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Free Radical Scavenging Activity of *Sida cordifolia* Linn. Extracts Measured by Hydrogen Peroxide, DPPH, ABTS and Ferric Reducing Antioxidant Methods

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Abstract

Antioxidants inhibit free radical reactions in our body and prevent cellular damage. Plant based polyphenols and flavonidic substances have been known as good antioxidants. *Sida cordifolia* Linn. exhibits many therapeutic pharmacological properties such as wound healing, anti-microbial, anti-inflammatory and hepato-protective due to presence of many polyphenols and flavonoids in its roots and seeds. Antioxidant capacities of aqueous and alcoholic (ethanol) extracts of *Sida cordifolia* roots were evaluated using *in vitro* standard procedures by assessing their free radical scavenging capacities. Total phenol content was assessed as 18.70 and 23.26 GAE/mg while the total flavonoid content was found to be 15.03 and 6.62 QE/mg for alcoholic and aqueous extract respectively. IC_{50} (µg/ml) of alcoholic extract, aqueous extract and ascorbic acid was found to be 36.189 ±0.993, 31.297 ±2.791 and 15.503 ±1.356 respectively in hydrogen peroxide radical scavenging, 1.520 ±0.075, 1.027 ±0.012 and 0.879 ±0.273 in DPPH radical scavenging assay, and 232 ±15.163, 179 ±53.894 and 6.627 ±0.061 during FRAP essay. Similarly, during ABTS radical scavenging assay, IC_{50} (µg/ml) was assessed as 332.960 ±2.177, 162.155 ±9.736 and 6.779 ±0.167 for alcoholic, aqueous extract and Trolox respectively. While both extracts showed significant free radical scavenging activity, the aqueous extract exhibits higher activity than alcoholic extract.

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Introduction

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exist both in enzymatic and non -enzymatic forms in the intracellular and extracellular environment. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions.

Normal biochemical reactions, increased exposure to the environment and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are responsible for the oxidative stress in different pathophysiological conditions. Cellular constituents of our body are altered in oxidative stress conditions, resulting in

various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants. Reactive oxygen species (ROS), including superoxide radicals ($\bullet O^{2-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($_1O_2$), are generated as byproducts of normal metabolism (Wang and Jiao, 2000; Živković et al., 2010).

To counteract the deleterious effects of ROS, phenolic compounds which are naturally distributed in plants are very effective (Chung et al., 1998). The food-derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention because they are known to function as chemo-preventive agents against oxidative damage. Polyphenols are considered to be plant chemical defenses against pathogens and herbivores, and these compounds can exert detrimental effects in a multitude of ways (Bernays et al., 1989). Polyphenols have many favourable effects on human health, such as the inhibition of the low density proteins oxidization (Frankel et al., 1993). They also have anti-inflammatory activity and anti-carcinogenic properties (Miyake et al., 1999).

Sida cordifolia Linn. belonging to the family, Malvaceae is one of the most useful medicinal plants in Ayurvedic literature. Also known as *Bala*, it is a small, erect, annual downy shrub. The leaves of the plant are chordate-oblong or ovate-oblong and fruits with a pair of awns on each carpel. The tap root of the plant is odourless with slightly bitter taste and grayish yellow colour which constitute a cluster 5-15 cm long with few lateral roots of smaller size. It has been used as a cooling, astringent, aromatic, stomachic, diuretic and tonic in Ayurvedic system of medicine for curing of diseases like asthma, cough, fever, wound, skin diseases, heart diseases, facial paralysis, muscle and joint pain, swelling, inflammation, urinary infection, skin diseases, lack of sexual desire and unwanted weight loss. Its roots and seeds contain alkaloid ephedrine, vasicinol, vasicinone, β-sitosterol and stigmasterol and N-methyl tryptophan while the leaves of *Sida cordifolia* contain small amounts of both ephedrine and pseudoephedrine. Its pharmacological actions include hypoglycaemic, wound healing, anti-microbial, antioxidant, anti-inflammatory, analgesic, adaptogenic and hepato-protective activities (Sharma, 1995; Jain et al., 2011; Sharma et al., 2001; Shastri, 1988; Chatterjee and Pakrashi, 1992; Kirtikar and Basu, 1989).

