Asian Journal of Chemistry; Vol. 33, No. 9 (2021), 2237-2243

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23413

Comparative Studies of Phytochemicals Analysis and *in vitro* Antioxidant Properties of Various Solvent Extracts of *Sphaeranthus indicus* Collected from three Different Climatic Zones of Central India

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Received: 14 July 2021; *Accepted*: 16 August 2021; *Published online*: 20 August 2021; AJC-20489

In present study, the total phenolic, flavonoid, tannin contents and the antioxidant activity of various solvent extracts of *Sphaeranthus indicus* collected from three different regions of central India were assessed. Plants extracts were prepared using Soxhlation method, while the total phenols, flavonoids and tannins were measured by the spectrophotometric method. DPPH, metal chelating, nitric oxide, superoxide oxide scavenging activity and FRAP, reducing power, total antioxidant assays were also evaluated. The highest phenolic contents 268.22 GAE equivalent, flavonoids 441.33 QE equivalents and tannin content was 120.32 tannic acid equivalents (mg/g) obtained from ethanolic extracts of sample SIEE-1 compared to other two plants extract. Similarly, the highest flavonoid contents was observed in SIAE-1 aqueous extract and lowest in SIAE-2 and SIAE-3. Extract of SIEE-1 possessed maximum antioxidant potentiality and SIAE-2 shown the least antioxidant activity in all assays. It could be concluded that different agroclimatic conditions have effects on the total phenolics, flavonoids, tannin contents and antioxidant potentiality of *S. indicus* plant.

Keywords: *Sphaeranthus indicus*, Antioxidant activity, Total phenolic, Total flavonoids, Total tannin, Climatic zone.

INTRODUCTION

The generation of oxygen free radicals is basic to any biochemical process and this constitutes a pivotal part of life processes. Generation of ROS like hydroxyl radical ($^{\bullet}OH$), superoxide ($O_2^{-\bullet}$), hydrogen peroxide and singlet oxygen ($^1O_2$) are associated with several degenerative diseases such as cancer, diabetes, cardiovascular diseases, Alzheimer's disease and inflammation [1,2]. A balance must exist between the generation of these free radicals and scavenging activities of antioxidants. Whenever there is a change in this balance, oxidative stress is generated [3,4]. Antioxidants provide protection against the oxidative stress in the cells and thus, useful in the treatment of many human diseases, such as cancer, cardiovascular diseases and inflammatory disorders [5].

Sphaeranthus indicus Linn. belonging to Compositae family is a branched herb with purple flowers. It grows plentiful in rice field and dispersed throughout India. It is used natively in the traditional system of medicine as anthelmintic. It possesses a broad range of medicinal properties and was used in jaundice, diabetes, fever, hemicranias, hernia, leprosy, pectoralgia, gastropathy, leprosy, hemorrhoids, helminthiasis, cough, dyspepsia, nerve tonic and skin diseases [6,7]. Various pharmacological activities were reported on this plant such as, antimicrobial [8,9], immunomodulatory [10], antibacterial [11,12], wound healing [13], anxiolytic action [7]. Many bioactive constituents isolated from this plant are isoflavonoids [14], eudesmanolides [15], 7-hydroxy eudesmanolides [16], essential oil (ocimene, cadiene, citral, *p*-methoxycinnamaldehyde, eugenol, geraniol and geranyl acetate) [17], eudesmanolides [18] and sterol glycoside [19].

The present study attempts to investigate novel plant materials as a rich source of antioxidants. This study focus on the comparison of total phenolic, flavonoids, tannins content and *in vitro* antioxidant potentiality of various solvent extracts of *S. indicus* collected from different climatic zones of Central India. India is a home to an extraordinary variety of climatic regions. These climatic variations can lead to a difference in the phytocomponents of plant species.

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EXPERIMENTAL

Plant material: The taxonomic identification of the whole plant of *S. indicus* collected from different locations (Rajgarh, Nagda and Rewa) of central India was confirmed and authenticated by Dr. H.B. Singh, Raw Materials Herbarium and Museum (RHMD) of NISCAIR, New Delhi. (Ref. letter No. of SI-1 from Rajgarh-NISCAIR/RHMD/Consult/-2017/3065-14-1), (Ref. letter No. of SI-2 from Nagda-NISCAIR/RHMD/Consult/-2017/3065-14-2), (Ref. letter No. of SI-3 from Rewa-NISCAIR/RHMD/Consult/-2017/3065-14-3)

Processing of plant material: *Sphaeranthus indicus* L. grows as a weed in paddy field and these plant samples were collected from three different regions of India for the proposed study. Sample 1 (SI-1) collected from Rajgarh, sample 2 (SI-2) from Nagda and sample 3 (SI-3) from Rewa of central India. The collected plant samples were cleaned thoroughly with distilled water to remove sticky dirt, naturally dried under shade and subjected to size reduction using a mechanical grinder. The coarsely dried, powder were stored in hermetically sealed containers for further use.

