Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria

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The antioxidant properties and antimicrobial potential of three ethnomedicinal plants, (Momordica charanta, Senna alata and Nauclea lafifolia) extracted with acetone were investigated. Polyphenols from the medicinal plants were screened for their antioxidant and antimicrobial activities against pathogenic microorganisms (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Candida albicans). The medicinal plants displayed different polyphenols contents and antioxidant activities. In addition, varying antimicrobial susceptibility patterns were exhibited. The highest amount of total phenolic compounds was shown by S. alata and the lowest one was M. charanta. The extract of S. alata showed the highest antioxidant activity. Some microorganisms (S. aureus and C. albicans) were susceptible to the polyphenol extracts with minimum inhibitory concentration values between 1.25 to 5.00 mg/ml while other microorganisms (S. pyogenes and E. coli) appeared to be resistant to the extracts. The results suggested that these plants are not only potential sources of phenolic antioxidants but also potentially, good source of antimicrobial agents.

Key words: Antimicrobials, antioxidants, ethnomedicinal plants, polyphenols.

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depend on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. It is on this basis that researchers keep on working on medicinal plants in order to develop the best medicines for physiological uses (Usman and Osuji, 2007). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies (Kirby, 1996; Hostellmann and Marston, 2002).

As a result of the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics (Cowan, 1999). There is need to develop alternative antimicrobial drugs. One approach is to screen local medicinal plants which represent a rich source of novel antimicrobial agents. The present study was carried out to investigate the antibacterial and antifungal properties of three medicinal plants found in the university town, Abraka, Nigeria. Their polyphenol contents and antioxidant activities were also assessed. This screening is of significant importance because of the urgent need for compounds that would be added to or replace the current antimicrobial agents to which microbes have become largely resistant (Chopra et al., 1997).

Polyphenols are a group of highly hydroxylated polyphenolic compounds present in the extractive fractions
of several plant materials. Polyphenols are well documented to have microbicidal activities against a large number of pathogenic bacteria and fungal species (Scalbert, 1991; Cowan, 1999). Oxidized polyphenols also have inhibitory activity against bacterial growth (Field and Lettinga, 1992; Cowan, 1999). The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (Protease and Carbohydrases) or other interactions to inactivate microbial adhesions, cell envelope transport proteins, non specific interactions with carbohydrates, among others (Cowan, 1999). The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also have inhibitory activity against bacterial growth (Field and Lettinga, 1992; Cowan, 1999). The mechanism of polyphenols toxicity against microbes may be related to inhibition of hydrolytic enzymes (Protease and Carbohydrases) or other interactions to inactivate microbial adhesions, cell envelope transport proteins, non specific interactions with carbohydrates, among others (Cowan, 1999). The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plants due to their nutraceutical effects (Zhu et al., 2004). Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of in vitro observations (Moosmann and Behl, 1999; Parr and Boolwell, 2000).

MATERIALS AND METHODS

Plant material collection

Three plants namely: *Momordica charantia*, *Senna alata* and *Nauclea latifolia* were collected from Abraka, Delta State, Nigeria for this study. Plants were chosen following the leads supplied by local herbal healers. The plants, parts used, ailments treated and preparations of plants are presented in Table 1. They were authenticated at the department of Botany, Delta State University, Abraka, Nigeria, by J. K. Ebigwai, a taxonomist.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Parts used</th>
<th>Ailment treated</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Momordica charantia</em></td>
<td>Leaves</td>
<td>Diabetes, piles, nervous disorders, antimicrobials, anthelmintic and jaundice.</td>
<td>Decoction</td>
</tr>
<tr>
<td><em>Senna alata</em></td>
<td>Leaves</td>
<td>Skin diseases, dysentery, abortifacient, anthelmintic, ringworm, measles and eczema.</td>
<td>Decoction</td>
</tr>
<tr>
<td><em>Nauclea latifolia</em></td>
<td>Leaves</td>
<td>Cough, jaundice, piles, emetic, menstrual disorders, measles and sore.</td>
<td>Decoction</td>
</tr>
</tbody>
</table>