During the present study, the aqueous and alcoholic extracts of the roots of *Sida cordifolia* were screened for their antioxidant capacities using in vitro standard procedures after assessing its phenolic and flavonoid contents. The objective of the research was to assess the medicinal potential of this plant, especially its antioxidant potential in the form of free radical scavenging activity, using scientific techniques and thus justify its traditional and folklore use as a substantial and efficacious reliable rejuvenator herbal formulation.

Materials and methods

Plant materials

The roots of *Sida cordifolia* Linn. were purchased from crude drug supplier of Katwa Chowrasta, Burdwan district and the plant samples were authenticated by the Research Officer, Botanical Survey of India, Howrah, India.

Chemicals

Potassium Sodium Tertrate, Aluminium chloride, Folin-Ciocalteu's reagent, sodium carbonate, Sulphuric acid, Hydrogen peroxide, Ferric Chloride (FeCl3), Di-Sodium hydrogen Phosphate and Butylated hydroxytoluene (BHT) were obtained from Merck Specialties Pvt. Ltd., Mumbai, India. 2, 2- azinobis (3 ethyl-benzothiazoline-6-sulfonic acid) [ABTS] was obtained from Roche diagnostics, Mannheim, Germany while 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Fluka, Buchs, Switzerland. Quercetin was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Gallic acid, Ascorbic acid, Potassium persulphate, Potassium ferricyanide and Trichloro acetic acid were obtained from Nice Chemicals Pvt. Ltd. Kochi, Kerala, India. Sodium phosphate was obtained from Sarabhai M. Chemicals Limited, Shantisadan, Ahmedabad, Gujarat, India and Ammonium molybdate was obtained from GFS chemicals, United States. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from MP Biomedicals, France.

Preparation of extracts

The roots of *Sida cordifolia* were washed, sun dried and crushed to particle size of 40 mesh. This coarse powder was sequentially extracted with petroleum ether (60°C-80°C), chloroform, acetone, ethanol and water using Soxhlet apparatus. These extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper at room temperature and concentrated at reduced temperature and pressure using rotary evaporator. All obtained extracts

were stored in refrigerator below 10°C for subsequent experiments. The aqueous and alcoholic (ethanol) extracts of the roots of *Sida cordifolia* were used in the study.

Total phenol content

Total phenol content was determined using the Folin-Ciocalteu reagent. To 0.5 ml aliquot of dried aqueous extract of *Sida cordifolia*, 2.5 ml of Folin-Ciocalteu's reagent (10 %) and 2 ml of 7.5% sodium carbonate were added. The absorbance was read after 30 min incubation period at room temperature at 760 nm colorimetrically. A standard calibration plot was generated at 760 nm using known different concentrations of Gallic acid (100, 200, 300, 400, and 500 µg/ml). The concentrations of phenol in the test samples were calculated from the calibration plot. Total phenolic content was expressed as mg Gallic Acid Equivalents (GAE). All determinations were performed in triplicates and the results were expressed as mg Gallic acid equivalents per gram sample extract (Baba and Malik, 2015; Harborne, 1984).

Total flavonoid content

Total Flavonoid content was determined using the Aluminum chloride [$AlCl_3$] method. Briefly, an aliquot of 0.5 ml of sample (1 mg/ml) was mixed with 1.5 ml of methanol, 0.1 ml of 1% aluminum chloride and 0.1 ml of potassium acetate solution (1 M). In the mixture, 2.8 ml of distilled water was added to bring up the total volume to 5 ml. The test solution was shaken vigorously and Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using different and known concentrations of Quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg Quercetin equivalent/g of sample (Chang et al., 2002; Usha Sri et al., 2012).

Hydrogen peroxide radical scavenging

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Keser et al. (2012). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Alcoholic and Aqueous extracts (24.94, 49.75 and 74.44 µg/ml) were added to hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined spectro-photometrically by UV-VIS (Shimadzu UV 2450) 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide and compared with ascorbic acid (12.42, 24.69, 36.81 and 48.78 and 74.44 µg/ml), the reference compound. The percentage of hydrogen peroxide scavenging of extracts and standard compounds were calculated as follows (Basniwal et al., 2009; Nishaa et al., 2012):

$$\% \text{ scavenged } [H_2O_2] = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

DPPH radical scavenging assay

DPPH [1, 1-di-phenyl-2-picryl hydrazyl] is a stable free radical with purple color, the intensity of which is measured spectrophotometrically at 517 nm wavelength. Antioxidants reduce DPPH to 1, 1-diphenyl-2-picryl hydrazine, a colorless compound. Ascorbic acid was used as standard due to its strong reducing power and weak metal-chelating ability.