Preparation of extract by successive solvent extraction method: Air-dried *Sphaeranthus indicus* Linn. (100 g) were extracted by successive soxhlet extraction method for 72 h were carried by using solvents with increasing polarity *viz.* petroleum ether, chloroform, ethanol and macerated to form an aqueous extraction. Extracts were filtered and solvents were evaporated by distillation process under reduced pressure and the resulting extracts obtained were dried. Dried extracts were stored in well closed air tight bottles for further use [20].

Quantitative phytochemical assays

Determination of total phenolic contents: Phenolic compound reacts with phosphomolybdic acid in presence of Folin-Ciocalteau phenol reagent (1:10) in an alkaline medium and produce a blue colored complex (with molybdenum blue), which can be estimated by colorimetric method. The total phenolic content of the plant extracts was determined by Folin-Ciocalteu's phenol method [21] with certain modifications. Contents of total phenolics in the ethanolic and water extract of whole plant of *S. indicus* collected from different regions of central India were estimated by a UV-visible spectrophotometer. Different concentrations of extracts and (gallic acid) standard were prepared. Basically, 1 mL of each SI-1, SI-2, SI-3 ethanolic and water extracts with different concentration 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) was added separately to 10 mL flask and mixed 1 mL of Folin-Ciocalteau phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the above mixture and volume made up to 10 mL using distilled water. The reaction mixture was kept in dark for 1 h. After 0.5 h until blue color was developed tube were centrifuged at 2000 rpm for 10 min, after which the absorbance was measured against prepared blank (reagent) at 725 nm. A calibration curve was generated by plotting concentration of gallic acid *versus* absorbance (Fig. 1). All the determinations were performed three times (n = 3) and the mean of three tests were used.

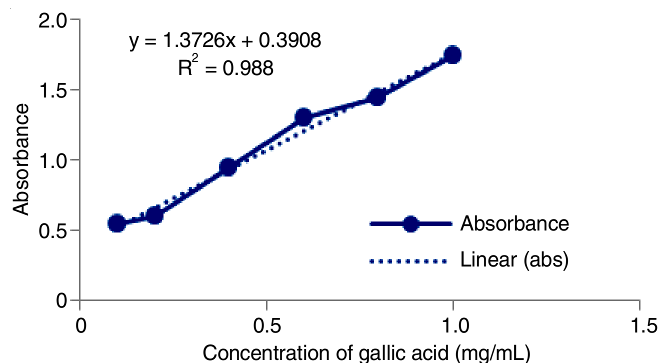

Fig. 1. Determination of phenolic contents using gallic acid as a standard

Determination of total flavonoid content: A 1 mL of each SI-1, SI-2, SI-3 ethanolic and water extracts with different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) and standard solution of quercetin (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) was added separately to 10 mL volumetric flask with 4 mL distilled water. To the above mixture 300 µL of 5% $NaNO_2$ was added, followed by the addition of 300 µL of 10% $AlCl_3$. After 5 min of incubation, 2 mL of 1 M NaOH solution was added and the total volume were increased upto 10 mL using distilled water. The above solution was thoroughly mixed and the absorbance was measured at 510 nm by spectrophotometer against blank [22,23]. A calibration curve was generated by plotting concentration of quercetin *versus* absorbance.

Determination of total tannins content: Total tannin contents of plant extracts was determined by Folin-Ciocalteu method [24]. Different concentrations of extracts and standard (tannic acid) solutios were prepared. Basically, 1 mL of each SI-1, SI-2, SI-3 ethanolic and water extracts with different concentration 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) was mixed with 8 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent (1:20) and 1.5 mL of Na_2CO_3 (20%). Absorption was recorded at 775 nm against blank.

