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Research Article

Antioxidant Activity of *Saraca asoca* (Roxb.) Wilde Flower: An *In Vitro* Evaluation

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ABSTRACT
The intension of the present study was to evaluate the total phenolic, flavonoid and *in vitro* antioxidant activity of different extracts of *Saraca asoca* flower. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Saraca asoca* flower were screened for *in vitro* antioxidant potential using models viz, 1,1-diphenly-2-picryl hydrazine (DPPH), hydroxyl, superoxide, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation scavenging activity and reducing power ability using standard procedures. Methanol extract of *Saraca asoca* flower extract exhibited strong antioxidant activity with DPPH (121.84%), hydroxyl (126.22%), superoxide (128.27%) and ABTS (99.22%) assays. The radical scavenging effect was found to increase with increasing concentrations. The IC_{50} values in all models viz, hydroxyl, DPPH, superoxide and ABTS were found to be 43.88, 42.83, 41.66 and 26.18μg/mL respectively in the methanol extracts. The results show that there was an increase in the reducing power of the plant extract as the concentration of the extract increases. Among those solvent extracts, ethanol extract of *Saraca asoca* flower exhibited maximum reducing ability. It can be accomplished that the methanol extracts of *Saraca asoca* flower is a potential source of natural antioxidants.

Keywords: *Saraca asoca*; DPPH; Hydroxyl; Superoxide; ABTS.

INTRODUCTION

Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radicals and hydrogen peroxide, which are generated by normal physiological processes and various exogenous factors initiate peroxidation of membrane lipids as well as a wide range of other biological molecules through a process that is believed to be implicated in the etiology of several disease conditions, including coronary artery disease, stroke, rheumatoid arthritis, diabetes and cancer[1,2]. Significant roles in inhibiting and scavenging radicals are played by the Antioxidants and they also give protection to humans against contaminations and degenerative diseases. However, these days people are more worried about the food practices and they are also concerned about the possible effect of synthetic additives on their health. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the two most commonly used synthetic antioxidants. It is to be noted that these antioxidants have been restricted because of their toxicity and DNA damage induction[3]. Recent research scholars have showed more interest to find out naturally occurring antioxidants to replace synthetic antioxidants, which are being restricted due to their carcinogenicity[4].

Saraca asoca (Roxb.) Wilde belongs to the family Caesalpiniaceae. This is an evergreen tree generally known as Ashoka. Ayurveda and Unani practitioners regarded it as a miracle tree for women because its medicinal properties solved many gynaecological issues[5]. Bark of *Saraca asoca* had profound antimicrobial activity against a wide range of bacterial pathogenic organisms[6]. The flowers were used to treat uterine toxicity, improper digestion, stomach pain, constipation, hemorrhagic dysentery, and diabetes. Flower and leaves of the plant were used in fever, colic, ulcers and pimples[7]. Ashokarishta is an ayurvedic preparation from *Saraca asoca* to cure various diseases in women. It reduces excessive bleeding, leucorrhoea and headache[8]. *Saraca asoca* has being investigated on a large scale by researchers for its antiinflammatory and analgesic properties[9,10] antipyretic activity[9], chemopreventive activity[11] and molluscicidal activity[12]. The anthelmintic activities of ethanolic and methanolic extracts of bark were also reported due to the presence of phytochemical constituent such as glycosides, alkaloids, tannin, flavonoids and terpenoids[13]. Gallic acid is one of the major compound in antimutagenic and antigenotoxic properties in *Saraca asoca* bark extract[14,15].

Saraca asoca though it has been expansively used in indigenous medicinal systems, the antioxidant property of its flowers are still unexplored. Hence in the current study flowers of *Saraca asoca* were subjected to evaluate the total phenolic content, flavonoids, antioxidant activity antioxidant properties of the different solvent extracts of *Saraca asoca* flower using DPPH, hydroxyl, superoxide and ABTS radical cation scavenging assays in order to

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exemplify the essential biochemical properties that impart such high medicinal values to this plant.

MATERIALS AND METHODS

Collection of Plant Material

Flowers of *Saraca asoca* (Roxb). Wilde was collected from Bryant Nagar, Tuticorin, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried flowers was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. For the extraction of active constituents of the plant material the final uniform powder was utilized.

