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Free radical scavenging capacity and antioxidant properties of leaf extract of *Senna alata* and *Senna podocarpa*

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ABSTRACT: Hydromethanolic and aqueous extract of two medicinal plants – *Senna podocarpa* and *Senna alata*-widely used in various herbal remedies, amongst the south western Nigerians, were studied for their total reducing power (TRP), total antioxidant capacity (TAC), total phenolic contents (TPC), DPPH radical scavenging activity (DRSA), as well as free amino acid content (FAA). The TRP (EC_{50}) varied from 49.47-61.45 µg/ml, TAC expressed as ascorbic acid equivalent varied from 298.78 - 958.19mg/g, TPC expressed as gallic acid equivalent was between 68.81-154.85mg/g, while the DRSA in terms of IC_{50} (inhibitory concentration) was similar for the hydromethanolic extract of both plants and FAA was highest (547.74mg/100g) in the aqueous extract of *S.podocarpa* and lowest (192,08mg/100g) in hydromethanolic extract of *S. alalta*. The aqueous extracts of both plants showed relatively low levels of antioxidant activity and reductive potential while there was positive correlation between TPC and TAC for all the extracts.

Introduction

Reactive oxygen species (ROS) are various forms of activated oxygen, which damage biomolecules within the cell; they include free radicals such as superoxide ions, hydroxyl radicals, as well as non-free radical species such as hydrogen peroxide. Major endogenous sources of antioxidants in living organisms, include, normal aerobic metabolism, stimulated polymorphonuclear leukocytes and macrophages, peroxisomes as well as metabolism of xenobiotics. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, pesticides and organic solvents (Mavi *et. al*., 2004).

The cell has developed several protective (antioxidant) mechanisms to detoxify or prevent the formation of ROS. In healthy individuals, equilibrium exists between free radical production and the antioxidative defense system (Lie-Fen *et. al*., 2005). Oxidative stress resulting from the imbalance between oxidants generation and destruction by antioxidant, may lead to damage in the structure and function of the cell membrane (Polidori *et. al*., 2000). Defense mechanism against oxidative stress in the human body includes antioxidant enzymes like peroxidases, catalases, superoxide-dismutases, proteins e.g albumin or transferring as well as small molecules like ascorbic acid, vitamin E or secondary metabolic plant products.

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Exogenous supplies of these small molecules (synthetic or naturally occurring) are paramount in the prevention and treatment of complex diseases like atherosclerosis (Benkebila, 2005), diabetes (Johansen *et. al*., 2005), cancer (Osman *et. al*., 2004) and sickle cell anemia (Takasu *et. al*., 2002). Due to the carcinogenicity of some synthetic antioxidants (El-Sayed 2009), interest has increased considerably in the search for naturally occurring antioxidants for use as foods, dietary supplements or drugs. Some secondary metabolic plant products, particularly phenolic compounds (Prakash *et. al*., 2007; Unver *et. al*., 2009) found in fruits, vegetables (Wangcharoen and Morasuk, 2009) and medicinal plants (Okigbo *et. al*., 2009) play a potential role in prevention of human diseases, due to their antioxidant activities.

The use of plants as a source of medicine, by majority of the lower social cadre in the south western population in Nigeria, in the treatment of a variety of disease is wide spread. *Senna alata* and *Senna podocarpa* are used either singly or in conjunction with other plants in herbal preparations. The leaves of *S. alata* have been used for the treatment of various intestinal disorders, skin diseases (eczema, ringworm) and jaundice (Awal *et al.*, 2004, Pieme *et al*., 2006). The leaves and flowers of *S.podocarpa* are employed in the treatment of sexually transmitted disease (gonorrhea)(Gomes *et al*., 1997), pile (Ogunkunle and Ladejobi, 2006), fever (Sofowora, 1982) and also used as laxative (Tan, 2001) or purgative (Akinremi, 2000). Phytochemical constituents of these plants include anthraquinones, flavonoids and saponins (Tan, 2001). This work focused on evaluating the antioxidant potential and free radical scavenging activity of these plants.

