combustion at elevated temperature in a laboratory electric furnace until the woodash was obtained. The woodash particles were shaken and sieved using a mechanical shaker with different mesh sizes. This was to remove detrital materials and to get the finest particle size of woodash. Sieved woodash particles were suspended in de-ionized sterile water at room temperature and centrifuged at 750 rpm for 3 minutes, to settle fine woodash particles using a laboratory centrifuge. This suspension was further centrifuged at 1000 rpm for 2 minutes to settle fine particle. Purification and fractionation of the woodash samples were done in triplicates after which all fractionate and collected woodash particles were dried at 600°C, sterilized by autoclaving at 1210°C for 1 hour, cooled and then stored in airight sample bottles until required for use (Washington, 1981).

The method of extraction used is the ethanol extraction method. ()
The powdered woodash of M.indica was weighed and 50g of each dispersed in triplicates into clean beakers and 200mL of 70% Ethanol added and allowed to stand for 72 hours. This solution was then filtered on Whatman No. 1 filter paper to get the ethanolic extract.

Exactly 10g of both woodash of M.indica was also placed in triplicates into clean beakers containing 100mL of distilled water, which was allowed to stand for 72 hours at room temperature and filtered using Whatman No.1 Filter paper, to obtain on aqueous extract (Konoman et al., 1992).

The filtered solvents were then placed on a laboratory hot plate and evaporated at 100C to complete dryness. The concentrate of each ethanolic and aqueous extracted woodash sample were kept in a sterile universal bottle, from which quantities were weighed to prepare various concentrations of both the aqueous and ethanol extracts which were used in the biological assay (Ellof, 1998b).Test solution made from both woodash samples of M.indica were prepared with care using well calibrated digital weighing balance. This was also done for the reference standard and control solutions (Ciprofloxacin) so as to ensure accurate potency estimation of the different wood ash samples (Humphrey et al., 1952). Data of colorimetric and spectrophotometric properties of the M.indica woodash concentrations of 1:9 dilutions in water at π=220 were collated.

The following bacterial species Staphylococcus aureus , Staphylococcus epidermidis, Streptococius species and Escherichia coli were isolated from different individuals within the Hostels of Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. Age, sex and occupation of volunteers were varied to obtain a representative sample. The skin isolates were collected using standard microbiological procedures and precautions. Bacterial samples were obtained by swabbing a given area of the lower arm and leg using a sterile swab stick. The swab stick was removed from its sterile tube and rubbed into the skin surface until; it was saturated with exudates and sweat from the skin surface. The swab stick was then inserted back into the culture tube and labelled appropriately before being transported back to the laboratory. Bacterial species in sampled skin isolates were involved, identified using cultural techniques, microscopy and biochemical methods.

The inoculums used for the agar diffusion assay consist of suspension containing 106 viable cells or CFU/mL from an overnight culture from a stock suspension that was preserved and used throughout the duration of the work (Innocent, 1989). Stock cultures of test microorganisms were maintained aseptically by culturing them in sterilized peptone water and maintained at 100C in refrigerator.

Assay test tubes and Bijou bottles used to establish the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the wood ash samples were sterilized by dry heat at 1500C for 1 hour. Sterile sampling bottles and tubes had sealed and screw caps.

Bacterial isolates were cultured on pre-dried nutrient, blood and MacConkey agar incubated for 16 hours. The agar well diffusion method was adopted as the most appropriate technique for the antimicrobial susceptibility test (AST) considering the range of concentrations of the different woodash samples used to determine the efficacy of the nanomaterials contained in the woodash (Vincent, 1989).

The 16 hours bacterial cultures were washed with diluted sterile physiological saline solution (0.85%) (w/v). To obtain a standard plate count of inoculum, reference was made to the McFarland’s standard.

Four wells were made on the inoculated plates using sterile 6mm diameter cork borer at well spaced points on the plate. Space between adjacent wells is at least 30mm and also the nearest Petri-dish edge (Hewitt, 1977). Applications of different concentrations of woodash solution to the agar well were done within 2 minutes to minimize atmospheric contamination.

Three drops of 25mg/mL, 50mg/mL, 100mg/mL and 200mg/mL concentrations of each woodash were aseptically placed into each agar well separately using a sterile hypodermic syringe. Inoculated cultures were allowed to stand for 1 hour at room temperature for samples to diffuse across the surface. The plates were then incubated at 370C for 24 hours and 48 hours, respectively.

Equal amounts of ciprofloxacin and sterile water were used as positive and negative controls in each series of independent experiments. Assays were done in triplicates. The diameter of each zone was measured to the nearest 0.1mm. This was done by viewing the plate illuminated from below against a dark background.

Zones of inhibition were measured from the circumference of the wells to the circumference of the inhibition zones. This is recorded as the difference in the diameter between the wells and the growth free zone around the wells. The diameters of the zones of inhibition were read after 6 to 8 hours of incubation and the results confirmed by taking readings again after overnight incubation. (Baron et al., 1994). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using the tube dilution method described by Stratton et al, 1990.