Preparation of Plant Extract

Petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 mL were used to extract the coarse powder (100 g) of flowers of *Saraca asoca* in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filters paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of Total Phenolic Content

Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay as previously described[16] with little modification. To 1 mL of each extract (100 µg/mL) in methanol, 5 mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4 mL (75 g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al*[17]. An aliquot of 0.5ml of sample (1 mg/mL) was mixed up with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1 M). 80% methanol was added to this mixture of 4.3 ml to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The optical density value was used to calculate the total flavonoid content present in the sample.

DPPH Radical Scavenging Activity

The DPPH is a stable free radical. It is extensively used to evaluate the radical scavenging activity of antioxidant component. This technique is based on the reduction of DPPH in methanol solution in the existence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H[18].

Making use of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) the free radical scavenging activity of all the extracts was assessed. This is carried out as per the previously reported method[18]. Briefly, an 0.1 mM solution of DPPH in methanol was prepared. 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (50,100,200,400 & 800 µg/mL). The mixtures were shaken forcefully and allowed to be at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

$\% \text{ inhibition} = \{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell [19]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The examination was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$**,** 0.1 mL H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of the extract of unlike concentration (50,100,200,400 & 800 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.9), 0.1 mL of ascorbic acid in succession. The mixture was then incubated at 37^0C for 1 hour. 1.0 mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The percentage inhibition was computed by comparing the results of the test with those of the control using the above cited formula.

Superoxide Radical Scavenging Activity

The superoxide anion scavenging activity was calculated as described by Srinivasan *et al*[20]. The superoxide anion radicals were made in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), having 0.5 mL of NBT (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1.0 mL extract of unlike concentration (50,100,200,400 & 800 µg/mL), and 0.5 mL Tris – HCl buffer (16mM, pH 8.0). to start with 0.5 mL PMS solution (0.12 mM) is added to the mixture. Further it is incubated at 25ºC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was estimated by matching up to the results of the test with those of the control using the above cited formula.

Antioxidant Activity by Radical Cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al*[21]. ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted. This is carried out with ethanol to an absorbance of 0.70+0.02 at 734 nm. The absorbance was determined after addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, at 734 nm by Genesys 10S UV-VIS (Thermo scientific). This is completed exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those

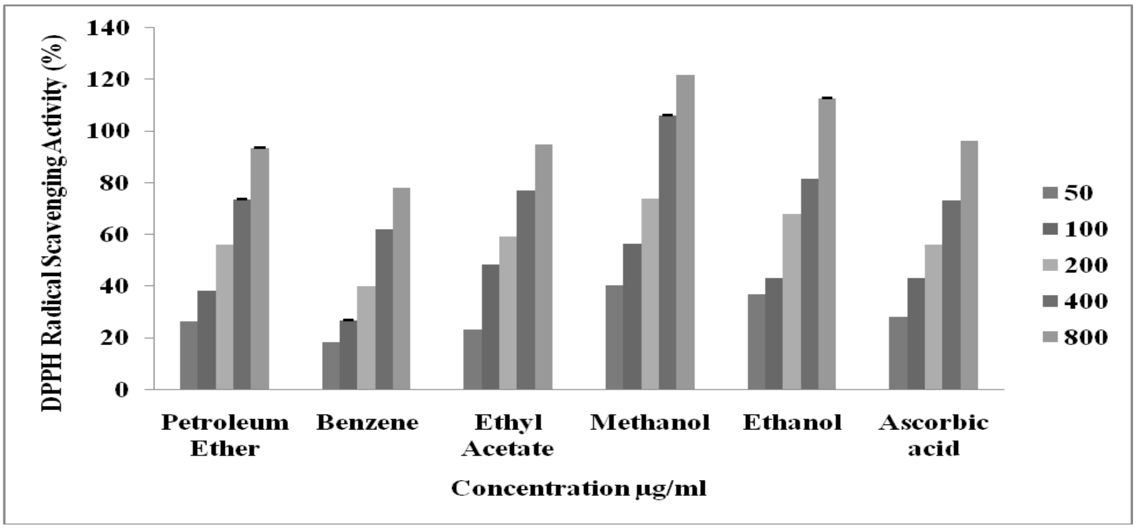

Figure 1: DPPH radical scavenging activity of different extracts of flowers of *Saraca asoca.*

