Journal of Applied Pharmacy

OPEN ACCESS Freely available online

Research Article

Effect of Extraction Solvents on Total Phenol, Flavonoid Content and Free Radical Scavenging Potential of *Saraca asoca*, an Indian Medicinal Plant

Shahin Taj RA, Sharath J, Bhagya M*

Endocrinology and Reproductive Physiology Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570006, India

ABSTRACT

Objective: The present study enumerates the assessment of the differences in biological activities among various solvent extracts of the bark of Ashoka. The aim was to estimate the total phenol content and flavonoid content of various solvent extracts of *Saraca asoca* bark and to establish the free radical scavenging potential of these solvent extracts.

Methods: A total of 7 solvent extracts was prepared by sequential extraction of *Saraca asoca* bark, which was screened for antioxidant activities by applying 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method, Ferric reducing antioxidant power assay and Total Antioxidant Activity.

Results: The maximum percentage yield of extract was drawn by utilizing methanol as solvent. The results showed that the ethanolic extract contains strikingly high phenol as well as flavonoid content, which also exhibited remarkably high free radical scavenging activity, in all the three *in vitro* assays performed.

Conclusion: The current work evidences that phenolic and flavonoids play a crucial role in the antioxidant potential of *S. asoca*. And that the immensity of radical scavenging behavior is also influenced by the solvent used.

Keywords: Antioxidants; Free radicals: *Saraca asoca*; Solvent extracts; Flavonoids; Phenolics

INTRODUCTION

Oxidative stress is a disparity between oxidants and antioxidants in favour of oxidants possibly leading to cell and tissue injury [1]. Reactive oxygen species (ROS) are chemically reactive compounds that include singlet oxygen, peroxides, superoxide, hydroxyl radicals, which are produced normally in cells as a by-product during the metabolic process [2]. They are highly reactive, noxious molecules which can cause serious damage to proteins, lipids, enzymes, and DNA with subsequent cell and tissue impairment [2], if surplus ROS are not wiped out by the antioxidant system [3]. However, undue production of these free radicals can be attributed to the onset of several diseases including cancer, hypertension, neurodegenerative disorders, etc and can also accelerate aging which have promoted the studies on the substances that can defend the body against ROS and lead to disease prevention [4].

Antioxidants are substances that significantly delay or inhibit the oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate [5]. Antioxidants can be categorized into endogenous and exogenous, where the former is synthesized within the body of living organisms while the latter is derived from sources outside the living system [6]. Butylated hydroxyanisole (BHA), propyl gallate (PG) and butylated hydroxyl toluene (BHT) are synthetic antioxidants that were found to cause adverse after-effects in experimental animals at high dose [7,8]. Hence there is a great concern on replacing the synthetic antioxidants with natural exogenous antioxidants owing to the fact that they are affordable, easily accessible, known to have minimal side effects and strong efficiency to scavenge free radicals [9].

Innumerous research has been conducted on the antioxidant activities of plants since they are the natural source of antioxidants. Some of the plants with high antioxidant capacities are *Emblica officinalis*, *Rheum ribes*, *Curcuma longa*, *Pelargonium endlicherianum*, *Mangifera indica*, *Momordica charantia*, *Uncaria tomentosa*, *Santalum*

Correspondence to: Bhagya M, Associate Professor, Endocrinology and Reproductive Physiology Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India, Tel: 0821 - 2411217; E-mail: mbhagyauom@gmail.com

Received: December 11, 2020; **Accepted:** March 22, 2021; **Published:** April 05, 2021

Citation: Shahin Taj RA, Sharath J, Bhagya M (2021) Effect of Extraction Solvents on Total Phenol, Flavonoid Content and Free Radical Scavenging Potential of *Saraca asoca*, an Indian Medicinal Plant. J Appl Pharm. 13:287.

album, *Salvia officinalis*, *Swertia chirata*, *Ficus microcarpa*, *Withania somnifera*, *Piper nigrum*, *Zingiber officinalis* [10-12].

Saraca asoca commonly known as the Ashoka, a member of Caesalpiniaceous family is used exhaustively as an herbal drug to cure several diseases since the entire plant is claimed to possess therapeutic properties [13]. It is an evergreen tree which is distributed throughout India and considered to be one among the sacred trees of India. In traditional medicine, Ashoka is used to managing various gynecological disorders like amenorrhea, dysfunctional uterine bleeding, leucorrhea, menorrhagia, uterine fibrosis [14,15]. It is also used to cure various other diseases/ ailments like diabetes, cancer, skin infections, Ulcers, urino-genital diseases, bacterial infections, inflammations, hemorrhagic dysentery, etc. [16,17].