The DPPH 0.1 mM solution in ethanol was prepared. This solution (3 ml) was added to 1 ml. of alcoholic and aqueous extracts at different concentrations (0.5, 1.0 and 2.0 mg/ml). The mixture was shaken vigorously and allowed to stand at room temp in dark for 30 min. and thereafter the absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu UV 2450) (Anil et al., 2011; Ozcelik et al., 2003). Reference standard compound being used was ascorbic acid with different concentrations (0.050, 0.100, 0.150 and 0.200 mg/ml) and experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. Linear graph of concentration vs. percentage inhibition was prepared using various concentrations of Ascorbic acid and IC_{50} values were calculated.

The percent DPPH scavenging effect was calculated by using the following equation:

$$\% \text{ inhibition} = \frac{\text{Blank} - \text{Extract or Standard}}{\text{Blank}} \times 100$$

Where, Blank was the absorbance of the control and the absorbance in the presence of the sample was denoted by Extract or Standard.

ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid) assay is based on the scavenging of

ABTS radicals. For ABTS assay, the working solution was prepared by mixing equal quantities of 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution and allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.7 to 0.8 units at 734 nm using the spectrophotometer (UV-VIS Shimadzu UV 2450). Fresh ABTS solution was prepared for each assay and used as Blank (Tupe et al., 2013; Re et al, 1999).

Alcoholic and aqueous extracts with different concentrations (200, 300 and 400 µg/ml) were allowed to react with 2.94 ml of the ABTS solution for 6 min in a dark condition. Trolox (water-soluble analog of vitamin E) in different concentrations (2.70, 4.05, 8.10 and 10.80 µg/ml) was used as a standard and the same procedure was used. Then the absorbance was measured at 734 nm using the spectrophotometer. Results were expressed in mM Trolox equivalents (TE)/g fresh mass. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve. IC_{50} value (concentration of sample where absorbance of ABTS decreases 50% with respect to absorbance of blank) of the sample was determined.

Scavenging ability relative to the reaction control (without plant extract) was calculated by using the formula:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract/standard}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical in Blank and $\text{Abs}_{\text{extract/standard}}$ is the absorbance of an ABTS radical solution mixed with extract/ sample.

Ferric reducing antioxidant power assay (FRAP)

The reducing power was determined by the modified Oyaizu method (1986). Substances which have reduction potential react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm.

The different concentrations of sample alcoholic and aqueous extracts (300, 600 and 900 µg/ml) were mixed with 1 ml of sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. After that, 1 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (1.5 ml) was mixed with 1.5 ml deionized water and 0.1 ml of 0.1% of ferric chloride, kept for 10 min and the absorbance was measured at 700 nm by UV-VIS Shimadzu UV 2450 (Hemalatha et al., 2010; Aparadh et al., 2012). Higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean value ± standard deviation. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid (4, 8, 12, 16, 20 µg/ml) was used as standard.

$$\text{Increase in reducing power (\%)} = \frac{\text{Abs}_{\text{test}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{blank}}} \times 100$$

Where, Abs_{test} is absorbance of test solution and $\text{Abs}_{\text{blank}}$ is absorbance of blank. Here reduction of Fe $[(CN)_6]_3$ to Fe $[(CN)_6]_2$ leads to the formation of the intense Perl's Prussian blue complex which gives strong absorbance at 700 nm with increasing reducing power.

Statistical analysis

The data generated for each mouse was considered for calculation of Mean ± S.E.M. for different groups. Statistical evaluation of data was done following Students' t-test. A difference was considered significant at $p \leq 0.05$.

Results

Total flavonoid content (TFC) and total phenol content (TPC)

The total flavonoid content (TFC) and total phenol content (TPC) was calculated from the absorbance calibration curve generated with different concentrations of Quercetin and Gallic acid respectively which is shown in Table 1.

Hydrogen peroxide radical scavenging

The HPS activity has usually been determined by following the rate of H_2O_2 consumption in an incubation system (H_2O_2 + scavenger) using the classical UV-method. The results of the hydrogen peroxide scavenging test of the alcoholic and aqueous extracts of *Sida cordifolia* in comparison with the standard (ascorbic acid) at 230 nm is shown in Table 2. The percentage of inhibition against different concentrations of both extracts of the research drug as well as ascorbic acid was used to plot the standard curve which was used to

calculate the IC_{50} (µg/ml) of each sample which was determined as 36.189 ±0.993, 31.297 ±2.791 and 15.503 ±1.356 for alcoholic extract, aqueous extract and ascorbic acid respectively.