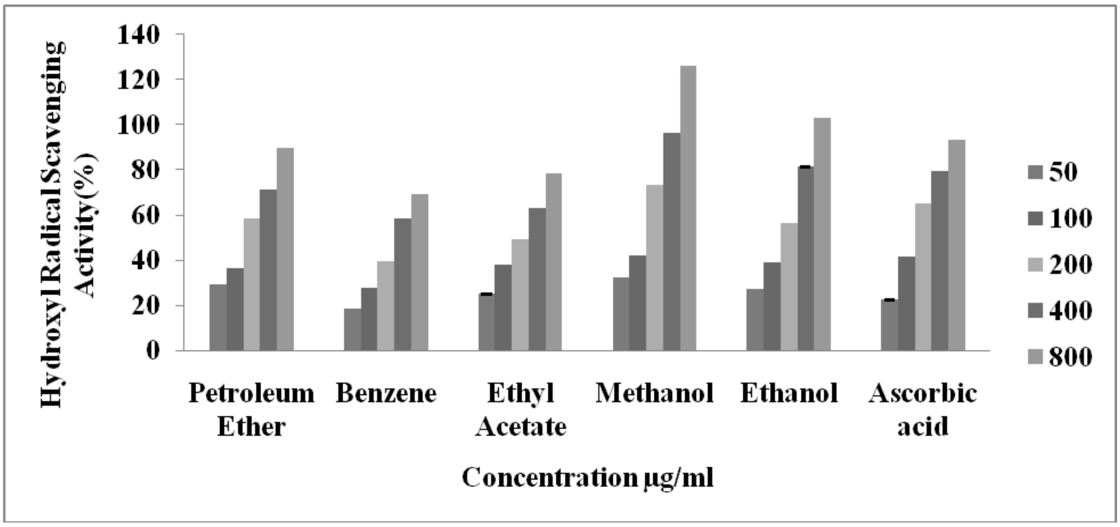

Figure 2: Hydroxyl radical scavenging activity of different extracts of flowers of *Saraca asoca.*

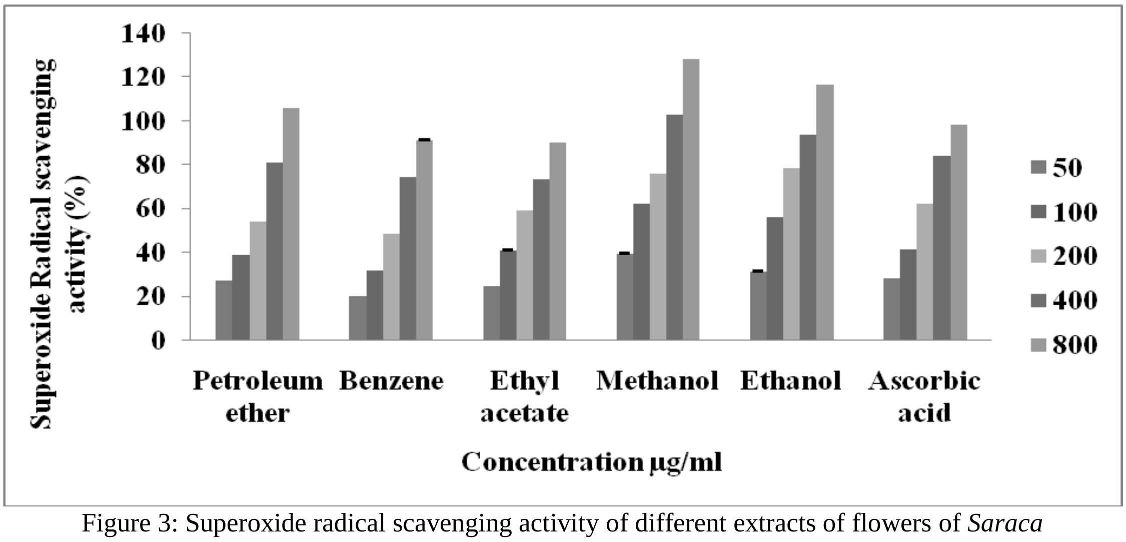

Figure 3: Superoxide radical scavenging activity of different extracts of flowers of *Saraca*