Although a considerable number of workers have explored the antioxidant property of one of the sacred trees of India, *S. asoca* the research is mainly restricted to leaves, seeds, and flowers. Only a little information is available on the antioxidant capacity which includes the Radical scavenging activity of ethanolic, hydro-alcoholic and acetone extracts of bark of *S. asoca*, studied by DPPH method and it was found that the acetone extract showed maximum free radical scavenging activity at 100 µg/ml concentration. Antioxidant properties were also demonstrated in 80% alcoholic crude extract and methanolic extract [18,19]. In Another study Ghatak et al., compared the antioxidant activities of four different extracts i.e., methanol, ethanol, distilled water and ethyl acetate and inferred that methanol is the best solvent system in comparison with the other solvents for the extraction of phytochemicals which is in agreement with Mohan et al., which showed that *S. asoca* has excellent compound activity to eliminate the free radicals [20].

The presence of secondary metabolites in an extract is very much dependent on the extraction solvent used [21]. There are no studies so far on the precise comparative antioxidant study on different sequential extracts of *Saraca asoca.* Therefore, the study was designed to carry out phytochemical screening, determine the total phenol, flavonoid content and antioxidant activities of different solvent extracts of *Saraca asoca* prepared by successive extractions.

MATERIALS AND METHODS

Plant processing, extraction by solvents and yield of extraction

The bark of Ashoka is obtained from Chandravana, Botanical garden in Mysuru. It was washed thoroughly with distilled water and 70% ethanol, shade dried at room temperature for 15 days and subsequently pulverized into a coarse powder using a mechanical grinder. The coarse powder was subjected to exhaustive sequential extraction using six different solvents based on increasing polarity viz., petroleum ether, benzene, chloroform, ethanol, cold water, and hot water by constant infiltration process in soxhlet apparatus. Each time after exhaustive extraction the material was dried completely and later extracted with the next solvent. Each extract was then concentrated by desiccating the solvent to dryness and the dried crude extract was then weighed and stored at 4°C. The coarse powder was also extracted exhaustively using absolute methanol as a solvent. The percentage yields of different extracts were calculated using the relation:

Percent yield = A/B x100 (Where A = weight of the material gained after sequential extraction of each solvent. B = weight of the plant material used for extraction).

Phytochemical analysis

Solvent extracts obtained from the exhaustive sequential extraction of *S. asoca* bark was subjected to different qualitative tests to identify the phytochemical constituents present using standard procedures.

Quantitative analysis

Total phenol content: Folin Ciocalteu method was used to calculate the total phenol content in different solvent extracts as modified by Okumu et al. The crude extract (0.2 mg/ml) was mixed thoroughly with Folin Ciocalteu reagent (diluted 10 times with distilled water) and aqueous sodium carbonate (7.5%) in the ratio of 1: 2: 2.5 and the final volume were made up to 10 ml using distilled water. The mixture was further incubated at 45°C for 15 min and the absorbance was measured at 765 nm. Gallic acid was chosen as standard and the total phenol content was expressed as Gallic acid equivalent (mg)/g of dry mass [22].

Total flavonoid content: The total flavonoid content in different solvent extracts was computed by aluminium chloride colorimetric method as modified by Okumu et al. Quercetin was used to plot the calibration curve.0.5 ml of crude extract (0.2 mg/ml) was added to a 5 ml vial containing 2 ml of distilled water. In addition to this 0.15 ml of Sodium nitrite (5%) was added and further 0.15 ml of aluminum chloride (10%) and 1 ml of sodium hydroxide (1M) was added with an interval of 6 min each and finally the volume was made up to 5 ml by adding 1.2 ml of distilled water. Immediately after the addition of water the absorbance was read at 510 nm. The total flavonoid content was expressed in terms of milligrams of Quercetin equivalents per gram of the dry plant material (mg. QE. g^{-1})

In vitro antioxidant assays

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: Blois spectrophotometric method was used to determine the free radical scavenging activity of different solvent extracts. Three ml of methanolic solution of DPPH was mixed with 0.5 ml of different extracts of concentration 0.4 - 2 mg/ml of *S. asoca*. Methanol was used as blank and an equal amount of methanol was taken as control as that of the extracts. The samples were then incubated in darkness for a time span of 20 mins and the later the absorbance was read at 517 nm. The percentage inhibition was estimated by using the relation given below and the antioxidant activity was expressed in terms of IC_{50}.

Percent inhibition = $(A_c - A_t)/A_c \times 100$

Where A_c=Absorbance of the control, A_t=Absorbance of the test samples (different solvent extracts).

