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Research Article

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITIES OF *SIDA RHOMBIFOLIA* EXTRACTS

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ABSTRACT
In the present study, different extracts of aerial part of the plant using solvents like 70% methanol, 100% methanol, 100% ethanol, 100% chloroform and 100% hexane were tested for DPPH and ABTS free radical scavenging activity along with the measurement of their total phenol content. Results showed that the inhibition DPPH radical was dependent on the concentration of the extract. The extent of percentage inhibition also increased from a low polarity solvent extract to a high polarity solvent extract. Thus the inhibition showed the pattern of 70% methanol > 1000% methanol> 100% >100% hexane respectively. The standards gallic acid displayed 100 % DPPH inhibition at the dose 5000ug/ml. Highest ABTS scavenging activity was seen in methanol extract having IC50 (µg/ml) of 50. Gallic acid showed IC50 (µg/ml) value as 25. Total phenol content showed the same pattern being highest in 70% methanol extract (560g GAE/g)) and lowest in 100% hexane extract (260mg GAE/g). Thus all SR extracts from solvent extraction of aerial parts showed free radical scavenging activity. Activity decreases as the polarity of solvent decreases. Hence extract from most polar solvent had greater activity than extract from less polar solvent. Free radical activity could be due to the phenol content.

Keywords: *Free Radicals, Anti-oxidants, Phenol Content, DPPH, ABTS, Oxidative Stress*

INTRODUCTION
Modern life style imposes various detrimental impacts on the human population. Present day population relies on taking fast food because of their busy schedule. They are used to take pain killers for relief of pain, sedatives and alcohols to overcome the mental stress. All these result into oxidative stress According to Karbownik and Lewinski (2003), oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, and chronic fatigue syndrome. Oxidative stress depletes the anti-oxidants present in the body and become the causative factors for above mentioned physiological disorders (Paul *et al.*, 2011). Therefore, there is a need to supplement the anti-oxidant system of our body. Best way to get rid of the imbalance is the supplementation of food with the diet rich in anti-oxidants. Green vegetables, leaves and fruits are the natural source of anti-oxidants. Apart from them, traditional medicines also play a significant role in alleviation of the oxidative stress conditions. *Sida rhombifolia* (SR) is one of those traditional medicines. In traditional African medicine decoctions of the roots and leaves are widely used as emollient (Burkill 1997). The leaves or the leaf sap are applied to the skin as an antiseptic and to treat abscesses, ulcers and wounds (Balkrishanan *et al.*, 2008). In Cameroon, macerated extract of leaves of the plant is used as anti hypertensive and to cure diarrhoea (Naumi *et al.*, 1999). Our previous study reported the hypoglycemic and hypolipedemic effects of extracts of aerial parts of SR (Chaturvedi *et al.*, 2009).
SR is a short-lived perennial subshrub (woody stem and herbaceous branches) commonly growing to 60 cm, but sometimes reaching 1.5 m in height. It is found throughout Botswana. The plant is predominantly found in eastern countries such as India where it is used in Ayurvedic system of medicine. Dhalwal *et al.*, (2007) has reported the anti-oxidant properties of leaf, root and whole aerial parts of SR. As per his report, whole aerial part could not prevent lipid peroxidation in liver and brain homogenate because of its little antioxidant properties. Plants of the same species growing in different environment may not have same potency with reference to their effect on particular biological system. Hepatoprotective effects of water extract of aerial part have already been reported by Rao *et al.*, (1997) and the protection is rendered only by the anti-oxidant effect. Even in the Ayurvedic system of medicine, whole aerial part is being used

for different formulation for different ailments. Because of these reasons, it was decided that the different extracts of the whole aerial part of SR should be studied for their anti-oxidant properties. Therefore the present study has been aimed to carry outthe free radical scavenging potency as well as the total phenol content of different extracts obtained after extraction with solvents of different polarities.

MATERIALS AND METHODS

Collection of Plant Materials

The aerial parts of SR were collected along the roadside and by the construction sites in the University of Botswana main campus. The whole plant was first washed with tap water and rinsed with distilled water and allowed to dry. The dried plant material was then ground to powder using a blender. Different types of extracts were then prepared from this powder SR material.