Calculation of phytoconstituents in plant extracts: The total content was calculated using the linear regression equation (1) and expressed in terms mg of standard equivalent per gm of extract.

$$y = mx + c \quad (1)$$

where y = absorbance, x = standard compound concentration (mg/mL), c = interception, m = slope.

Now, the concentration of total contents in sample was determined as milligram of standard sample equivalent (eqn. 2):

$$C = c\frac{V}{m} \quad (2)$$

where C = total content (mg/g plant extract), c = concentration of sample established from the calibration curve of standard (mg/mL), v = volume of extract (mL), m = weight of pure plant extract (g).

in vitro antioxidant assay

DPPH free radical scavenging assay: Free radical scavenging analysis of various extracts of *S. indicus* collected from different regions was measured by DPPH method [25,26] with

few modifications. A 1 mL of different concentrations of extracts and standard (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were added to the separate test tube and then an equal amount of DPPH (0.0001 M) methanol solution was added. The mixture was shaken vigorously and allowed to stand at room temperature for 0.5 h in a dark place. Decreasing absorbance was measured against the blank at 517 nm using a standard spectrophotometer (UV-Vis) in the presence of sample extracts and at different concentrations. A blank reading was taken using ethanol in place of sample extract.

Metal chelating scavenging assay: A 1 mL of different concentrations of extract and standard (disodium EDTA) (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were added to the separate test tube and allow to react with 0.1 mL $FeCl_3$ and 3.7 mL distilled water. To a above reaction mixture, 0.2 mL ferrozine was added. The mixture was agitated vigorously and allow to stand at room temperature for 20 min. The absorbance was noted at 562 nm against reagent blank [27].

Nitric oxide scavenging assay: A 0.5 mL of different concentrations of extracts and standard (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were added followed by the addition of 0.5 mL of phosphate buffer (pH = 7.4) and then 2 mL of sodium nitroprusside solution (10 mM). This reaction mixture was shaken gently and allowed to stand for 150 min at room temperature. About 0.5 mL reaction mixture solution was separated from above solution and diluted with 1 mL of sulphanilic acid (0.33% in 20% GAA) and allowed to stand for 5 min for completing the diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand again for 30 min at room temperature. A pink colored chromophore was formed in diffused light. Absorbance was recorded at 540 nm against blank [28].

Superoxide scavenging assay: A 1 mL solution of NBT (156 µM nitro blue tetrazolium prepared in 0.1 M phos-phate buffer, pH 7.4), 1 mL nicotinamide adenine dinucleotide solution (468 µM NADH prepared in 0.1 M phosphate buffer, pH 7.4) and 1 mL of different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) of extracts and standard (ascorbic acid) were added to the separate test tube and mixed thoroughly. The reaction begins by adding 100 µL of phenazine methosulfate solution (60 µM PMS prepared in 0.1 M phosphate buffer, pH 7.4) to the mixture. The reaction mixture solution was incubated for 5 min at room temperature. Decreasing absorbance was measured against the blank at 560 nm [29].

Percentage scavenging (inhibition) capacity and IC_{50} values: The free radical scavenging and chelating activity of the different extracts of *S. indicus* were calculated according to eqn. 3:

$$\text{Scavenging (\%)} = \frac{A_o - A_t}{A_o} \times 100$$

where, A_o = Absorbance of the control (without test sample), A_t = Absorbance of the test sample.

The IC_{50} values were calculated by regression equation, which was obtained from the standard curve. The IC_{50} (inhibitory concentration) value is inversely proportional to the antioxidant scavenging activity.

Reducing power assay: A 1 mL of different concentrations of extracts and standard (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were mixed with 2.5 mL of 0.2 M $Na_2HPO_4{\cdot}2H_2O$ buffer. In the above mixture, 2.5 mL of 1% potassium ferricyanide was added. The reaction mixture was agitated vigorously and allowed to stand at room temperature for 20 min at 50 ºC. To the above incubated mixture, 2.5 mL of 10% trichloroacetic acid was added for the reaction termination and centrifuged at 6000 rpm for 10 min. The supernatant was collected in a tube then 2.5 mL distilled water and 0.5 mL 0.1% $FeCl_3$ was mixed in the solution. Increasing absorbance was measured using spectrometer against the blank at 700 nm [30].