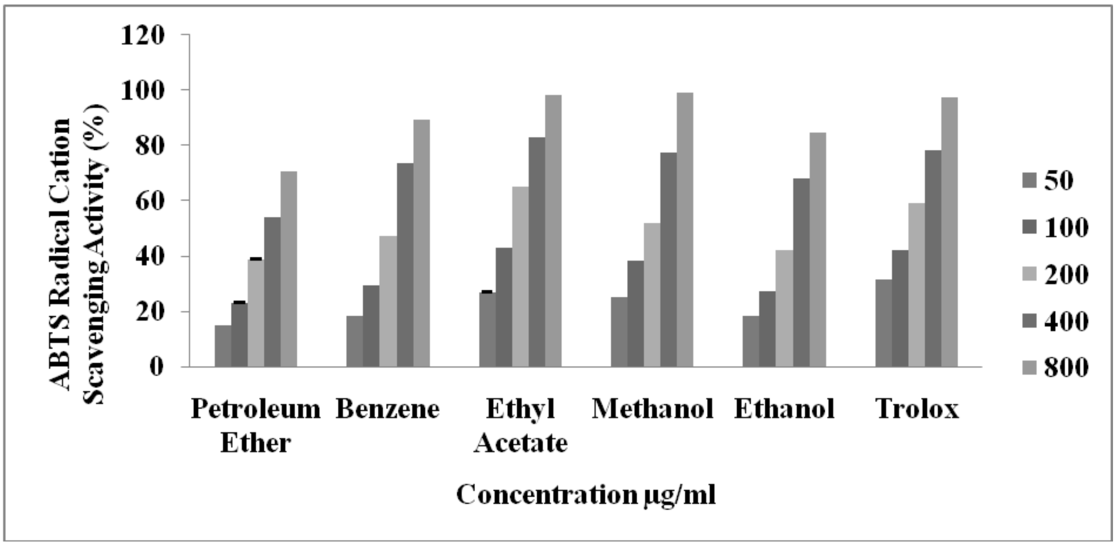

Figure 4: ABTS Radical scavenging activity of different extracts of flowers of *Saraca asoca*.

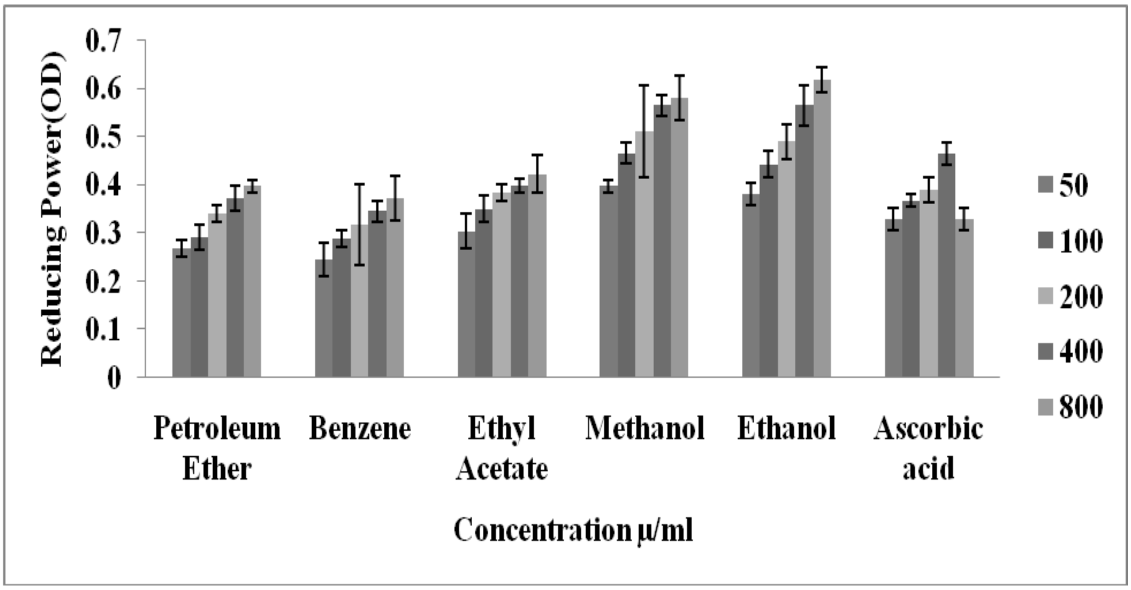

Figure 5: Reducing Power of different extracts of flowers of *Saraca asoca*.

of the control using the above formula.

Reducing Power

The reducing power of the extract was determined by the method demonstrated by Kumar and Hemalatha[22]. 1.0 mL of solution containing 50,100,200,400 & 800 µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. This experiment was replicated thrice and the results are averaged.