STATISTICAL ANALYSIS

Data derived from the antimicrobial susceptibility assays, minimum inhibition concentration (MIC) was analyzed using a one-way analysis of variance (ANOVA) to determine the
difference in the mean diameters of the zone of inhibition and significance statistically (Daniels, 1990).

**RESULTS**

Based on these susceptibility experiments performed in triplicates with woodash extract from M.indica resulted in a maximum zone of inhibition of 12.01mm in Staphylococcus aureus, 12.69mm in Staphylococcus epidermidis, 19.76mm in Streptococcus spp., and 13.02mm in Escherichia coli (table 1) Positive and negative controls were used in the antimicrobial susceptibility assay.

**TABLE 1:** Mangifera indica woodash extract

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>S. aureus</th>
<th>S.epidermidis</th>
<th>Streptococcus spp</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean S.D</td>
<td>Mean S.D</td>
<td>Mean S.D</td>
<td>Mean S.D</td>
</tr>
<tr>
<td>200</td>
<td>12.01 ± 1.23</td>
<td>12.69 ± 1.50</td>
<td>19.76 ± 1.437</td>
<td>13.02 ± 2.01</td>
</tr>
<tr>
<td>100</td>
<td>8.95 ± 0.218</td>
<td>8.36 ± 3.041</td>
<td>18.56 ± 0.835</td>
<td>10.72 ± 1.722</td>
</tr>
<tr>
<td>50</td>
<td>8.97 ± 0.800</td>
<td>9.42 ± 1.507</td>
<td>15.91 ± 1.501</td>
<td>9.91 ± 1.484</td>
</tr>
<tr>
<td>25</td>
<td>6.74 ± 0.468</td>
<td>6.01 ± 0.990</td>
<td>9.78 ± 0.641</td>
<td>6.72 ± 0.825</td>
</tr>
<tr>
<td>Control</td>
<td>13.51 ± 0.510</td>
<td>27.34 ± 2.086</td>
<td>17.71 ± 3.055</td>
<td>15.62 ± 1.022</td>
</tr>
</tbody>
</table>

Values in the table above are the mean ± standard deviation of three replicate samples. Values with the same superscripts are not significantly different using Duncan Multiple Range Test (DMRT) at 0.05 significance level.

**TABLE 2:** Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of Mangifera indica woodash extract on test organism

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Concentrations of woodash extract (mg/mL)</th>
<th>Ciprofloxacin (mg/mL)</th>
<th>Distilled water negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.25 12.5 25 50 100 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.0 ± 0.75 6.75 ± 8.97 ± 8.95 ± 12.01</td>
<td>13.51 ± 10.51 0.00 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>0.0 ± 0.74 ± 0.0 ± 0.09 ± 5.01 ± 14.92</td>
<td>27.34 ± 2.09 0.0 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>0.0 ± 1.24 ± 0.0 ± 0.09 ± 10.0 ± 15.91</td>
<td>17.71 ± 3.06 0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.0 ± 0.81 ± 0.0 ± 0.13 ± 6.72 ± 1.48</td>
<td>15.62 ± 1.02 0.0 ± 0.84 ± 1.44</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The uniqueness of woodash of M.indica is evident considering its pharmacology and shown to significantly decrease the growth of isolated skin bacteria. However, it’s a combination of constituents of the woodash and not a single component of woodash that makes it posses’ antibacterial properties may be responsible for the observed inhibitory effects of the woodash extracts. Woodash can affect bacterial metabolism indirectly by altering the physiochemical properties of a specific environment or directly through surface interaction. It was demonstrated that the nano-particle size of M. Indica posses inhibitory activities on all test bacterial species at a given concentration.

Therefore, it is hypothesized that the physical-chemical properties of the ethanolic woodash extract of M.indica at specified concentrations inhibits bacterial growth, by generating an unfavorable environment. Thermal activation and hydration further aided the extraction of critical components that confers antimicrobial properties of woodash extracts of M. indica Reactive oxygen ions and radicals are byproducts of aerobic bacterial metabolism with demonstrated toxic effects on bacteria (ImLay et al 1998). Numerous transition elements which are inherent in woodash in combination with elevated levels of reduced numerous macro elements and microelements in woodash with excessive free radical production in the presence of oxygen could cause oxidative stress and damage to bacterial cells, resulting in death (Aruoma, 2003).

**CONCLUSION**

This study confirms that the wood ash extract of M. indica have antimicrobial properties, however the result should be further strengthened by fractioning and assessment of constituents and particle size interference with bioactivity it was noticed that woodash extract of M. indica showed different inhibitions on all test bacteria with varying minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) which includes:

S. aureus with MIC =12.5 mg/mL and MBC= 6.25 mg/mL
S. epidermidis with MIC=13.0 mg/mL and MBC=7.0 mg/mL
Streptococcus spp with MIC = 12.5 mg/mL; MBC=6.5 mg/mL

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E. coli with MIC=12.5 and MBC=7.0 mg/mL. These inhibitory activities may be considered sufficient for further studies aimed at isolating and identifying active principles and evaluating possible antibacterial activity (Lampinen, 2005).

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