Table 1. Estimation of total flavonoid content (TFC) and total phenol content (TPC) in *Sida cordifolia* extracts.

Tests (TFC and TPC)	Extract of *Sida cordifolia*	
	Alcoholic	Aqueous
Flavonoid content (µg Quercetin equivalent / mg of extract) following the standard curve (R^2 =0.979)	15.03	6.62
Phenol content (µg Gallic acid equivalent / mg of extract) following the standard curve (R^2 =0.979)	18.70	23.26

Table 2. Hydrogen peroxide scavenging activity of extracts of *Sida cordifolia*.

Sample tested	Concentration (µg/ml)	% of inhibition	IC_{50} (µg/ml) from standard curve
Alcoholic extract of *Sida cordifolia*	24.94	17.02	36.189 ±0.993 (R^2=0.9754)
	49.75	83.40	
	74.44	199.57	
Aqueous extract *Sida cordifolia*	24.94	33.62	31.297 ±2.791 (R^2=0.9996)
	49.75	95.74	
	74.44	153.19	
Ascorbic acid (Standard)	12.42	31.50	15.503 ±1.356 (R^2=0.989)
	24.69	93.50	
	36.81	145.50	
	48.78	182.50	

Values are represented as Mean ±SEM.

DPPH radical scavenging assay

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picryl hydrazine at 517 nm. The results are expressed as the IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%). The results of the DPPH radical scavenging test of alcoholic and aqueous extracts of *Sida cordifolia* in comparison with the standard (ascorbic acid) are shown in Table 3. The percentages of inhibition against different concentrations of both extracts of the research drug as well as ascorbic acid were used to plot the standard curve which was used to calculate the IC_{50} (µg/ml) of each sample which was determined as 1.520 ±0.075 in alcoholic extract, 1.027±0.012 in aqueous extract of *Sida cordifolia* and 0.879±0.273 in Ascorbic acid respectively.

Table 3. DPPH radical scavenging activity of extracts of *Sida cordifolia.*

Sample tested	Concentration (mg/ml)	% of inhibition	IC_{50} (mg/ml) from standard curve
Alcoholic extract of *Sida cordifolia*	0.500	16.18	1.520±0.075 (R^2=0.9911)
	1.000	36.27	
	2.000	64.38	
Aqueous extract of *Sida cordifolia*	0.500	30.59	1.027±0.012 (R^2=0.9422)
	1.000	55.33	
	2.000	75.93	
Ascorbic acid (Standard)	0.05	3.59	0.879±0.273 (R^2=0.9142)
	0.10	9.97	
	0.15	10.78	
	0.20	14.54	

Values are represented as Mean ±SEM.

ABTS radical scavenging assay

In this assay, ABTS is converted to its radical cation by addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and vitamin C. During this reaction, the blue ABTS radical cation is converted back

to its colorless neutral form. The reaction may be monitored spectrophotometrically. This assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay.

The results of the ABTS radical scavenging test of the alcoholic and aqueous extracts of *Sida cordifolia* in comparison with the standard (Trolox) are shown in Table 4. The percentage of inhibition against different concentrations of both extracts of the research drug as well as Trolox as Standard was used to plot the standard curve. This curve was used to calculate the IC_{50} (µg/ml) of each sample which was determined as 332.960 ±2.177 for alcoholic extract, 162.155 ±9.736 for aqueous extract of *Sida cordifolia* and 6.779 ±0.167 in case of Trolox respectively. It is also observed that inhibition percentage values go on increasing with increases in concentration of research plant extracts in the assay mixture.

Table 4. Percentage of ABTS radical scavenging activity shows by Trolox.

Sample tested	Concentration (µg/ml)	% of inhibition	IC_{50} (µg/ml) from standard curve
Alcoholic extract of *Sida cordifolia*	200	5.86	332.960 ±2.177 (R^2=0.9502)
	300	49.14	
	400	67.86	
Aqueous extract of *Sida cordifolia*	200	57.61	162.155 ±9.736 (R^2=0.858)
	300	61.42	
	400	83.25	
Trolox (Standard)	2.70	14.29	6.779 ±0.167 (R^2=0.9973)
	4.05	25.00	
	8.10	64.00	
	10.8	84.00	

Values are represented as Mean ±SEM.