Materials and Methods

Preparation of Extracts

All plants were sourced and authenticated by the Forestry Research Institute of Nigeria, Ibadan, and voucher specimens of *Senna alata* leaves (No.FHI. 106929) and *Senna podocarpa* leaves (No.FHI. 106995) were deposited at FRIN herbarium.

Plant material (200g) was exhaustively extracted with water (Aq) or aqueous methanol(Hm) (3:1 v/v) using Soxhlet apparatus, concentrated under reduced pressure to a viscous liquid, freeze dried and stored at 4^{o}C until use.

Total Antioxidant Capacity

The total antioxidant capacity of the extract(s) was determined with slight modifications to the method of Prieto *et. al*.(1999) The assay is based on the formation of a green phosphate- molybdenum (v) complex by the reduction of molybdenum (vi) at acidic pH. Aliquots of 0.1ml of each extract or ascorbic acid (100µg/ml) was combined with 3ml of working reagent (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were incubated at 95^{o}C for 90 minutes, cooled to room temp and the absorbance of each solution was measured at 695nm against a blank (working reagent plus solvent) using a spectrophotometer. The experiment was performed in triplicates. The total antioxidant capacity was expressed as milligram equivalent of ascorbic acid.

Total Reducing Power

The reducing power of the crude extracts, gallic acid and ascorbic acid was determined by assessing the ability to reduce ferric chloride solution. Aliquots of 1ml graded concentration (25-100µg/ml) of the crude extract, gallic acid or ascorbic acid was mixed with equal volume of 1% ferriccyanide and 200mM phosphate buffer, pH. 6.6. the mixture was incubated at 50^{o}C for 20 minutes. An equal volume of 10% trichloroacetic acid was then added and the resulting solution centrifuged at 6000 rpm for 10 minutes. The resulting supernatant, distilled water and 0.1% ferric chloride were mixed in a ratio 1:1:2 and the absorbance measured at 700nm. A higher absorbance indicated higher reducing power.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts or ascorbic acid was assayed with slight modification of the method of Shiwalker *et. al*., (2006) which depends on the production of yellow coloured diphenylhydrazine via reduction of purple DPPH (maximum absorbance at 517nm). Graded concentrations (25-100µl) of the extract or vitaminC were prepared, 2ml of each test solution was then added to 0.5ml of 1mM DPPH solution in methanol and the mixture incubated in the dark for 15minutes at 37^{o}C, after which the absorbance was measured at 517nm against a blank containing methanol and DPPH. The experiment was performed in triplicate. The DPPH radical scavenging activity was calculated using the equation:

% DPPH radical scavenging activity = 1-(A_s/A_b) x100

Where As and A_b are absorbance of the sample and blank respectively. Decrease in absorbance indicates increase in DPPH radical scavenging activity.

Total Phenolic Content

The total phenolic content of the plant extract(s) was determined using the folin-ciocalteau reagent (FCR) which is reduced by phenols to a mixture of blue oxides with maximal absorbance at around 750nm. Equal volumes (100µl) of plant extract or gallic acid(0-100µg) were mixed with 500µl of FCR and 1.5ml of 20% sodium carbonate. The resulting mix was thoroughly shaken, made up to 10ml with distilled water and incubated at room temperature for 2hours. The absorbance at 760nm was determined against a reagent blank. The experiment was performed in triplicate and the total phenolic content expressed as gallic acid equivalents (GAE)

Results and Discussion

Table1. Total amount of phenolic compounds, free amino acids and total anti oxidant capacity

Plant	Total phenolic (mgGAE/gplant extract)		Free amino acids (mg/100g)		Total antioxidant capacity (mgAAE/gplant extract)	
	Hydro-methanolic	Aqueous	Hydro-methanolic	Aqueous	Hydro-methanolic	Aqueous
S.alata	102.21±0.11	68.81±0.03	192±0.12	542.92±0.63	855.58±0.44	298.78±0.38
S.podocarpa	154.85±0.34	79.28±0.06	418.31±0.99	547.74±0.45	958.19±0.59	559.74±0.07

