Phytochemical screening, Antioxidant and Antimicrobial activities of Senna occidentalis (L.) leaves

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Abstract
Phytochemical screening, antimicrobial and antioxidant activities of the hexane, ethylacetate and methanol crude extracts of Senna occidentalis (L.) leaves were studied in this work. The preliminary screening of the various extracts revealed the presence of tannins, alkaloids, reducing sugar, phenols, anthraquinones, resins, saponins and glycosides. The antimicrobial screening was carried out using the following organisms; Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Candida albicans, Aspergillus niger, Penicillium notatum and Rhizopus stolonifer. The free radical scavenging capacity using hydrogen peroxide was equally determined to evaluate the antioxidant activities of the extracts. The results obtained showed that Senna occidentalis (L.) leaf extracts have interesting pharmacological active compounds with great radical scavenging and antimicrobial effects and as such could be used in ethno medicine for treatment of some infections and ailments.

Keywords: Antioxidant, Antimicrobial, Senna occidentalis (L.), Phytochemicals, Organisms, ethnomedicine.

1. Introduction
Plants are important source of drugs; especially in traditional medicine [1]. It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to WHO, over 70% of the world populations rely on medicinal plants for primary health care and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties [2-4]. Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [5]. The active principle of many drugs found in plants is biochemical substance that produces definite physiological action on the human body [6]. Some of the valuable ones include; alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, and body building [7]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages [8, 9]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [10]. Interest has also increased recently in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity [11]. Antioxidants have become synonymous with good health; they are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by a variety of sources including, smoking, pesticide and fumes from exhaust. Destroying free radicals may help fight cancer, heart diseases, stroke and other immune compromising diseases [12, 13]. Senna occidentalis (L.) a small shrub about 3 ft. high belong to Leguminosae family. It is native to the tropical regions of America and naturalized in Australia, eastern Africa, southern and eastern USA [14]. Plants belonging to the family have been extensively investigated because of their rich medicinal (anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-plasmodial, anti-rheumatic and hepatoprotective) and economic uses [15, 16, 17, 18, 19]. It is part of our continuing effort and mandate to investigate Nigeria medicinal floras, since studies on this plant showed that the...
nature and amount of the phytochemicals varies according to the season, geographical location and because much work has not been done on this particular genus [20]. This paper reports on the phytoconstituents, antimicrobial and antioxidant activities of Senna occidentalis.

2. Material and method

2.1 Plant Material

Fresh leaves of Senna occidentalis (L.) were collected from botanical garden of University of Ibadan, Ibadan. Specimens were identified by Mr. Donatus Eratus and authenticated by Dr. Ayodele of the Department of Botany, University of Ibadan, Oyo State, Nigeria. The leaves were chopped into pieces using knife and then air dried under shade for 10 days and ground into mesh size and kept in a non-absorptive nylon for subsequent use.

2.2 Preparation of Extracts

Dried and milled leaf materials were extracted successively with Soxhlet extractor at temperature of 80 °C. Each of the solvent; hexane, ethylacetate and methanol were allowed to remain in contact with the plant material for 12 hours; the extracts were evaporated to dryness using rotary evaporator.

2.3 Phytochemical analysis

The extracts were analyzed for the presence of alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatannins, reducing sugars [21, 22, 23, 24].

2.3.1 Test for Alkaloids

0.2 g of extracts was shaken with 1% HCl for two minutes. The mixture was filtered and drops of Dragendorff’s reagent added. Formation of a precipitate indicated the presence of alkaloids.

2.3.2 Test for Saponins

0.2 g of extracts was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins.

2.3.3 Test for Tannins

0.2 g of extracts was stirred with distilled water and filtered. Ferric chloride was added to the filtrate. A blue-black, green or dirty green precipitate was taken as an evidence for the presence of tannins.

2.3.4 Test for Steroids

0.2 g of the extracts was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycoside.

2.3.5 Test for Cardiac-active Glycoside

0.2 g of the extracts was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycoside.

2.3.6 Test for Reducing Sugars

0.2 g of the extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling’s solution A and B for two minutes. An orange precipitate on boiling with the Fehling’s solution indicated the presence of reducing sugars.

2.3.7 Test for Flavonoids

A little amount of magnesium powder and few drops of concentrated hydrochloric acid were added to 3 ml of the extracts. A red or intense red colouration indicated the presence of flavonones.

2.3.8 Test for Resins

5 ml of copper acetate solution was added to 5 ml of the extracts. The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin.

2.3.9 Test for Anthraquinones

0.2 g of the extracts was shaken with 4 ml of benzene. The mixture was filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammoniacal (Lower) phase indicated the presence of free anthraquinones.

2.3.10 Test for Phenols

0.2 g of extracts was dissolved in Ferric chloride solution. A green or dirty green precipitate indicated the presence of phenolic compound.