Total antioxidant capacity (TAC): An aliquot of 1 mL of different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) of extracts and standard gallic acid were treated with 1 mL of total antioxidant reagent which comprised of 0.6 M H_2SO_4, 28 mM $Na_2HPO_4{\cdot}2H_2O$ buffer and 4 mM $(NH_4)_2MoO_4$. The tubes were capped and incubated at 95 ºC for 90 min in a water bath. After incubation period, the mixture was cooled to room temperature and absorbance was observed at 695 nm against blank [31]. The total antioxidant activity of plant extract is expressed as the number of equivalents of gallic acid (mg/g).

Ferric reducing antioxidant power (FRAP) assay: Different concentrations of extract and standard ($FeSO_4$) were prepared. A 2.85 mL working solution of FRAP was mixed with 150 µL test sample and then incubated for 30 min in the dark condition. Absorbance of the colored product (ferrous-2,4,6-tripyridyl triazine complex) were observed at 593 nm [32]. Results were expressed in term µM of Fe^{2+} equivalent per gram of extract (µM Fe(II)/g dry mass).

RESULTS AND DISCUSSION

Secondary metabolites like phenol, flavonoids and tannins were analyzed in ethanol and aqueous extracts of various samples of *S. indicus* (S1-1, SI-2 and SI-3), which are present in the extracts are shown in Table-1. Total flavonoid, phenolic and tannin contents in different ethanolic and aqueous extract samples of *S. indicus* (S1-1, SI-2 and SI-3) was calculated as gallic acid equivalents (GAE), quercetin equivalents and tannin acid equivalents using regression equations obtained from standard graphs (Figs. 1-3).

The phenolic content obtained from the extracts of *S. indicus* collected from three different regions of central India was found to be different. The highest phenolic content was 268.22 GAE equivalents (mg/g) obtained from ethanolic extracts of

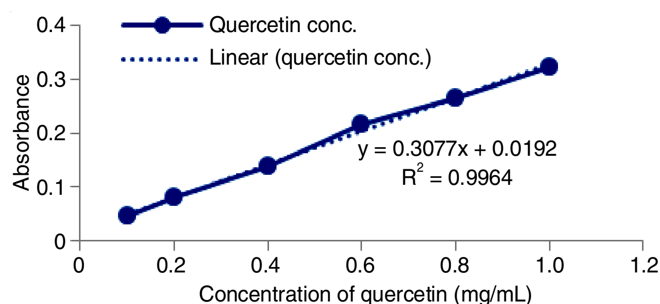

Fig. 2. Determination of flavonoid content using quercetin as a standard

TABLE-1
COMPARATIVE QUANTITATIVE ANALYSIS OF VARIOUS BIOACTIVE COMPOUNDS IN ETHANOLIC AND AQUEOUS EXTRACTS OF *S. indicus* S1-1, SI-2 AND SI-3

Extracts	Total phenol (mg in gallic acid equivalent/g of dried plant extract)	Total flavonoid (mg in quercetin equivalent/g of dried plant extract)	Total tannin (mg in tannin acid equivalent/g of dried plant extract)
SIEE-1	268.22 ± 0.002	441.33 ± 0.0080	120.32 ± 0.0060
SIEE-2	12.39 ± 0.006	109.84 ± 0.0010	1.05 ± 0.0005
SIEE-3	74.31 ± 0.004	233.34 ± 0.0120	8.62 ± 0.0010
SIAE-1	–	22.74 ± 0.0010	–
SIAE-2	–	3.24 ± 0.0010	–
SIAE-3	–	9.75 ± 0.0005	–

All values are expressed as Mean ± SD of 3 observations; SIEE = *S. indicus* ethanolic extract; SIAE = *S. indicus* aqueous extract