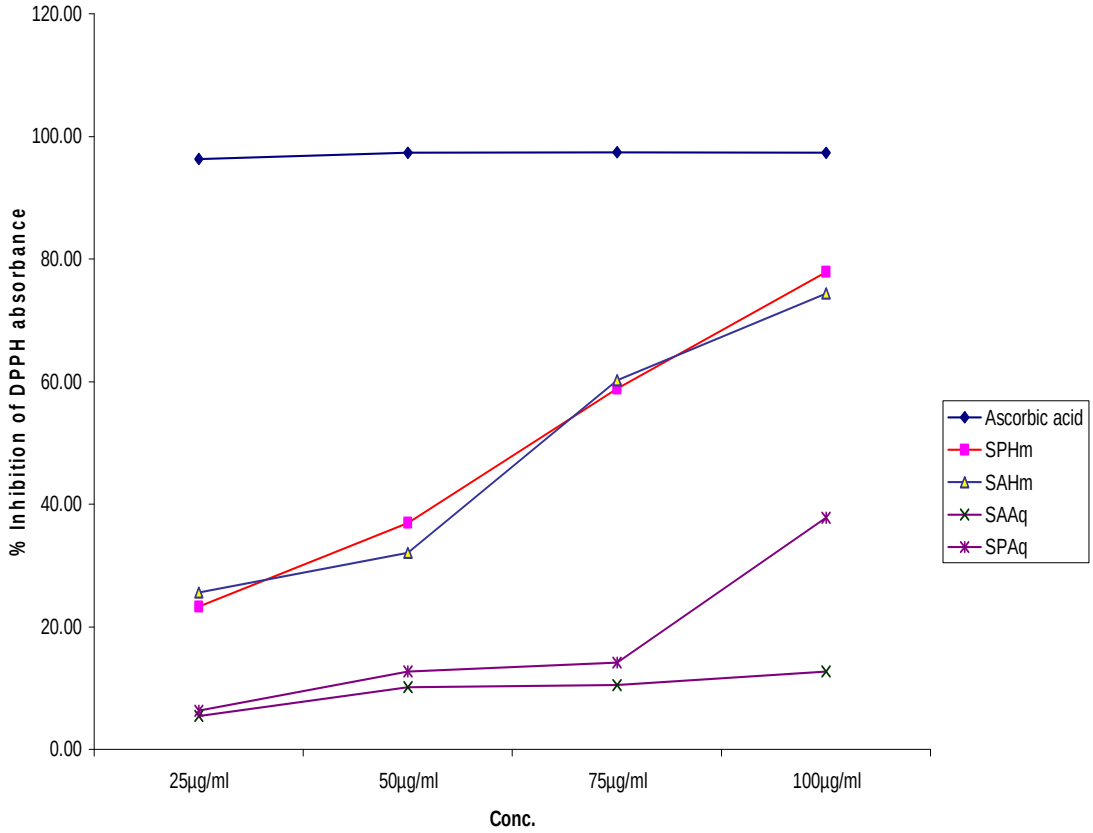

Fig.1 DPPH radical scavenging activity of hydromethanolic(Hm) and aqueous extract of *S.alata* and *S.podocarpa*