Ferric reducing antioxidant power (FRAP) assay: The method described by Benzie and Strain was adopted to estimate the total antioxidant potential using the ferric reducing ability. The sample mixture consisted of 100 µl of solvent extracts (0.2 – 1 mg/ml), 300 µl of distilled water and 3 ml of FRAP reagent. Freshly prepared FRAP reagent was used which was a mixture of TPTZ (10 mmol/L) dissolved in HCl (40 mmol/L), Ferric chloride (20 mmol/L) and acetate buffer (300 mmol/L, pH 3.6) in the ratio of

1:1:10. The sample mixture was then incubated for a time span of 30 mins at 37°C and later the absorbance was recorded at 593 nm. Ferrous sulfate was chosen as the standard and a standard curve was plotted using absorbance against concentration (0.2 – 2 mM). The concentration of ferrous sulphate was calculated using the equation obtained from the standard curve [23-26].

Total antioxidant activity: The total antioxidant activity in different solvent extracts was estimated by phosphomolybdenum method as described by Prieto et al. 100 µl of solvent extracts were mixed with reagent solution containing 28 mM sodium phosphate, 0.6M Sulphuric acid, and 4 mM ammonium molybdate. The sample mixture was then incubated for 90 min at 95°C and after cooling the mixture to room temperature OD values were recorded at 695 nm against blank using methanol. Ascorbic acid was used as standard to plot the standard curve and the total antioxidant activity was expressed as ascorbic acid equivalents (AAE).

Statistical analysis

Mean value of each parameter was computed considering values of 3 assays and expressed as mean ± SE. the mean values of different groups of each parameter were compared by one way analysis of variance (ANOVA) followed by Duncan's multiple test and judged significant $p < 0.05$.

RESULTS

Yield of extraction

Based on the extraction solvent and plant material used, varying quantity of yields was detected among the different successive extracts of *S. asoca*. The yield of extraction was found to decrease in the order of methanol > ethanol > chloroform > hot water > cold water > petroleum ether > benzene (Table 1).

Phytochemical analysis

Preliminary phytochemical analysis was conducted on the various solvent extracts; the findings are shown in Table 2. The presence of all phytochemicals except proteins was evidenced by the ethanolic and methanolic extracts. All the extracts showed the presence of steroids, terpenoids, carbohydrates, diterpenes, glycosides, alkaloids, flavonoids, tannins and phenolic compounds. Proteins were absent in all the extracts while saponins were present only in ethanolic and methanolic extracts and absent in all the other extracts.

Table 1: Effect of different extraction solvents on the percentage yield of the bark of *S. asoca*.

Solvents Used	Yield of extraction (%)
Petroleum ether	0.398 ± 0.005 ^{d}
Benzene	0.330 ± 0.006 ^{d}
Chloroform	0.756 ± 0.009 ^{cd}
Ethanol	16.294 ± 0.48 ^{b}
Cold water	0.697 ± 0.003 ^{cd}
Hot water	1.142 ± 0.01 ^{c}
Methanol	23.97 ± 0.21 ^{a}
F – value	2360.13
Sig	p > 0.05

Tests were performed in triplets and average was taken ± standard error.

Quantitative analysis

Total phenol content: Table 3 shows the total phenol content in the different solvent extracts of *S. asoca*. It reveals that ethanolic extract (32.01 ± 0.88 mg GAE/g of dry extract) possess maximum phenol content among the other extracts. Significantly high phenol content was seen in ethanol followed by methanol (26.20 ± 0.65 mg GAE/g of dry extract) and chloroform (10.616 ± 0.75 mg GAE/g of dry extract). However, no significant difference was seen in the phenol content in hot water (4.06 ± 0.04), petroleum ether (3.414 ± 0.31), cold water (2.585 ± 0.32) and benzene (2.191 ± 0.19) extracts. The total phenol content in all the solvent extracts was expressed in terms of Gallic acid equivalent mg/g of dry mass extract.

Total flavonoid content: Our results concerning the total flavonoids in the different extracts of *S. asoca* show that the ethanolic extract (141.5 ± 7.00) is a better source of flavonoids compared to the other extracts, which is statistically significant, as seen in case of TPC as well. The other extracts with significantly high flavonoid contents were methanolic extract (120.66 ± 1.96), chloroform extract (66 ± 5.00) and petroleum ether extract (42.16 ± 2.24). The flavonoid content was found to decrease in the order of ethanol > methanol > chloroform > petroleum ether > benzene (26.33 ± 1.09) > cold water (24.166 ± 1.48) > hot water (19.66 ± 1.01 mg QE/g of dry extract) (Table 3).