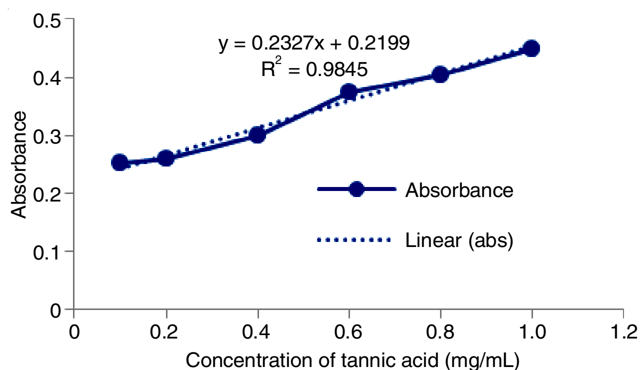

Fig. 3. Determination of tannin content using tannic acid as a standard

SIEE-1. The next highest 74.31 GAE equivalents (mg/g) was observed in SIEE-3 ethanolic extracts and the lowest phenolic content was found to be about 12.39 GAE equivalents (mg/g) in SIEE-2 ethanolic extract. Total phenolic content was not found in the aqueous extracts of all three samples as shown in Fig. 4.The highest flavonoid content was 441.33 QE equivalents (mg/g) obtained from ethanolic extracts of SIEE-1. The next highest 233.34 QE equivalents (mg/g) was observed in SIEE-3 ethanolic extracts and the lowest flavonoid content was found to be about 109.84 QE equivalents (mg/g) in SIEE-2 ethanolic extract. Whereas, SIAE-1, SIAE-3 and SIAE-2 were found to the lowest flavonoid content, which are 22.74, 9.75 and 3.24 QE equivalents (mg/g), respectively (Fig. 4). The highest tannin content was 120.32 tannic acid equivalents (mg/g) derived from ethanolic extracts of SIEE-1. The lowest tannin was found to be about 1.05 tannic acid equivalents (mg/g) in SIEE-2 and 8.62 in SIEE-3, indicating that there is little or no tannin content in it. Total tannin content was not found in the aqueous extracts of all three samples (Fig. 4).

DPPH free radical scavenging assay: The results obtained from the free radical scavenge activity against DPPH free radical are shown in Fig. 5. The variation observed between scavenging activities of the same extract depends upon the regions of a plant used in this study. This difference can be attributed to the uneven distribution of antioxidant molecules such as polyphenols, flavonoids, present in plants collected from different locations.All the three ethanolic extracts of *S. indicus* plants revealed a good DPPH radical scavenging activity than aqueous extract. The DPPH scavenging activities of various extracts of *S. indicus* depends on the extract concentrations. Maximum DPPH scavenging activity and minimum IC_{50} value were shown by ethanolic extract using the standard (ascorbic acid), which contained the highest amount of phenol, flavonoid and tannin. Aqueous extract has shown the minimum scavenging activity and maximum IC_{50} value as well as less amount of phenolic content. As comparison to SIEE-2 and SIEE-3, it can be seen that the percentage of free radical scavenge activity of ethanolic extract of SIEE-1, at various concentrations exhibited high percentage inhibition at upper concentration it, which was found to be 74.47% and its IC_{50} value is 0.46 ± 0.005 (Fig. 5). The ascorbic acid (standard) shown maximum scavenging potential at very low concentration which is 85.24% percent and its IC_{50} value is 0.19 ± 0.01. Whereas aqueous extract of SIAE-2 has shown low scavenging effect up to 1 mg/mL, it was found to be 24.49% and its IC_{50} value is 3.27 ± 1.00. Thereafter, the effect of DPPH scavenging becomes greater with the concentration of sample extracts. The potential to scavenge 50% of DPPH was found to be in order of IC_{50}, 0.46 ± 0.005 mg/mL for SIEE-1 > SIEE-3 (0.6 ± 0.03 mg/mL) > SIAE-1 (0.64 ± 0.005 mg/mL) > SIEE-2 (1.05 ± 0.04 mg/mL) > SIAE-3 (1.42 ± 0.10 mg/mL) > SIAE-2 (3.27 ± 1.00 mg/mL).

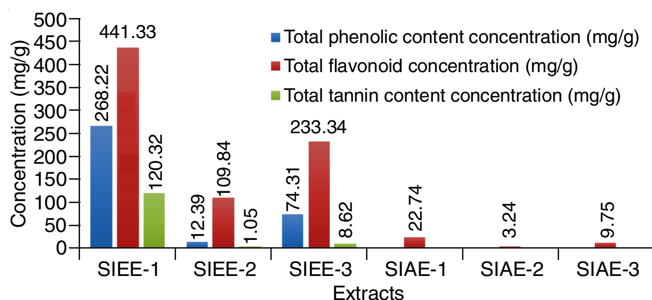

Fig. 4. Comparative diagrammatic representation of total phenolic, flavonoid and tannin content in ethanolic and aqueous extracts of *S. indicus* (SI-1, SI-2 and SI-3)