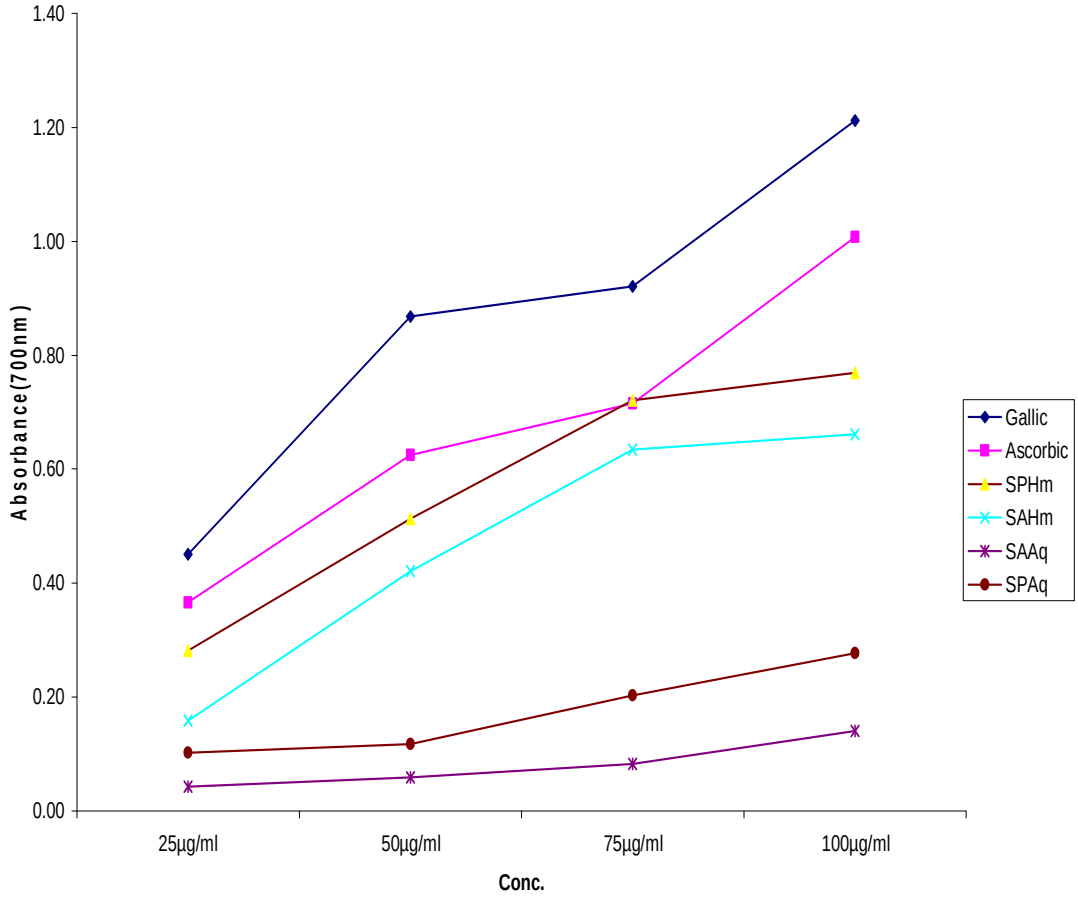

Fig.2 Reducing Power of gallic acid, ascorbic acid, hydromethanolic(Hm) and aqueous(Aq) extract of *S.alata* ***and*** *S.podocarpa*

Plant phenolics constitute one of the major groups of compounds, possessing a wide spectrum of chemical and biological activities including radical scavenging properties and may thus act as primary antioxidant free radical terminators. The total phenolic content (Table 2) of the four extracts expressed as mg gallic acid equivalent per gram dry weight of extract was highest in the hydromethanolic extract of *S.podocarpa* (154±0.34) followed by hydromethanolic extract of *S.alata* (102.21± 0.11), then aqueous extract of *S.podocarpa* (79.28±0.06) and lastly aqueous extract of *S.alata* (68.81±0.03). There were significant differences ($p<0.05$) amongst the four extracts in total phenolic compounds content. Both *S.podocarpa* and *S.alata* hydromethanolic extract had higher total phenolic content than their corresponding aqueous extracts. Plant polyphenolic compounds play an important role in stabilizing lipid oxidation and are associated with antioxidant activity of vegetables or medicinal plants. Several studies have described the antioxidant properties of medicinal plants, foods and beverages which are rich in phenolic compounds (Krings and Berger, 2001; Wang *et. al.*,2008).