Statistical Analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS

Total Phenolic and Total Flavonoid Content

The total phenolic and flavonoid content of the methanol extract of *S. asoca* flower were found to be 0.98 g 100 g^{-1} and 1.01 g 100 g^{-1} respectively.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S. asoca* flower were depicted in figure 1. The scavenging activity increased with the concentration of standard ascorbic acid and flower extracts. Among the solvent tested, methanol extract exhibited highest DPPH radical scavenging activity. At 800 µg/mL concentration methanol extract of *S. asoca* flower possessed 121.84 %

Table 1: IC_{50} values of different extracts of flowers of *Saraca asoca.*

Solvent	IC_{50} (µg/mL)			
	DPPH Radical Scavenging Activity	Hydroxyl Radical Scavenging Activity	Superoxide Radical Scavenging Activity	ABTS Radical Cation Scavenging Activity
Petroleum Ether	34.16	32.16	37.28	20.36
Benzene	30.28	21.80	34.13	23.18
Ethyl Acetate	30.88	26.13	30.13	27.88
Methanol	42.83	43.88	41.66	26.18
Ethanol	38.43	39.16	38.96	22.13
Ascorbic Acid	31.75	31.22	34.84	-
Trolox	-	-	-	37.84

scavenging effect. The concentration of methanol extract of *S. asoca* flower needed for 50 % inhibition (IC_{50}) was found to be 42.53 µg/mL, whereas 31.75 µg/mL needed for ascorbic acid (Table 1).

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S. asoca* flower were compiled in figure 2. Methanol extract confirmed potent activity. At 800 µg/mL concentration *S. asoca* flower possessed 126.22 % scavenging activity on hydroxyl radical. The concentration of methanol extract of *S. asoca* flower needed for 50 % inhibition (IC_{50}) was found to be 43.88 µg/mL, whereas 31.22 µg/mL needed for ascorbic acid (Table 1).

Superoxide Radical Scavenging Activity

The *S. asoca* flower extracts were subjected to superoxide radical scavenging activity and the results were represented in figure 3. It indicates that methanol extract of *S. asoca* flower (800 µg/mL) exhibited maximum superoxide radical scavenging activity of 128.27% which is higher than the standard ascorbic acid whose scavenging effect is 98.22%. The quantity of methanol extract of *S. asoca* flower required to produce 50% inhibition of superoxide radical was 41.66 µg/mL, whereas 34.84 µg/mL was needed for ascorbic acid (Table 1).

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ABTS Radical Cation Scavenging Activity

The *S. asoca* flower extracts were analyzed for its ABTS radical cation scavenging activity and the results were demonstrated in figure 4. The methanol extract exhibited strong ABTS radical cation scavenging activity in concentration dependent manner. At 800 µg/mL concentration *S. asoca* flower exhibited 99.22% scavenging effect on ABTS which is higher than the standard trolox whose scavenging effect is 74.39%. The IC_{50} values of methanol extract of *S. asoca* flower on ABTS radical were found to be 26.18 µg/mL and 37.84 µg/mL for trolox correspondingly (Table 1).

Reducing Power

Figure 5 pictured the data of reducing power ability of different solvent extracts of *S. asoca* flower and standard ascorbic acid. The absorbance of the solution increased as the concentration increases. A higher absorbance indicates higher reducing power. Elevated reducing power was observed in ethanol extract when compared to other solvent extracts tested.

DISCUSSION

Phenolic compounds, such as flavonoids, phenolic acid and tannins, possess anti-inflammatory, anticarcinogenic, antiatherosclerotic, and other properties that may be related to their antioxidant activities[23]. Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups[24]. It is a reality that phenolic compounds are components of many plants. These phenolic compounds have attracted a great deal of civic and scientific interest. This is due to their health supporting effects as antioxidants[25]. The phenolic compounds show substantial free radical scavenging actions. This is done through their reactivity as hydrogen or electron donating agents, and metal ion chelating properties[26]. The phenolic compounds in herbs act as antioxidants. This is due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators.

The most varied and extensive group of natural compounds and are possibly to be the most important natural phenolics are the Flavonoids. These compounds have a broad spectrum of chemical and biological activities including radical scavenging activity. It has been long-established that pharmacological effects of flavonoids is correlating with their antioxidant activity[27]. Therefore, it would be valuable to determine the total phenolic and flavonoid contents of the plant extracts.

Free radicals and other reactive species are thought to play an important role in many human diseases. Due to the deleterious role of free radicals in biological systems radical scavenging activities are very important. Many secondary metabolites which include flavonoids, phenolic compounds etc serve as resources of antioxidants. They also perform scavenging activity[28,29]. In this study, it is evident that the extract of the study species, *Saraca asoca* flower possess effective antioxidant activity.