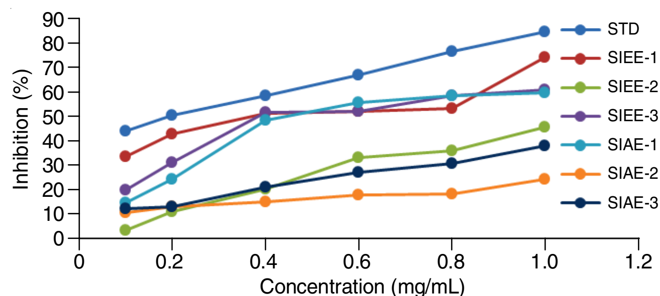

Fig. 5. Comparative free radical scavenging activity (percentage inhibition) of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

Metal chelating scavenging assay: The results obtained from the metal chelating scavenge activity are shown in Fig. 6. All three aqueous extracts of *S. indicus* revealed a good metal chelating scavenging activity than ethanolic extracts. Metal chelating activities of various extracts of *S. indicus* depends on the concentration of the extracts. Maximum metal chelating scavenging activity and minimum IC_{50} value were shown by aqueous extract. Ethanolic extracts have shown the minimum scavenging activity and maximum IC_{50} value. As compared to SIAE-2 and SIAE-3, it can be seen that the percentage of metal chelating scavenging activity of aqueous extract of SIAE-1, at various concentrations shown high percentage inhibition at upper concentration (84.81%) and its IC_{50} value is 0.20 ± 0.06, which is lower than the positive standard EDTA as shown in Fig. 6. Whereas the ethanolic extract of SIEE-2 showed low scavenging effect up to 1 mg/mL and found to be 67.72% (IC_{50} 0.69 ± 0.02); thereafter, metal chelating scavenging effect higher with the concentration of sample extract. The Na_2EDTA standard has shown the minimum scavenging potential as compared to other extract, which shows that the metal chelating potential in *S. indicus* has higher than the standard. The potential to scavenge 50% of metal chelating scavenging effect was found to be in order of SIAE-1 > SIAE-3 (0.31 ± 0.01 mg/mL) > SIEE-1 (0.28 ± 0.04 mg/mL) > SIEE-3 (0.41 ± 0.003 mg/mL) > SIAE-2 (0.54 ± 0.03 mg/mL) > SIEE-2 (0.69 ± 0.02 mg/mL).

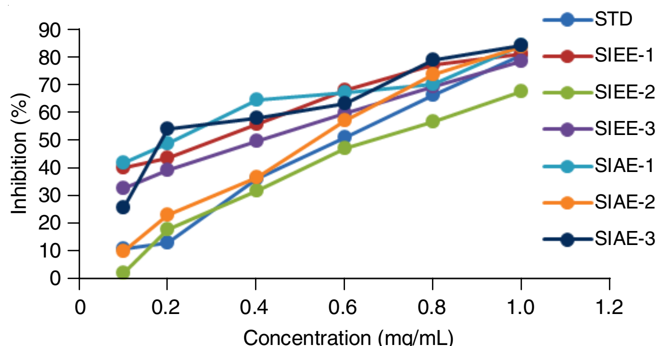

Fig. 6. Comparative metal chelating scavenging activity (percentage inhibition) of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

Nitric oxide scavenging assay: In this study, nitrite NO_2 produced by incubation of sodium nitroprusside solutions in a standardized phosphate saline buffer at room temperature was reduced by three different ethanolic and water extracts of *S. indicus*. The scavenging activity of ethanolic and water extracts was found to be concentration-dependent. All the three ethanolic extract of *S. indicus* revealed a good nitric oxide scavenging activity than aqueous extract. Maximum nitric oxide scavenging activity and minimum IC_{50} value were shown by ethanolic extract after the standard (ascorbic acid), which contained the highest amount of phenol, flavonoid and tannin. aqueous extract shown minimum scavenging activity and maximum IC_{50} value as well as less amount of phenolic content. As compared to SIEE-2 and SIEE-3, it can be seen that the percentage of nitric oxide scavenging activity of ethanolic extract of SIEE-1, at different concentrations has shown high percentage inhibition at higher concentration (63.24% and IC_{50} = 0.05 ± 0.004) (Fig. 7), whereas aqueous extract of SIAE-2 exhibit low nitric oxide scavenging activity up to 1 mg/mL and found to be 6.51% (IC_{50} 8.59 ± 0.32). The potential to scavenge 50% of nitric oxide scavenging effect was found to be in order of SIEE-1 (IC_{50} 0.05 ± 0.004 mg/mL) > SIEE-3 (0.47 ± 0.005 mg/mL) > SIAE-1 (1.67 ± 0.01 mg/mL) > SIEE-2 (1.76 ± 0.15 mg/mL) > SIAE-3 (7.03 ± 0.80 mg/mL) > SIAE-2 (8.59 ± 0.32 mg/mL). The present study has proven that the extracts contains a powerful nitric oxide scavenging activity and can be useful for the management of diseases in which radical nitrite is directly involved.