Preparation of 70% Methanol, 100% Methanol, 100% Chloroform and 100% Hexane Extracts

Soaking the plant in a solvent, such as water, methanol and chloroform, dissolves the active ingredients and separates them from the plant material. The powered plant (375grams) was first soaked in 1000ml of hexane. The plant material was allowed to soak in hexane for 24 hours, after 24 hours, Hexane soaked with the plant extract was then filtered in an empty bottle. Chloroform was added to the same bottle containing the powdered plant material, it was allowed to soak for the minimum of 24 hours before filtering into another empty bottle. The same procedure was carried out with 100% methanol and then with 70% methanol. Both the extracts were made solvent free by evaporation using a Butchi Type Rotary evaporizer under reduced pressure to yield concentrated extracts. The percent yield was 2.4% for 100% hexane extract, 4.1% for 100% chloroform extract, 5% for 100% methanol extract and 5.8% for 70% methanol extract.

Free Radical Scavenging Activity by TLC- DPPH Method (Semi-quantitative Assay)

Thin layer chromatography was used to determine the capacity of scavenging of the free radical DPPH by the plant extracts as described by Kwape and Chaturvedi (2011). First of all, grids with 10mm spacing were made on a thin layer chromatography plate. Using a micro liter syringe, different concentrations of the extracts/ standard (Gallic acid) ranging from 0.5-20mg/ml were spotted on different labeled grids. For the control, methanol was used. The volume of the spoted samples was kept constant for the different extracts. The spots were allowed to dry for at least 2 hours before spraying with 0.02% DPPH solution. A photograph of the chromatogram was taken after 30 minutes. The inhibition of DPPH by different extract was noted.

Spectrophotometric Measurement of Free Radical,2, 2- diphenyl-1-picryl Hydrazyl Radical (DPPH) Scavenging Activities of Extracts by using

For the determination of scavenging properties of different extracts of SR using DPPH (2, 2-diphenyl-1-picrylhydrazlyl), the procedure was carried out as described by Yeboah and Majinda (2009). Different concentrations of Gallic acid or plant extracts were prepared ranging from 0.5- 5mg/ml. 0.02% of DPPH was prepared and stored in the refrigerator until ready for use. One milliliter of the plant extract or standard was added to an equal volume of DPPH solution. A control mixture containing 1ml of the solvent and DPPH was prepared and all the mixtures were kept in darkness for 30 minutes, hence absorbance was measured at 517nm using methanol as the blank. Using the equation 1 below, inhibition curve versus concentration was plotted, and the concentration of each sample required to reduce DPPH radical by 50% was extrapolated.

I= {(Absorbance control – Absorbance sample)/ Absorbance control} * 100

2, 2-Azobis-3-ethyl Benzothiazoline-6-sulphonic Acid (ABTS) Radical Scavenging Activity

The antioxidant capacity was estimated in terms of the ABTS+ radical scavenging activity following the procedure described by Pellegrini *et al.*, (1999). To obtain ABTS+ radical, 7 mM ABTS stock solution was incubated with 2.45 mM potassium persulfate in the dark at room temperature for 16 hours before use. The ABTS+ radical solution was diluted with distilled water to an absorbance of 1.000. Inhibiting concentrations of extracts were tested at 6.25, 12.5, 25, 50 and 100µg/ml. Gallic acid was tested at 1.56, 3.13, 6.25, 12.5 and 25µg/ml.

The ABTS+ radical-scavenging activity of the samples was expressed as the percent inhibition of the free radical by using following equation:

% inhibition =[(Absorbance of control – Absorbance of test sample)/Absorbance of control*100

The concentration of each sample required to reduce ABTS radical by 50% (I50) was also determined.

Determination of the Phenol Content

Method described by Stoilova *et al.*, (2007) was used to measure the total phenolic content of all four extract using Folin-Ciocalteu reagent. Gallic acid was used as standard. Folin Ciocalteu reagent was diluted 10 times with distilled water. 0.1 ml Gallic acid or SR (0-50mg/ml) were mixed with 0.75 mL of the diluted Folin–Ciocalteu reagent and incubated at room temperature. Then, 0.75 mL of 2% sodium carbonate solution was added to the mixture. The mixture was allowed to stand in darkness for an hour before measuring the absorbance at 765 nm using a UV–Visible spectrophotometer. Total Phenol Content values were determined from a calibration curve prepared with a series of Gallic acid standards. Results are expressed as mg of Gallic acid equivalents/g fresh weight (mg GAE/g).

RESULTS AND DISCUSSION

Results

TLC/DPPH Antioxidant Activity of Different Extracts of SR

Results of TLC/DPPH semi quantitative assay are shown in photograph of TLC plate after experimentation (Figure 1). Various concentrations (0.5mg/ml to 20mg/ml) of whole plantextracts from different solvents spotted on the TLC sheet showed high activity, indicated by theyellow color over the purple DPPH background. The extentof yellow color showed the antioxidant activities of the extracts against DPPH free radicals. The TLC sheet showed the highest activities with 70% methanol extract followed by 100% methanol and 100% chloroform extract. The extent of yellow coloration produced by scavenging DPPH free radicals was comparable with yellow color produced by the activities of Gallic acid. 100% hexane extract produced the least effect.