2.3.11 Test for Phlobatannins

The extracts (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of Phlobatannins.

2.3.12 Test for glycosides

The extracts was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling’s solution A and B were added. Red precipitate indicates the presence of glycosides.

2.4 Scavenging of Hydrogen Peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi et al., 2008a and 2008b [25, 26]. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 285 nm using a UV/Vis spectrophotometer. The samples at ‘1mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml’ were added to H2O2. The decrease in absorbance of H2O2 at 285 nm was measured spectrophotometrically after ten minutes (10 min) against a blank solution containing the test sample in phosphate buffer solution (PBS) without H2O2 and blank solution containing phosphate buffer without hydrogen peroxide (control). All the tests were performed in triplicate. The percentage of hydrogen peroxide scavenged by the extracts was calculated as follows:

\[
\% \text{ Scavenged } [\text{H}_2\text{O}_2] = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

Where \( A_c \) is the absorbance of the control and \( A_s \) the absorbance in the presence of the sample of extract and standard [25, 26]. The values of % inhibition were obtained from Equation 1. For the 50% Inhibitory Concentration (IC50) evaluation of the extract, graphs showing the concentration of the test samples (hexane extract, ethyl acetate extract,
The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi et al.; 2008a and 2008b. In the hydrogen peroxide scavenging radical method, the percentage inhibition of the methanolic extract was in the range of 28.29% – 86.03%. The lowest concentration (0.0625 mg/ml) showed the highest percentage inhibition value (86.03%). There is a characteristic increase in inhibition as the concentration decreases. The ethylacetate extract also showed similar trend as revealed in Table 2 (28.31% at 1.0 mg/ml, 52.95%, 84.64%, 88.45% at 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml respectively, and 93.38 % at 0.0625 mg/ml). The hexane extract followed similar trend, showing 87.77% at 1 mg/ml and 91.73%, 91.87%, 96.95% at 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml respectively and 97.80% at 0.0625 mg/ml. At the lowest concentration of 0.0625 mg/ml the percentage inhibition was the highest for all the extracts. The IC$_{50}$ (the concentration of the samples required to scavenge 50% of the peroxide radicals) was actually used to examine the antioxidant effectiveness of the samples. The lower the IC$_{50}$, the greater the overall effectiveness of the suspected antioxidant sample in question. From the results obtained, it was revealed that the synthetic antioxidant ($\alpha$-tocopherol), showed the best antioxidant effectiveness with IC$_{50}$ of 0.24. However, the test samples; MSO, ESO and HSO had IC$_{50}$ of 0.38, 0.66 and 4.73 respectively. Therefore, the $\alpha$-tocopherol is more effective than the test samples, although they are good antioxidants. The trend thus: $\alpha$-tocopherol > MSO > ESO > HSO (The order of decreasing antioxidant effectiveness).

### Table 2: The percentage inhibition (antioxidant levels) of the different concentrations of the three different extracts.

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<tr>
<th>Conc. (mol/dm³)</th>
<th>HSO</th>
<th>ESO</th>
<th>MSO</th>
<th>$\alpha$-Tocopherol</th>
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A plot of % inhibition against concentration for HSO showing the IC$_{50}$
The three different extracts were effective antibacterial and antifungal agents with methanol extract showing the greatest activity. The presence of phytochemicals has been attributed to be the bioactive principle responsible for the antimicrobial activities of most medicinal plants [27]. The methanol extract contains tannins, saponins, alkaloids, anthraquinones, and resins which majorly were absent in the hexane and ethyl acetate extracts. These secondary metabolites which have been reported to offer great pharmacological activities both in traditional and orthodox medicine are responsible for the enhanced activity of the methanol extract as shown in Table 3. Among all the bacterial organisms screened, the growth of *Staphylococcus aureus* and *Escherichia coli* were majorly inhibited in the methanol extract; *Klebsiella pneumoniae* and *Staphylococcus aureus* in hexane extract while *Escherichia coli* and *Salmonella typhi* in the ethylacetate extract. Comparatively, multicellular metabolism of the *Candida albicans* and *Aspergillus niger* were the most hindered by the extracts placing them at a minimum inhibitory concentration of 25 mg/ml with few exceptions as shown in Table 3.

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3.1 Conclusion
From the results obtained, this plant has the ability to scavenge for free radicals and contain bioactive compounds that can inhibit the growth of microorganisms. The methanol extract proved to be the most effective among the three extracts used and thus support the use of the plant in formulation of new antimicrobial and antioxidant drugs.
Further investigations on the chemical compositions and possible isolation of the active ingredient for specific functions in order to standardize the formulation for efficient medical use would be carried out.

4. References
http://lifesciencesleaflets.ning.com/