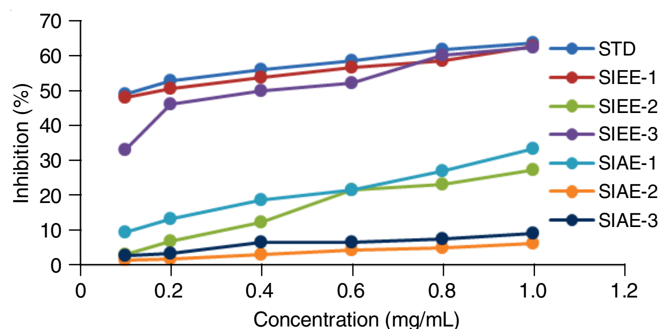

Fig. 7. Comparative nitric oxide scavenging activity (percentage inhibition) of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

Superoxide scavenging assay: The present study shows that all the extracts were found to exhibit strong antioxidant activity against the superoxide anion radical, when compared to standard (ascorbic acid). All the three aqueous extract of *S. indicus* revealed a good Superoxide scavenging activity than ethanolic extracts (Fig. 8). Superoxide scavenging activity of various extracts of *S. indicus* depends on the concentration of the extracts. Maximum superoxide scavenging activity and minimum IC_{50} value were shown by aqueous extract. Ethanolic extracts have shown the minimum scavenging activity and maximum IC_{50} value. As compared to SIAE-2 and SIAE-3, the percentage of superoxide scavenging activity of aqueous extract of SIAE-1 at different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) exhibit high percentage inhibition and found to be 59.29% (IC_{50} = 0.67 ± 0.005). The ascorbic acid as standard has shown the maximum scavenging potential at low concentration (76.28% and IC_{50} = 0.37 ± 0.01) as shown in Fig. 8. Whereas the ethanolic extract of SIEE-2 has shown low scavenging effect up to 1 mg/mL (21.29%, IC_{50} = 2.63 ± 0.02). The ascorbic acid standard showed maximum scavenging potential as comparisons to all extract's samples of *S. indicus*. The potential to scavenge 50% of Superoxide scavenging effect was found to be in order of SIAE-1 (IC_{50} 0.67 ± 0.005 mg/mL) > SIAE-3 (0.79 ± 0.005 mg/mL) > SIEE-1 (1.74 ± 0.02 mg/mL) > SIAE-2 (1.86 ± 0.01 mg/mL) > SIEE-3 (2.49 ± 0.01 mg/mL) > SIEE-2 (2.63 ± 0.02 mg/mL).

Reducing power assay: All the three ethanolic extracts of *S. indicus* revealed a good reducing power activity than the aqueous extracts. All the ethanolic extracts of *S. indicus* plants showed some degree of electron donation. In this assay, similar

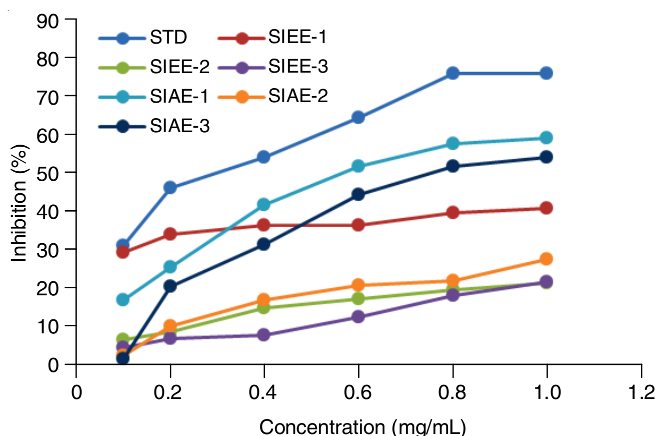

Fig. 8. Comparative Superoxide scavenging activity (percentage inhibition) of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

pattern has been observed, when compared to SIEE-2 and SIEE-3, the ethanolic extract of SIEE-1 at various concentrations showed the high reducing power effect whereas, ascorbic acid showed the maximum reducing power than all extracts of *S. indicus* (Fig. 9). The reducing power capacity of ethanolic and aqueous extracts was found to be in order of SIEE-1 > SIEE-3 > SIAE-1 > SIAE-3 > SIEE-2 > SIAE-2.