Determination of amount of total phenolic compounds

The amounts of total phenolic content of the extracts were determined by the method described by Singleton et al. (1999). 500 µl of the extract was transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by addition of distilled water. 1 ml of Folin-Ciocalteau reactive solution was added and incubated at room temperature for 3 min. 3 ml of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

Antioxidant activity

The antioxidant activity was determined by ammonium thiocyanate assay (Lee et al., 2002). 500 µl of the extract, 200 µl of diluted linoleic acid (25 mg/ml 99 ethanol) and 400 µl of 50 mM phosphate buffer (pH 7.4) was mixed and incubated at 40°C for 15 min. Aliquot (100 µl) from the reaction mixture was mixed with reaction solution containing 3 ml of 70% ethanol, 100 µl of ammonium thiocyanate (300 mg/ml distilled water) and 100 µl of ferrous chloride (2.45 mg/ml in 3.5% hydrochloric acid). Final reaction solution was mixed and incubated at room temperature for 3 min. Absorbance was measured at 500 nm. Linoleic acid emulsion without extract served as control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

\[\% \text{ Inhibition} = \left(\frac{\text{control OD} - \text{sample OD}}{\text{control OD}}\right) \times 100.\]

Organisms

The bacterial strains used in this study were, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*. The only fungus utilized was *Candida albicans*. The isolates were obtained from the laboratory of Eku Baptist Hospital, Delta State, Nigeria. These organisms were characterized to confirm their identity.

Screening for antimicrobial activity

The disc diffusion method as used by Basri and Fan (2005) was used to evaluate the antimicrobial activity. Mueller Hinton agar plates were prepared as the test medium. Sterile filter paper discs (Whatman No. 1, 6 mm) were impregnated with 100 µl of each of the extracts (10 mg/ml) to give a final concentration of 1 mg/disc and left to dry under the laminar flow cabinet overnight. The microbial inoculum was spread evenly onto the surface of the Mueller Hinton agar plates using a sterile cotton bud before the extract discs were positioned on the inoculated agar surface. Each
Table 2. Polyphenols content and antioxidant activities of some Nigerian ethnomedicinal plants.

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Total poly phenols (mg/g) of the plants&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antioxidant Activities (ammonium thiocyanate assay % of plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Momordica charanta</td>
<td>10·40 ± 0·96</td>
<td>28·39 ± 1·22</td>
</tr>
<tr>
<td>Senna alata</td>
<td>23·19 ± 0·89</td>
<td>37·02 ± 0·45</td>
</tr>
<tr>
<td>Nauclea lafifolia</td>
<td>17·55 ± 0·77</td>
<td>33·42 ± 0·48</td>
</tr>
</tbody>
</table>

<sup>a</sup> Gallic acid equivalents.

* Values are in terms of Mean ± SEM (n = 3) for both total phenolic content (mg/g) and antioxidant activity (% inhibition).

Table 3. Antimicrobial activities expressed as inhibition zone diameter (mm).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>M. charanta</th>
<th>S. alata</th>
<th>N. lafifolia</th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>3.00</td>
<td>-</td>
<td>20.00</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>3.00</td>
<td>4.00</td>
<td>3.00</td>
<td>17.00</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.00</td>
</tr>
</tbody>
</table>

RESULTS

The results of the total phenolic content and antioxidant activities of the three ethnomedicinal plants using gallic acid as standard for the antioxidant activities are shown in Table 2, while Table 3 shows the results of the antimicrobial activities expressed as inhibition zone diameter. The MIC values of the aqueous acetone extracts from the medicinal plants (*M. charanta*, *S. alata* and *N. lafifolia*) against *S. aureus*, *S. pyogenes*, *E. coli* and *C. albicans* are shown in Tables 4, 5, 6 and 7.