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S. asoca* flower were investigated in the present study by DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities. These methods have proven the effectiveness of the extracts in comparison to that of the reference standard antioxidants ascorbic acid and trolox.

A stable free radical, DPPH is at room temperature and admits an electron or hydrogen radical to become a stable diamagnetic molecule[30]. This is executed with an absorption maximum band around 515-528 nm. So it is a useful reagent for evaluation of antioxidant activity of compounds[31]. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow coloured compound, diphenylpicrylhydrazine. The extent of this reaction will depend on the hydrogen donating ability of the antioxidants. There was a reduction in the concentration of DPPH. This is due to the scavenging ability of extract of *S. asoca* flower. Methanol extract showed reasonable effect in inhibiting DPPH. At a concentration of 800 µg/mL, the scavenging effects of five extracts of flower on the DPPH radical increased in the order: benzene extract (78.36%) < petroleum ether extract (92.63%) < ethyl acetate extract (94.88%) < ethanol extract (112.86%) < methanol extract (121.84%). Among the solvent extracts analyzed for DPPH scavenging activity, methanol extract of *S. asoca* flower showed higher radical inhibition activity which is comparable to standard ascorbic acid (96.22%) at 800 µg/mL concentration.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. It is to be distinguished here that Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. Among the reactive oxygen species, the hydroxyl radical is the most reactive. This also induces severe damage to the adjacent biomolecules[32]. When *S.asoca* flower extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. At a concentration of 800 µg/mL, the scavenging effects of five extracts of flower on the hydroxyl radical increased in the order: benzene extract (69.46%) < ethyl acetate extract (78.31%) < petroleum ether extract (89.62%) < ethanol extract (103.16%) < methanol extract (126.22%). Among the solvents tested methanol extract possessed more hydroxyl radical scavenging activity when compared with standard ascorbic acid.

Superoxide anion plays an important role in formation of ROS. Even though superoxide is a relatively weak oxidant, it is decomposed to form stronger ROS, such as singlet oxygen and hydroxyl radicals. These initiate peroxidation of lipids. The *S. asoca* flower extracts showed superoxide radical scavenging effects. At a concentration of 800 µg/mL, the methanol extract of flower proved maximum inhibitory effect of about 128.27%. Superoxide radical scavenging activities of flower extracts on the decreasing order: methanol extract (128.27%) > ethanol extract (116.92) > petroleum ether extract (106.22%) > benzene extract (91.46%) > ethyl acetate extract (90.22%). Further, superoxides are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals. These results clearly suggest that the antioxidant activity of *S. asoca* flower is also related to its ability to scavenge superoxides.

The trolox equivalents antioxidant capacity (TEAC) was measured using the improved ABTS radical decolorization assay; one of the most frequently employed methods for antioxidant capacity, which measures the ability of a compound to scavenge ABTS radical. ABTS assay is an excellent tool to determine the antioxidant activity in hydrogen donating antioxidants (scavenging aqueous phase radicals) and of chain breaking antioxidants (scavenging lipid peroxy radicals). The *Saraca asoca* flower extracts demonstrated a dose-response inhibition of ABTS radical cation. The methanol extract of *S. asoca* flower exhibited good ABTS radical cation scavenging activity at higher concentration. Among the five solvent tested for ABTS radical cation scavenging activity, higher activity was shown by methanol extract when compared with other extracts.

Daffodil and Mohan[33] reported that reducing power was associated with antioxidant activity. High reducing power also indicates a high antioxidant activity. The reducing power of extracts increased with increase in concentration. At the concentration of 800 µg/mL the reducing power of *S.asoca* flower was in the increasing order: benzene extract (0.373) < petroleum ether extract (0.398) < ethyl acetate extract (0.423) < methanol extract (0.582) < ethanol extract (0.618). Among the five flower extracts, ethanol extract showed higher reducing ability.

The present study disclosed that the flower extract of *S. asoca* exhibited satisfactory scavenging effect in all the radical scavenging examination. This is the first report on the antioxidant property of *Saraca asoca* flower extracts. The results obtained by these methods provide some insight into the important factors responsible for the antioxidant potential and the mechanism of action. However, methanol extract of *Saraca asoca* flower possessed good antioxidant activity. Further studies on isolating and characterizing the antioxidant substances and their potential as antidiabetic agents are in progress.

CONFLICT OF INTEREST STATEMENT

We affirm that we have no conflict of interest.

ACKNOWLEDGEMENTS

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