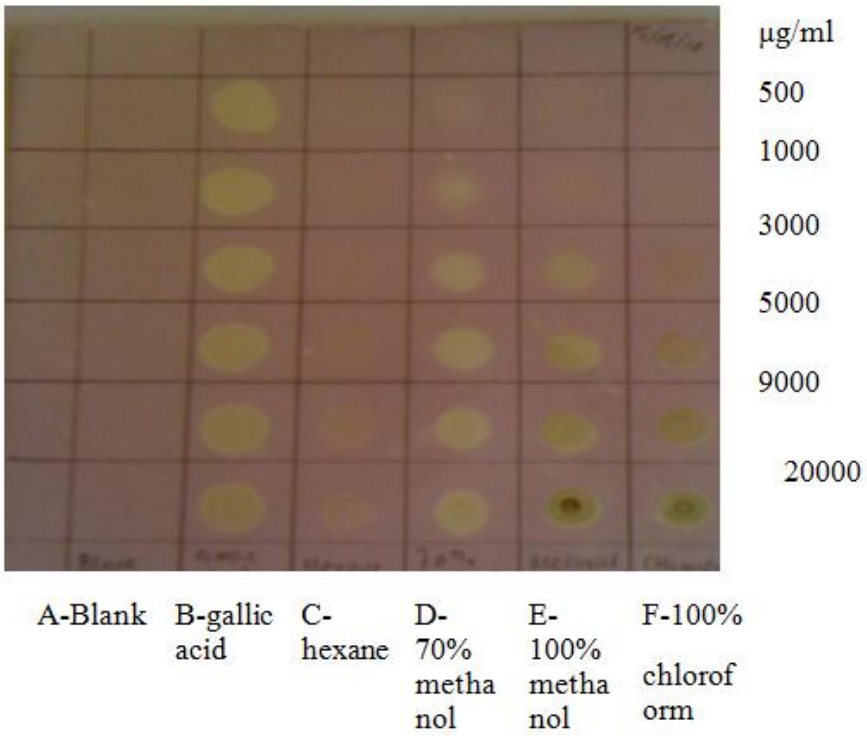

Figure 1: Free radical scavenging activity by semi quantitative method (after 30 minutes of spraying with DPPH)

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Spectrophotometric Assay of DPPH Radical Scavenging Activities of Different Extracts

Results of Quantitative measurement of DPPH radical inhibition by different types of SR extracts presented in Figure 2. It indicated that the inhibition activity was concentration dependent. The extent of percentage inhibition also increased from a low polarity solvent extract to a high polarity solvent extract. Thus the inhibition showed the pattern of 70% methanol > 1000% methanol> 100% >100% hexane respectively. The standards Gallic acid displayed 100 % DPPH inhibition at the dose 5000ug/ml.