DISCUSSION

The total phenolic compounds contents in the plants extracts are shown in Table 2. All the extracts exhibited antioxidant properties. *S. alata* showed the highest antioxidant activity (37·02 ± 0·45), the least antioxidant activity was shown by *M. charanta* (28·39 ± 1·22). Amount of total polyphenols were high in *S. alata* (23·19 ± 0·89) and *N. lafifolia* (17·55 ± 0·77). The least polyphenols activity was shown by *M. charanta* (10·40 ± 0·96). From these tables, it was indicated that antioxidant activity may be affected by different parameters, such as...
Table 5. Determination of MIC values of acetone extracts of some Nigerian medicinal plants against 
*Streptococcus pyogenes*.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>M. charanta</em></th>
<th><em>S. alata</em></th>
<th><em>N. laffolia</em></th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.50</td>
<td>-</td>
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</tr>
<tr>
<td>1.25</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

*+ = Absence of growth, positive control; - = presence of growth, negative control.*

Table 6. Determination of MIC values of acetone extracts of some Nigerian medicinal plants against 
*Candida albicans*.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>M. charanta</em></th>
<th><em>S. alata</em></th>
<th><em>N. laffolia</em></th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.50</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1.25</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ = Absence of growth, positive control; - = presence of growth, negative control.*

Table 7. Determination of MIC values of acetone extracts of some Nigerian medicinal plants against 
*Escherichia coli*.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>M. charanta</em></th>
<th><em>S. alata</em></th>
<th><em>N. laffolia</em></th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>2.50</td>
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<td>+</td>
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<tr>
<td>1.25</td>
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</tr>
</tbody>
</table>

*+ = Absence of growth, positive control; - = presence of growth, negative control.*

the amount of phenolic compounds which is in line with 
the suggestion of Yildrim et al. (2001) that there may be 
relationship between phenolic compounds and reducing 
powers. Presence of phenolic compounds might be the 
reason for reducing power. The antioxidative effect was 
mainly due to phenolic components, such as phenolic 
acids and phenolic diterpenes (Shahidi et al., 1992). The 
antioxidant activity of phenolic compounds is mainly due 
to their redox properties, which can play an important 
role in absorbing and neutralizing free radicals, quenching 
singlet and triplet oxygen, or decomposing peroxides 
(Osawa, 1994). Phenolic compounds are also thought to 
be capable of regenerating endogenous α-tocopherol, in 
the phosphor lipid bilayer of lipoprotein particles, back to 
its active antioxidant form. They are also known to inhibit 
various types of oxidizing enzymes. These potential 
mechanisms of antioxidant action make the diverse group 
of phenolic compounds an interesting target in the search 
for health beneficial phytochemicals (Halliwell and 
Gutteridge, 1989; Hall and Cuppett, 1997). Polyphenols 
have been reported to exhibit antibacterial activities with 
distinguished characteristics in their reactivity with 
proteins related polyamides polymers (Haslam, 1996). Of 
the bacterial strains used in this study (*S. aureus*, *S. 
pyogenes* and *E. coli*), only *S. aureus* was inhibited by *S. 
alata*, while the only fungus utilized, *C. albicans*, was 
inhibited by all three plants extracts with *S. alata* showing 
the highest inhibition, even at low concentration of 1.25 
mg/ml. The inhibition of microorganisms by phenolic 
compounds may be due to iron deprivation or hydrogen 
binding with vital proteins such as microbial enzymes 
(Scalbert, 1991). Phenolic compounds notably proantho-
cyanidins (often called condensed tannins) are vulnerable 
to polymerization in air through oxidation reactions. 
Therefore, an important factor governing their toxicity is 
their polymerization size. Oxidized condensation of 
phenols may result in the toxification of microorganisms. 
On the other hand, polymerization can result in the 
detoxification of phenols (Scalbert, 1991; Fiel and 
Lettinga, 1992). These support the fact that polyphenols 
may be responsible for the antimicrobial activities of the 
extracts of the screened plants. Results from this 
investigation showed the rationale behind the use of 
these plants in traditional medicine. These plants are not 
only interesting sources for antimicrobial activities but 
also potential sources of phenolic antioxidants. The 
present study showed that the extracts from the plants 
inhibited the Gram-positive bacteria better than Gram-
negative bacteria. Generally, plant extracts are usually 
more active against Gram positive bacteria than Gram-
negative bacteria (Lin et al., 1999).

In conclusion, the extracts of the plants have high
potential as antimicrobial agent. This finding provides an insight into the usage of these plants in traditional treatment of foot infections, subcutaneous parasitic infection, intestinal parasitism, venereal diseases and other diseases associated with bacterial and fungal infections.

REFERENCES