In vitro antioxidant assays

DPPH radical scavenging activity: The IC50 value i.e., the ability to scavenge 50% of free radicals is inversely proportional to the antioxidant capacity, which is derived from the rectilinear regression of the percent inhibition against the concentration of the plant extract used. All the solvent extracts exhibited free radical scavenging activity at all the concentrations, but the ethanol extract showed high free radical scavenging activity with IC50 value 0.018 µg/ml, whereas methanol, chloroform, benzene, and hot water extracts showed moderate activity with IC50 value 0.078, 0.16, 0.481 and 0.549 µg/ml respectively, while petroleum ether and cold water extracts showed very poor radical scavenging activity with IC50 value 0.91 and 1.834 µg/ml respectively. The antioxidant activity was found to decrease in the order of ethanol > methanol > chloroform > benzene > hot water > petroleum ether > cold water (Figure 1).

FRAP assay: The ethanolic extract of *S. asoca* showed significantly high antioxidant activity with 0.84 ± 0.05 µmol/l of $FeSO_4$ followed by the methanol extract (0.698 ± 0.05), chloroform extract (0.361 ± 0.03) and low in other solvents. No significant difference was observed between the antioxidant potential of benzene (0.156 ± 0.006), hot water (0.138 ± 0.009), petroleum ether (0.103 ± 0.007) and cold water (0.08 ± 0.007 µmol/l of FeSO4) (Figure 2).

Total antioxidant activity: The Figure 3 clearly indicates that the ethanolic extract (0.4568 ± 0.03) shows a significantly high total antioxidant capacity when compared to other six extracts. The methanolic (0.325 ± 0.01) and the hot water extract (0.1799 ± 0.02) also show significant antioxidant activity, while the cold water (0.1357 ± 0.01), chloroform (0.1324 ± 0.01) and petroleum ether (0.0955 ± 0.01) extracts do not exhibit any significant difference in the antioxidant activity. However, the benzene (0.0156 ± 0.009) extract is found to have significantly poor antioxidant potential among all the other solvent extracts.

Table 2: Phytochemical constituents present in the various solvent extracts of bark of *S. asoca*.

Phytoconstituents	Tests performed	Petroleum ether	Benzene	Chloroform	Ethanol	Cold water	Hot water	Methanol
Alkaloids	Hagers test Wagners test	+	+	+	++	+	+	++
Flavonoids	Alkaline reagent test	+	+	+	++	+	+	++
Saponins	Foam test	-	-	-	+	-	-	+
Tannins	Lead acetate test Ferric chloride test	+	+	+	+	+	+	+
Phenolic compounds	Lead acetate test Ferric chloride test	+	+	+	++	+	+	++
Steroids	Libermann-Burchard test Salkowski's test	+	+	+	+	+	+	+
Terpenoids	Libermann-Burchard test Salkowski's test	+	+	+	+	+	+	+
Proteins	Biuret test	-	-	-	-	-	-	-
Carbohydrates	Benedicts test Fehling's test	+	+	+	+	+	+	+
Glycosides	Keller Killiani test	+	+	+	+	+	+	+
Diterpenes	Copper acetate test	+	+	+	+	+	+	+

Here +, ++ refers to present and - refers to absent.

Table 3: Results showing total phenol and flavonoid content of different extracts of *S. asoca*.

Solvents Used	Total Phenol Content (Gallic acid equivalent (mg)/g of dry mass extract)	Total Flavonoid content (Milligrams of Quercetin equivalents per gram of the dry plant material (mg. QE. g^{-1})
Petroleum ether	3.414 ± 0.31^{d}	42.16 ± 2.24^{d}
Benzene	2.191 ± 0.19^{d}	26.33 ± 1.09^{e}
Chloroform	10.616 ± 0.75^{c}	66 ± 5.00^{c}
Ethanol	32.01 ± 0.88^{a}	141.5 ± 7.00^{a}
Cold water	2.585 ± 0.32^{d}	24.166 ± 1.48^{e}
Hot water	4.06 ± 0.04^{d}	19.66 ± 1.01^{e}
Methanol	26.20 ± 0.65^{b}	120.66 ± 1.96^{b}
F – value	165.858	195.541
Sig	$p > 0.05$	$p > 0.05$

Tests were performed in triplets and average was taken ± standard error.