The medicinal value of amino acids-plant primary metabolite- in form of supplements can be very beneficial. The aqueous extracts of *S.podocarpa* and *S.alata* had a significantly higher content of free amino acids than their hydromethanolic extracts (Table2). These plants may serve as a reservoir of available amino acid when consumed as teas. Hydrogen and electron transfer from antioxidant analytes to Mo (VI) complex and DPPH occur in the phosphomolybdenum and DPPH assay methods. The hydromethanolic extracts of *S.podocarpa* and *S.alata* showed (Table 2) a significantly higher antioxidant capacity than their respective aqueous extracts, but the hydromethanolic extract of *S.podocarpa* showed overall highest total antioxidant capacity, which may be attributed to its higher phenolic content. The DPPH radical scavenging activity (fig.1) showed a dose-response relationship. The hydromethanolic extract of *S podocarpa* and *S.alata* had similar IC_{50} values of 64.56µg/ml and 65.16µg/ml respectively which were about five times less potent than ascorbic acid (13.59µg/ml). The aqueous extract of both plants showed very weak antioxidant activity since the DPPH absorbance was not lowered by 50% under the experimental conditions. The aqueous extracts showed poor reducing power, but gallic acid, ascorbic acid, hydromethanolic extracts of *S.podocarpa* and *S.alata*, exhibited EC_{50} (effective concentration) value extrapolated from fig.2, of 28.13, 38.02, 49.47 and 61.45 µg/ml respectively.

The presence of phenolic compounds might be the reason for reducing power since a positive correlation ($r^2=0.78$) existed between TPC and TAC, which is in accordance with previous studies which showed that high TPC increased antioxidant activity ( )

The antioxidative activity of the phenolic compounds may be attributed to its redox properties which allow them act as a reducing agent, hydrogen donors, singlet oxygen quenchers, metal chelating properties (Huang *et. al.*, 2004), inhibit various oxidizing enzymes and regenerate endogenous alpha tocopherol in the lipid bilayer (Letelier *et. al*., 2009) According to Osman *et. al*., (2004) reducing power has a direct positive correlation with antioxidant properties. Little quantities of different active principles are present in these extracts which could provoke multiple and synergestic antioxidant effects (Letelier et. al., 2009)

Different values of each antioxidant assay from the same sample are found in various papers (Okoro *et al*., 2010, Surrasmo *et al*., 2005) which could be caused by variation in geographical location, environmental conditions or the mechanism of the compounds in the natural sample.

The increased DPPH and TAC values of hydromethanolic over aqueous extract could be due to the solvent of extraction which may have selectively solubilised more phenolic compounds as shown by the increase in phenolic content. The hydromethanolic extracts contain more apolar compounds than the water extract, increase in the polarity of a compound causes an increase of solubility of the compound in the apolar phases in which peroxidation occur. The free radical scavenging property may be one of the mechanisms by which these plants are effective in traditional medicine. Phenolic compounds may be responsible for antioxidant properties of many plants (Uddin *et. al*., 2008)Therefore methanolic extract of *S.podocarpa* and *S. alata* can be more effective antioxidants than their water extract.

Food and medicinal herbal compounds processing relevant antioxidant properties are xenobiotics for human being. These plants have various compounds (Ogundare, 2009, Okwu and Uchenna, 2009) which have antioxidant properties (Mavi *et. al*., 2004). The health benefit of these plants (phytochemicals), may therefore be due, to their content, of diverse group of phenolic compounds with different mechanisms of antioxidant action.

Conclusion

The aqueous extracts showed relatively low values for the antioxidant activity and the reductive potential which gives credence to the strong positive association between total phenolic content and the total reductive potential as well as DPPH radical scavenging activity. The high free amino acid contents, however, suggests a nutritional benefit. Conversely the results obtained from this study demonstrate that the phytochemicals in the leaf extract of *S.podocarpa* and *S.alata* may have a significant radical scavenging activity, reducing potential and antioxidant capacity in the following order, hydrmethanolic extract of S.podocarpa> hydromethanolic extract of S.alata > aqueous extract of S.podocarpa > aqueous extract of S.alata. The use of these species, in the therapy and prevention of diseases in which free radicals or oxidants have been implicated as major contributing factors to disease etiology, requires more studies to ascertain their mechanism of action.

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