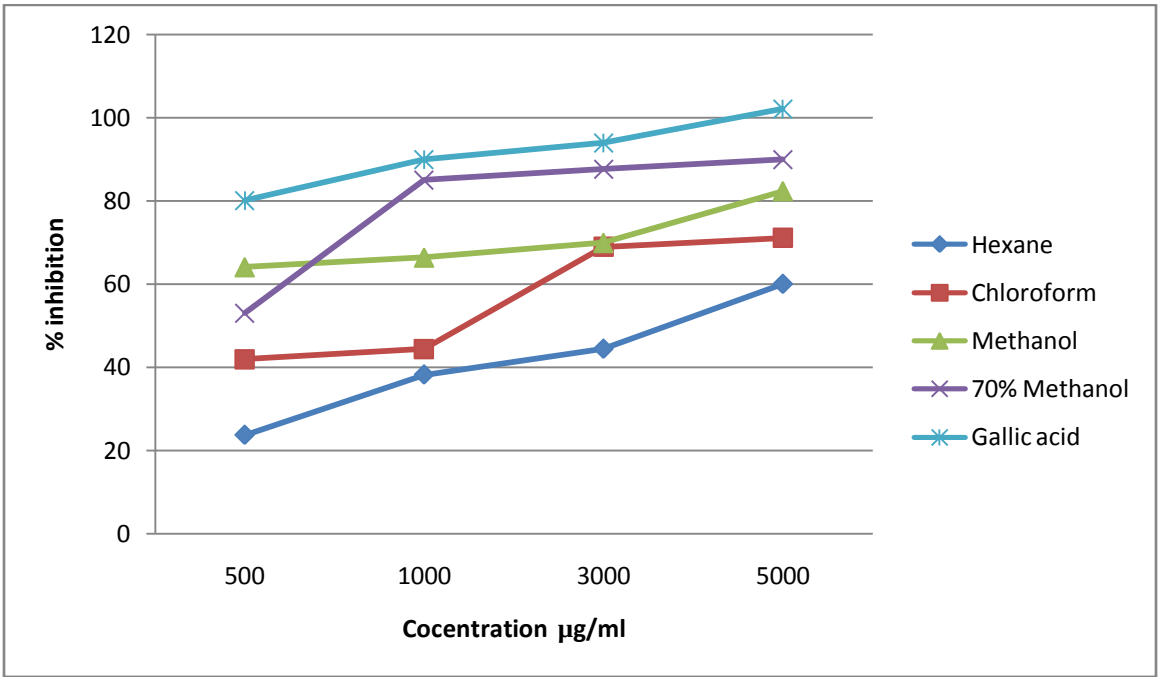

Figure 2: Percent DPPH inhibition versus concentration of different SR extracts

Phenol Content

As presented in Table 1, the highest amount of phenol content was present in 70% methanol extract (562 mg GAE/g)) followed by 100% chloroform and 100% methanol extract. Least amount was present in 100% hexane extract (230mg GAE/g).

Table 1: Total phenol content of SR

Plant extract	Total phenol content(mg GAE/g)
70% methanol	562±2.4
100% methanol	520±3.1
100% chloroform	466.6±6.7
100% hexane	230±2.8

ABTS Radical Scavenging Activity

Table 2 showed the results of ABTS free radical scavenging activities of different SR extracts. Effect of ABTS free radical scavenging activity of 70% methanol, 100% methanol, 100% chloroform and 100 %hexane extracts of SR was tested at varying concentrations of 6.25, 12.5, 25, 50 and 100µg/ml. The method was based on the ability of antioxidant molecules to quench the long lived ABTS radical action. Significant ABTS IC50 was determined as shown in the table.Highest ABTS scavenging activity was seen inmethanol extract having IC50 (µg/ml)of 50. Gallic acid showed IC50 (µg/ml) value of 25.

Table 2: In vitro ABTS free radical scavenging activity of different extracts of SR.

Plant part	Tested extract	% inhibition	IC50 (µg/ml)
Whole plant extract	70% methanol	63.8	50
	100% methanol	66.7	50
	Chloroform	55.1	100
	Hexane	51.5	100
Reference standard	Gallic acid	94.4	25

Discussion

Above results indicate that all the extracts of aerial parts of SR have antioxidant properties. Any substance can be considered as anti-oxidant if it scavenges free radicals. DPPH is considered as proton free radical and scavenging of proton free radical is one of the mechanisms to reduce oxidants in the biological system (Krystyna and Anna, 2013). In the present study, different extracts of SR scavenged DPPH radicals at different degrees. As per TLC-DPPH study, 70% methanol extract has shown maximum scavenging followed by 100% methanol extract. At the dosage, 500 and 1000ug/ml, 100% methanol extract shows higher inhibition of DPPH radical than 100% chloroform extract. But at the dosage 3000ug/ml, both the extracts show similar inhibition. Hexane extract has shown least scavenging. This result is further supported by quantitative measurement of percent inhibition of DPPH radical where highest inhibition was shown by 70% methanol extract and least inhibition by 100% hexane extract. Thus Percentage inhibition increased from a low polarity solvent extract to a high polarity solvent extract. In ABTS assay method, ABTS reacts with potassium persulphate to produce ABTS + which gives a blue green chromogen.

Antioxidant reductant, the blue color becomes colorless the absorbance of which is measured at 734 nm. In the present study, the percentage inhibition of ABTS + radical by different extracts showed the same pattern like DPPH scavenging. Here 70 methanol extract shows maximum inhibition followed by 100% methanol extract, 100%chloroform extract. The anti-oxidant effects of plants are attributed in part, because of their phenol content. These compounds interrupt the propagation of chain reaction of lipid per-oxidation by contributing a hydrogen atom with the formation of relatively stable compound that prevents the propagation of oxidation process. Here in this study, 70% methanol extract has the highest phenol content and hexane extract had least amount of phenol content. High content of phenol content could be the reason for high free radical scavenging activities of all extracts.

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