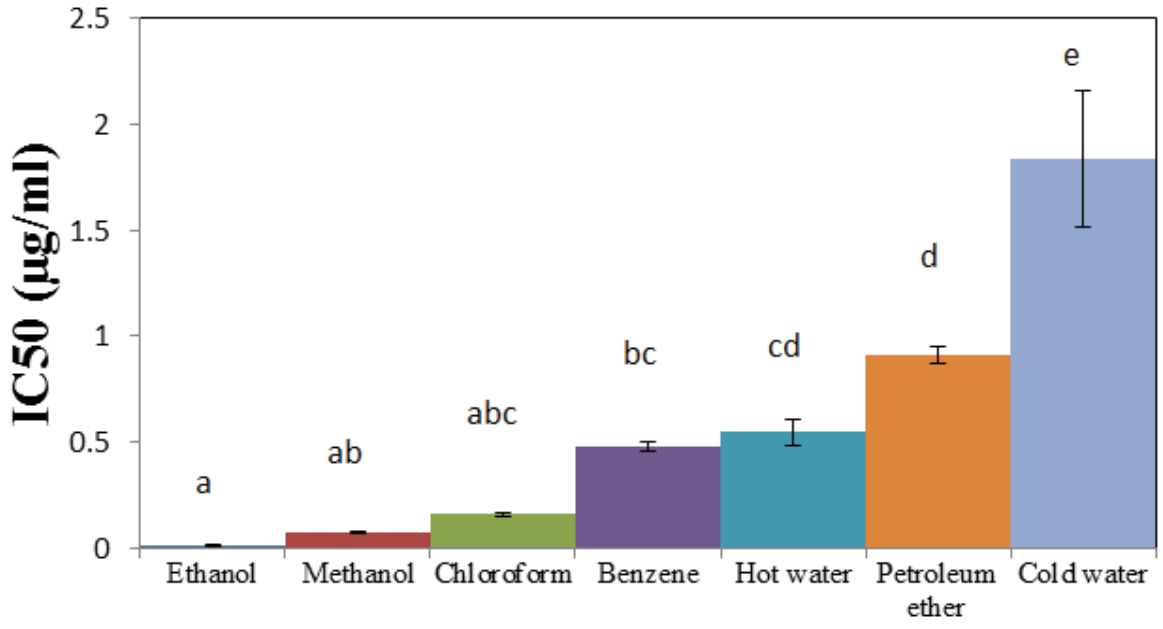

Figure 1: Effect of different solvent extracts of *S. asoca* on DPPH radical scavenging activity.