Ferric reducing antioxidant power (FRAP) assay

In this assay the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each compound. The presence of radicals (i.e. antioxidants) causes the conversion of the Fe^{3+} / ferricyanide complex used in this method to the ferrous form indicated by the formation of pearls Prussian blue at 700 nm, a higher absorbance indicating a higher reducing power.

The results of the FRAP test of the alcoholic and aqueous extracts in comparison with the standard (ascorbic acid) at 700 nm is shown in Table 5. The percentage of inhibition against different concentrations of both extracts of the research drug as well as ascorbic acid was used to plot the standard curve which was used to calculate the IC_{50} (µg/ml) of each sample, which was determined as 232 ±15.163, 179 ±53.894 and 6.627 ±0.061 for alcoholic extract, aqueous extract of *Sida cordifolia* and Ascorbic Acid respectively. It is also observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture.

Table 5. Ferric reducing antioxidant power assay with *Sida cordifolia* extracts.

Sample tested	Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml) from standard curve
Alcoholic extract of *Sida cordifolia*	300	66.207	232 ±15.163 (R^2=0.9992)
	600	142.431	
	900	211.691	
Aqueous extract of *Sida cordifolia*	300	109.00	179 ±53.894 (R^2=0.998)
	600	264.10	
	900	440.60	
Ascorbic acid (Standard)	4	20.78	6.627 ±0.061 (R^2=0.997)
	8	63.64	
	12	114.29	
	16	174.03	
	20	224.68	

Values are represented as Mean ±SEM.

The obtained IC_{50} values in respect of the two extracts of *Sida cordifolia* as well as the standard drug using the various techniques discussed above have been shown in Fig. 1. It is evident that aqueous extract shows higher level of antioxidant activity as compared to the alcoholic extract in all the methods of analysis.

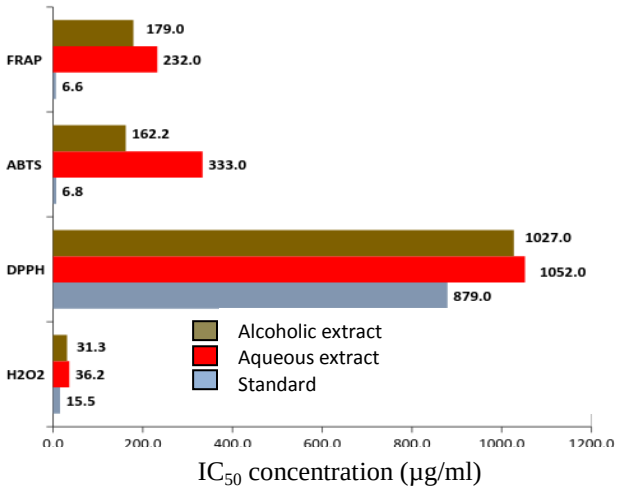

Fig. 1: Comparative IC_{50} values of *Sida cordifolia* extracts and standard.

Discussion

The total phenolic content of the alcoholic root extract was 18.70 Gallic acid equivalents/mg while it was 23.26 for the aqueous extract. Similarly, the total flavonoid content was assessed as 15.03 and 6.62 Quercetin equivalent /mg for these two extracts respectively. Thus, the alcoholic extract of *Sida cordifolia* shows more Quercetin equivalent content indicating higher flavonoids than its aqueous extract. However, its aqueous extract shows more Gallic acid equivalents (higher total phenol content) than its alcoholic extract. Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee et al., 2005). As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* (Geetha et al., 2003; Shimoi et al., 1996). Plants rich in secondary metabolites, including phenolics, flavonoids and carotenoids, have antioxidant activity due to their redox properties and chemical structures.

Examination of the IC_{50} values obtained during Hydrogen Peroxide Radical Scavenging test indicate that when compared with the standard drug ascorbic acid, more than twice the amount of concentrations of the aqueous and alcoholic extracts of the research drug are required for scavenging 50% Hydrogen peroxides. The free radical scavenging effect in respect of Hydrogen Peroxide was found higher in the aqueous extract at 24.94 and 49.75 µg/ml concentrations than in alcoholic extract while it was lower at 74.44 µg/ml concentration of *Sida cordifolia.* However, the radical scavenging effect at all observed concentrations for both aqueous and alcoholic extracts of the research drug was quite low in comparison to ascorbic acid.

