African Journal of Biotechnology Vol. 9 (20), pp. 2989-2993, 17 May, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria

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Accepted 30 December, 2009

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 micro organisms (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Esherichia 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 micro organisms (*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, micro-organisms 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

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Table 1. Names, parts used, ailments treated and preparations of plants.

Botanical name	Parts used	Ailment treated	Administration
Momordica charanta	Leaves	Diabetes, piles, nervous disorders, antimicrobials, anthelmintic and jaundice.	Decoction
Senna alata	Leaves	Skin diseases, dysentery, abortifacent, anthelmintic, ringworm, measles and eczema.	Decoction
Nauclea lafifolia	Leaves	Cough, jaundice, piles, emetic, menstrual disorders, measles and sore.	Decoction

of several plant materials. Polyphenols are well documented to have microbicide 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 Carbo-hydrolases) 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 charanta*, *Senna alata* and *Nauclea lafifolia* were collected from Abraka, Delta State, Nigeria for this study. Plants were chosen following leads supplied by local herbal healers. The plants, parts used, ailment treated and administration adopted by herbal practitioners are presented in Table 1. They were authenticated at the department of Botany, Delta State University, Abraka, Nigeria, by J. K. Ebigwai, a taxonomist.

Plants extraction

The leaves of the plants were dried in the laboratory of the department of Biochemistry, Delta State University Abraka, at room temperature (20 - 25°C), for four weeks. Afterwards, samples were ground to pass a sieve of 0.3 mm and were extracted with aqueous acetone (70%, v/v) as described by Yu and Dahlgre (2000). The extracts were then washed with hexane to remove chlorophyll and other low molecular weight compounds. Acetone was evaporated and the extracts were lyophilized and stored at - 22°C prior to biological tests. For the biological tests, lyophilized samples were dissolved in water at the desired concentration; this being referred to as freshly prepared extracts.

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:

$$\% \text{ Inhibition} = [(\text{control OD} - \text{sample OD})/\text{control OD})] \times 100.$$

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.

Plant Extracts	Total poly phenols (mg/g) of the plants[a]	Antioxidant Activities (ammonium thiocyanate assay % of plants)
Momordica charanta	10·40 ± 0·96	28·39 ± 1·22
Senna alata	23·19 ± 0·89	37·02 ± 0·45
Nauclea lafifolia	17·55 ± 0·77	33·42 ± 0·48

[a] 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).

Microorganisms	*M. charanta*	*S. alata*	*N. lafifolia*	Gentamicin
Staphylococcus aureus	-	3.00	-	20.00
Streptococcus pyogenes	-	-	-	-
Candida albicans	3.00	4.00	3.00	17.00
Escherichia coli	-	-	-	3.00

Table 4. Determination of MIC values of acetone extracts of some Nigerian medicinal plants against *Staphylococcus aureus*.

Concentration (mg/ml)	*M. charanta*	*S. alata*	*N. lafifolia*	Gentamicin
5.00	-	+	-	+
2.50	-	-	-	+
1.25	-	-	-	+

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

extract was assayed in triplicate. Sterile distilled water served as negative control. Gentamicin (10 µg/disc) was used as positive control. All the plates were incubated for 24 h at 37°C. The anti-microbial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimetre (mm).

Determination of MIC values

The minimum inhibitory concentration (MIC) of the extracts was determined as described by Basri and Fan (2005) for organisms using the twofold serial dilution method with saline at a final concentration ranging from 5 to 1.25 mg/ml. The tested extracts were added to sterile Mueller Hinton broth into microtiter plates before the diluted microbial suspensions (final inoculums of 10^5 CFU/ml) were added. Each extract was assayed in triplicate. The microbial suspensions were used as positive control and extracts in broth were used as negative control. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 h of incubation at 37°C. The turbidity of the wells in the microtiter plates were interpreted as visible growth of the microorganisms.

Statistical analysis

All tests and analyses were run in triplicate. The experimental data were subjected to an analysis of variance for a completely random design to determine the least significant difference at the level of 0.05.

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 anti-microbial 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*.

Concentration (mg/ml)	*M. charanta*	*S. alata*	*N. lafifolia*	Gentamicin
5.00	-	-	-	-
2.50	-	-	-	-
1.25	-	-	-	-

+ = 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*.

Concentration (mg/ml)	*M. charanta*	*S. alata*	*N. lafifolia*	Gentamicin
5.00	+	+	+	+
2.50	-	+	-	+
1.25	-	-	-	+

+ = 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*.

Concentration (mg/ml)	*M. charanta*	*S. alata*	*N. lafifolia*	Gentamicin
5.00	-	-	-	+
2.50	-	-	-	+
1.25	-	-	-	+

+ = 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 bounding with vital proteins such as microbial enzymes (Scalbert, 1991). Phenolic compounds notably proanthocyanidins (often called condensed tannins) are vulnerable to polymerization in air through oxidization 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.

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