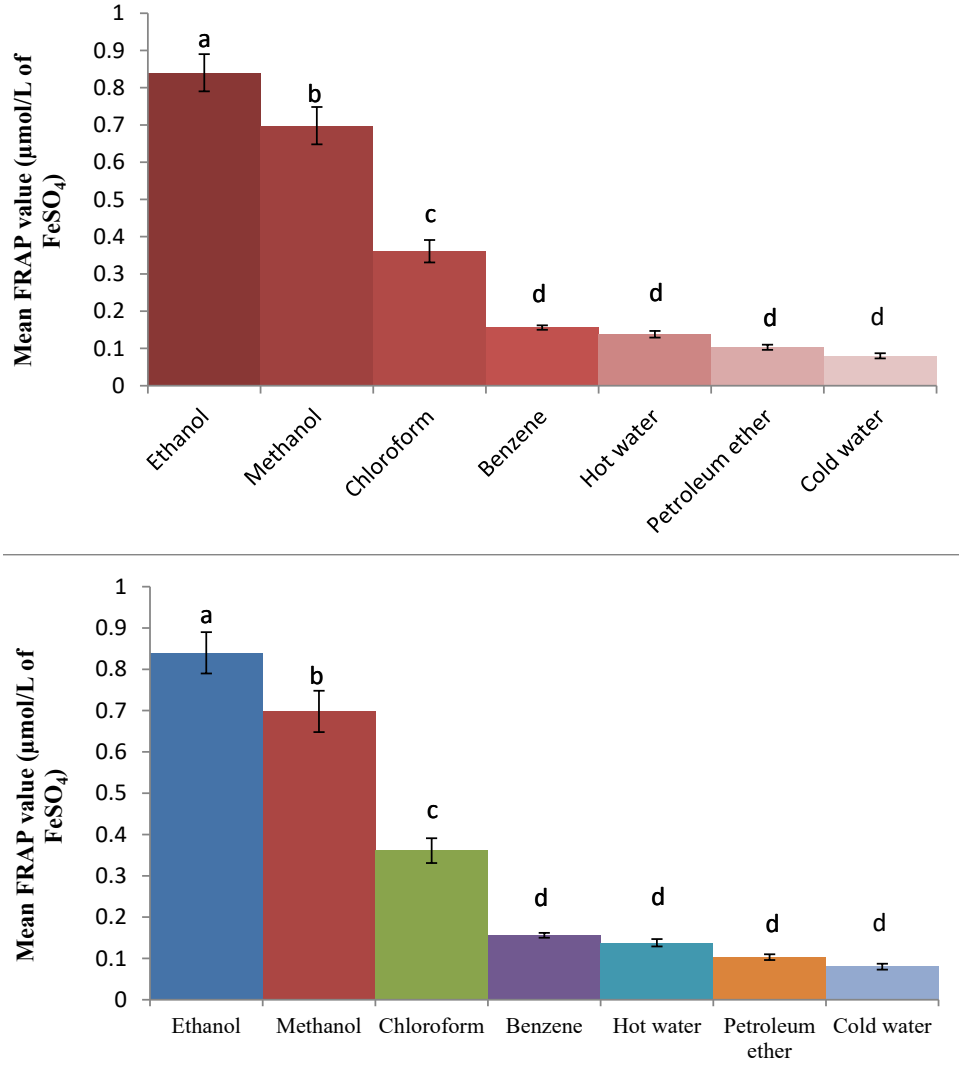

Figure 2: Effect of different solvent extracts of *S. asoca* on Ferric reducing antioxidant power (FRAP).

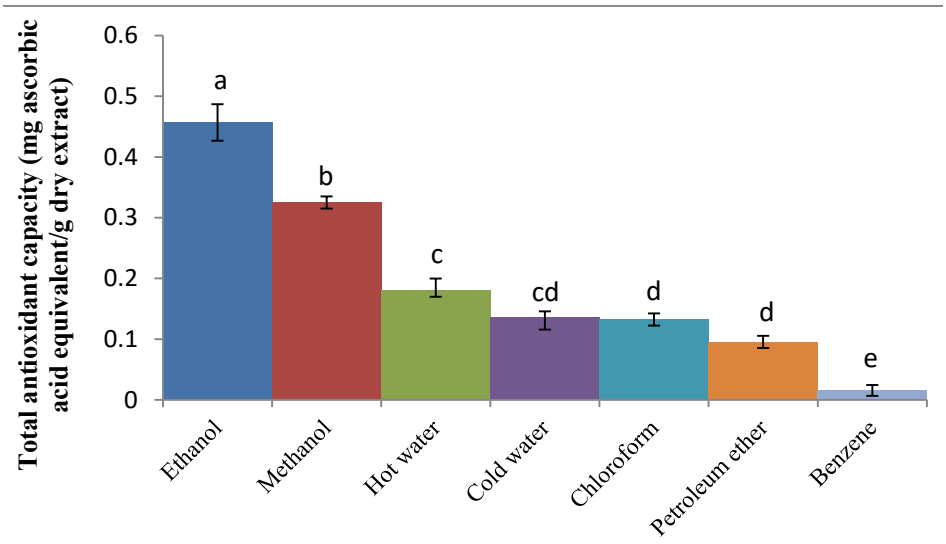

Figure 3: Effect of different solvent extracts of *S. asoca* on total antioxidant activity.

DISCUSSION

An organism's antioxidant defense system safeguards the body against the physiological stress caused by the free radicals produced as a consequence of necessary regular metabolic processes in the body or due to extraneous determinants like exposure to radiations, environmental pollutants, chemicals or cigarette smoke, etc. Methodology is important, even the medium plays a significant role in extraction of solvent. In the present study the yield of extraction solely depends on the solvent used since all the other factors like temperature, extraction time, material used and pH were constant. According to Do et al., the extraction is directly

proportional to the polarity of the solvent used in the extraction, which is also true in the present study where methanol yielded the highest percentage of extract followed by ethanol, chloroform and benzene and petroleum ether showing insignificant difference. Water extracts were an exception which showed less percentage of extraction, the most likely reason could be the absorption of various phytochemicals by the preceding extraction solvents as explained in the material and methods.

The existence of electron donating and the electron accepting constituents or hdroxyl group in the cyclic structure of phenolic compounds have been implicated to their scavenging ability of free radicals, hence are said to be antioxidant in nature [27,28]. These compounds lose hydrogen atoms/electrons from their hydroxyl groups in order to scavenge the free radicals and form stable phenoxyl radicals [29]. In the present study, the ethanolic extract contains significantly high phenol content among the six other extracts. The ethanolic extract also exhibits high antioxidant potential which clearly indicates that antioxidant compounds are in phenolic forms which plays an important role in free radical scavenging 2. According to Do et al., there was a decrease in phenolic compounds and antioxidant potential with an increase in water content which is also true in the present study where the hot and cold water extracts showed the presence of least phenolic compounds and minimal antioxidant potentials. The current study contradicts the report of Ghatak et al., who showed that methanolic extract comprised of the highest phenolic content, and the phenol content decreases in the order of methanol> ethanol> distilled water.