The high phenolic and flavonoid content is responsible for the bioactivity of these crude extracts. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases Hydrogen peroxide (H_2O_2), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes, but there has been limited information regarding its scavenging by polyphenolic antioxidants. Among reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) is a relatively stable, non-radical oxidant, which can diffuse across biological membranes. It is produced by 2-electron reduction of molecular oxygen or by dismutation of the superoxide anion radical (Shukla et al., 2009; Bravo, 1998; Agati et al., 2012).

Ascorbic acid or citamin C has been used as the standard in this research since it is a water-soluble free radical scavenger. Moreover, it regenerates vitamin E in cell membranes in combination with compounds capable of donating reducing equivalents. Vitamin C changes to the ascorbate radical by donating an electron to the lipid radical in order to terminate the lipid peroxidation chain reaction (Nimse et al., 2015). The research plant extracts reduce the DPPH radical to corresponding hydrazine when they react with hydrogen donors. It is noticed that aqueous and alcoholic extracts of the research drug are required in higher concentrations than the ascorbic acid standard for performing antioxidant activity measured by assessing the concentration required for 50 % scavenging of the DPPH radical, i.e. IC_{50} (µg/ml). The aqueous extract having higher phenolics exhibits higher scavenging activity during this test as compared to the alcohol extract. The free radical scavenging effect assessed as percentage inhibition was found to be 75.93 in the aqueous extract at 2.0 mg/ml concentration as compared to 64.38% at same concentration in the

alcoholic extract. It is also observed that inhibition percentage increases commensurate with increases in concentration of research plant extracts in the assay mixture during DPPH test.

An antioxidant has the ability to donate a hydrogen atom that will quench the stable free radical which is associated with a change in absorption which can be followed calorimetrically. The pre-formed radical mono-cation of ($ABTS^{\bullet+}$) is generated by oxidation of ABTS with potassium per-sulfate and is reduced in the presence of hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction are taken into account when determining the antioxidant activity. It is observed that for any particular level of antioxidant activity, higher concentrations of aqueous and alcoholic extracts of the research drug are required than the standard ascorbic acid. This phenomenon is clearly observed when measuring the antioxidant activity for scavenging 50% ABTS radicals as evaluated in the form of IC_{50} (µg/ml).

The free radical scavenging effect measured as percentage inhibition in case of the stable ABTS was found to be 61.42 in the aqueous extract at 300 µg/ml concentration as compared to 49.14 in alcoholic extract at the same concentration of *Sida cordifolia*. The standard drug Trolox showed high inhibition at very low values of concentration in comparison to both the aqueous and alcoholic extracts. Since Trolox is a water-soluble analog of vitamin E, it is used as an antioxidant like vitamin E in biological or biochemical applications to reduce oxidative stress or damage.

FRAP is an electron transfer based total antioxidant assay, also called redox linked colorimetric method. There is an increase in absorbance at a pre specified wavelength as an antioxidant reacts with chromogenic reagent (Fe (II)) (Everest and Ozturk, 2005). The percentage of inhibition at 300 µg/ml concentrations was found to be 66.207 in case of alcoholic and 109.00 for aqueous extract, while it was 142.431% and 264.10% in case of alcoholic and aqueous extracts respectively at 600 µg/ml concentration. Thus, the aqueous extract of the research drug consistently showed higher inhibition and, therefore, higher antioxidant activity during this study. The percentage inhibition as well as the IC_{50} values clearly indicated that the standard (ascorbic acid) showed very low IC_{50} levels indicating its very high antioxidant capacity in comparison with both the alcoholic and aqueous extracts.

Conclusion

The evaluation of in vitro antioxidant activity of the aqueous and alcoholic extracts of *Sida cordifolia* showed substantial and significant free radical scavenging activity in both the extracts which is compared to that of the standard drug during the study. The aqueous extract exhibits a higher level of activity than the alcoholic extract in all the antioxidant assessment techniques. This significant pronounced antioxidant activity of aqueous extract may be due to the presence of higher concentrations of phenolic compound in it as compared to the alcoholic extract. This in vitro assessment of the antioxidant properties of Sida cordifolia validates and confirms its free radical scavenging activity also known as *Rasayan Karma* as described in the Ayurvedic system of medicine.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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