Flavonoids are other principal secondary metabolites that include flavones and flavanols that act as scavengers of many oxidizing compounds and free radicals. Their bioactivities rely on the presence of free hydroxyl groups and a double bond between the second and third carbon atom [30]. A study by Shimoi et al., have pointed out that flavonoids which display *in vitro* antioxidant potential also serve as antioxidants *in vivo*. The current study portrait that the ethanolic extract contained the highest flavonoid contents and also demonstrates maximum antioxidant potential in comparison to the other solvent extracts. A contradictory result was shown in two reports, one by Mohan et al., who have shown the absence of flavonoids in ethanolic extract while the methanolic extract showed its presence. Another report by Ghatak et al., who showed that methanolic extract comprised of the highest flavonoid content. A positive correlation was detected between total phenol and total flavonoid content (R=0.610), these results are in agreement with Do et al. Hence it can be concluded that phenolic and flavonoids are chief antioxidant compounds that display free radical scavenging abilities.

DPPH is a stable free radical which in the presence of an antioxidant molecule gets reduced from a purple colored 2, 2-diphenyl-1-picrylhydrazyl to a yellow colored 2, 2-diphenyl-1-picrylhydrazine by accepting an electron from the antioxidant molecule and pairing off the electron on its nitrogen molecule. The intensity of the color change can be measured at 517 nm which depicts the scavenging capability of an extract/sample. In the present study ethanolic extract of S. asoca showed a significantly high free radical scavenging activity, which is in accordance with the work carried out by Ghatak et al., who compared the antioxidant activities of four extracts i.e., methanol, ethanol, distilled water and ethyl acetate in bark and leaves and found that ethanolic extract showed maximum scavenging potential in case of bark. This indicates that S. asoca comprises electron donating molecules to eradicate the unpaired electron of the free radical to inhibit the process of oxidation.

FRAP assesses the potentiality of an antioxidant to reduce the radical cation. It is based on the reduction of ferric 2, 4, 6-Tri (2-pyridyl)-s-triazine (III) complex to ferrous 2,4,6-Tri (2-pyridyl)-s-triazine (II) complex in an acidic condition of pH 3.6 aided by antioxidants. Here antioxidant activity may be considered analogous to reducing the capability of antioxidants/reductants. In the present study ethanolic, methanolic, chloroform extracts were detected to be better reductants in comparison to the other four extracts, which did not show any significant changes. A similar result was pointed out by Ghatak et al., showing ethanol extract to be a better ferric reducing ability in comparison with methanol and distilled water.

The total antioxidant capacity of different solvent extracts of *S. asoca* was quantified spectrophotometrically at 695 nm based on the formation of green colored phosphomolybdenum (V) complex by the reduction of Mo (VI)to Mo (V) in acidic conditions assisted by antioxidants. There is only one study reporting the total antioxidant capacity of *S. asoca* bark so far. The total antioxidant capacity was noticed to be significantly high in the ethanolic extract of *S. asoca*, which can be ascribed to the existence of high phenolics and flavonoids.

In the three different *in vitro* assays performed, several solvent extracts used in this study showed varying potency of antioxidant capacity. In all the assays ethanol was proved to be the most efficient solvent for the extraction of antioxidant compounds which is clearly noticed with the presence of the highest measure of total phenolic and flavonoids and displayed the maximum antioxidant potential. A similar trend was seen in most of the assays as well as phenol and flavonoid quantification where ethanol was most effective followed by methanol and chloroform (except total antioxidant activity), while the other four i.e., water extracts, petroleum ether, and benzene did not show any particular trend/pattern but showed significantly poor radical scavenging activity depleted amount of phenols and flavonoids [31].

This study for the first time evaluates the differences in biological activities among different solvent sequential extracts in the bark of *S. asoca*. The solvent is one of the major factors in describing the nature of phytochemicals in an extract, though the different extraction techniques increase efficiency. The current study evidently proves that different solvent extracts of *S. asoca* show antioxidant potential and its magnitude of radical scavenging behaviour very much dependent on the solvent extract.

CONCLUSION

The present study clearly indicates that *Saraca asoca* is a potent source of natural antioxidants which could be productively used as a therapeutic agent, however advanced studies are required to identify the dominant antioxidant molecules in the bark of *Saraca asoca* and to unveil its efficacy *in vivo*. Furthermore, study to conserve the species has to be emphasized so that it can be used as an herbal drug for time immemorial.

ACKNOWLEDGEMENTS

Authors express their sincere thanks UGC, New Delhi, India for the financial support.

REFERENCES

1. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. J. King Saud Univ. Sci. 2015;27(3):224-32.
2. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Journal of Taibah University for Sciences. 2015;9(4):449-454.
3. Nagulendran KR, Velavan S, Mahesh R, Begum VH. In vitro antioxidant activity and total polyphenolic content of Cyperus rotundus rhizomes. J. Chem. 2007;4(3):440-449.
4. Hossain MA, Shah MD. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. Arab. J. Chem. 2015;8(1):66-71.
5. Halliwell B, Gutteridge JM. Ageing, nutrition, disease and therapy: a role for antioxidants. Free Radicals in Biology and Medicine. 1999;784-859.
6. Bouayed J, Bohn T. Exogenous antioxidants—double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative Medicine and Cellular Longevity. 2010;3(4):228-37.
7. Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity. Life sciences. 2003;73(2):167-79.
8. Borneo R, León AE, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their in vitro testing in a model food system. Food Chemistry.2009;112(3):664-70.
9. Tadhani MB, Patel VH, Subhash R. *In vitro* antioxidant activities of Stevia rebaudiana leaves and callus. J. Food Compos. Anal. 2007;20(3-4):323-329.
10. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J. Ethnopharmacol. 2000;71(1-2):23-43.
11. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 2011;89(3):217-33.
12. Sharma SK, Singh L, Singh S. A review on medicinal plants having antioxidant potential. Ind J Res Pharmac Biotechnol. 2013;1(3):404.
13. Pradhan P, Joseph L, George M, Kaushik N, Chulet R. Pharmacognostic, phytochemical and quantitative investigation of Saraca asoca leaves. J Pharm Res. 2010;3(1):776-80.
14. Mukhopadhyay MK, Nath D. Phytochemical screening and toxicity study of Saraca asoca bark methanolic extract. Int J Phytomed. 2011;3(4):498.
15. Borokar AA, Pansare TA. Plant profile, phytochemistry and pharmacology of Ashoka (Saraca asoca (Roxb.) D. Wild)-A comprehensive Review. Int J Ayur Herb Med. 2017;7(2):2524-2541.
16. Panchawat S, Sisodia SS. In vitro antioxidant activity of Saraca asoca Roxb. De Wilde stem bark extracts from various extraction processes. Asian J Pharm Clin Res. 2010;3(3):231-233.
17. Saha J, Mukherjee S, Gupta K, Gupta B. High-performance thin-layer chromatographic analysis of antioxidants present in different parts of Saraca asoca (Roxb.) de Wilde. J pharm res. 2013;7(9):798-803.
18. Yadav NK, Saini KS, Hossain Z, Omer A, Sharma C, Gayen JR, et al. Saraca indica bark extract shows in vitro antioxidant, antibreast cancer activity and does not exhibit toxicological effects. Oxid Med Cell. 2015;1-15.
19. Mohan C, Kistamma S, Vani P, Narshimha Reddy A. Biological activities of different parts of Saraca asoca an endangered valuable medicinal plant. International Journal of Curr Microbiol Appl Sci. 2016:5(3):300-8.
20. Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K, et al. Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of Saraca asoca (Roxb.) De. Wilde. Int J Adv Res. 2015;3(5):318-327.
21. Vargas RA, Malacara CF, Petricevich VL. Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea × Buttiana holttum and standl, (Var. rose) extracts. Antioxidants. 2016;5(4):45.
22. Okumu MO, Mbaria JM, Kanja LW, Gakuya DW, Kiama SG, Ochola FO. Phytochemical profile and antioxidant capacity of leaves of Moringa oleifera (Lam) extracted using different solvent systems. J Pharmacog Phytochem. 2016;5(4):302-308.
23. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200.
24. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-76.
25. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999: 269(2):337-341.
26. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal. 2014;22(3):296-302
27. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Compl Alternative Med. 2012;12(1):221.
28. Zhang HY, Sun YM, Wang XL. Substituent Effects on O□ H Bond Dissociation Enthalpies and Ionization Potentials of Catechols: A DFT Study and Its Implications in the Rational Design of Phenolic Antioxidants and Elucidation of Structure–Activity Relationships for Flavonoid Antioxidants. Chem Eur J. 2003;9(2):502-508.
29. Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. Saudi J Biol Sci. 2013;20(3):235-239.
30. Sen S, De B, Devanna N, Chakraborty R. Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of Meyna spinosa Roxb, an Indian medicinal plant. Chin J Nat Med. 2013;11(2):149-157.
31. Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. Mutation Research/Fundamental and Molecular mechanisms of mutagenesis. 1996;350(1):153-161.