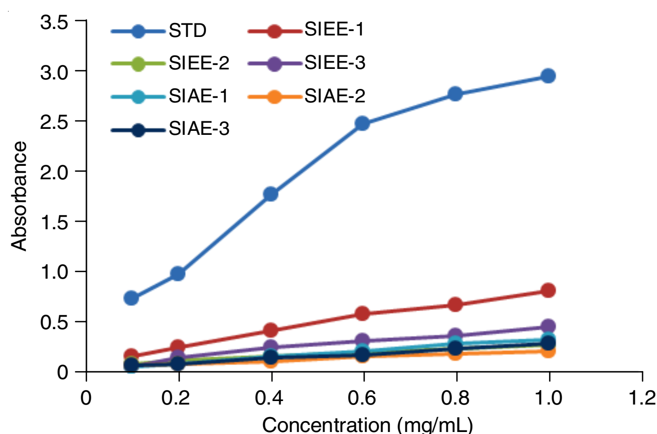

Fig. 9. Comparative reducing power activity of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

Total antioxidant capacity (TAC): Using phosphomolybdenum assay, the antioxidant potential values of all the extracts of *S. indicus* are given in Table-2. All the ethanolic and aqueous extracts showed increasing antioxidant activity with increasing concentration. All the extracts of *S. indicus* showed good total antioxidant capacity. The ethanolic extracts possess the maximum total antioxidant capacity in comparison to the aqueous extracts. Total antioxidant capacity (mg/g) of various extracts of *S. indicus* was found to be in order of SIEE-1 > SIEE-3 > SIEE-2 > SIAE-1 > SIAE-3 > SIAE-2.

Ferric reducing antioxidant power (FRAP) assay: In this study (Fig. 10), the absorbance of ethanolic extracts of *S. indicus* increased due to the formation of Fe^{2+}-TPTZ complex with increasing concentration as seen also in the standard antioxidant. The ethanolic extracts of *S. indicus* demonstrated a higher FRAP than aqueous extracts. FRAP (µM Fe(II)/g dry extract) of various extracts of *S. indicus* was found to be in order of: SIEE-1 > SIEE-3 > SIEE-2 > SIAE-1 > SIAE-3 > SIAE-2.

TABLE-2
COMPARATIVE TOTAL ANTIOXIDANT CAPACITY AND FERRIC REDUCING ANTIOXIDANT POWER OF ALL THREE ETHANOLIC AND AQUEOUS EXTRACTS SAMPLES OF *S. indicus* (SI-1, SI-2, SI-3)

Extracts	TAC (mg in gallic acid equivalent/g dry extract)	FRAP (µM Fe(II)/g dry extract)
SIEE-1	934.94 ± 0.0005	9.770 ± 0.0400
SIEE-2	505.88 ± 0.0010	6.720 ± 0.0230
SIEE-3	644.69 ± 0.0030	8.020 ± 0.0009
SIAE-1	341.83 ± 0.0120	6.580 ± 0.0130
SIAE-2	183.39 ± 0.0050	5.162 ± 0.0010
SIAE-3	194.61 ± 0.0060	5.389 ± 0.0020

All values are expressed as Mean ± SD of 3 observations.
SIEE = *S. indicus* ethanolic extract; SIAE = *S. indicus* aqueous extract

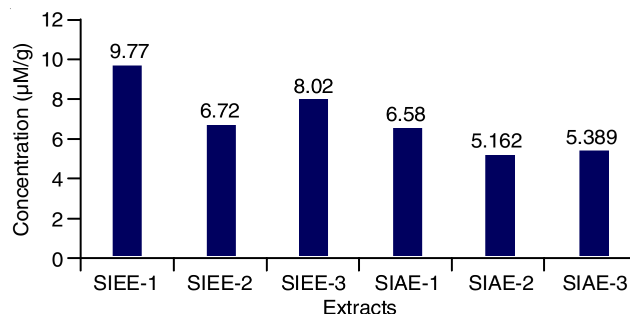

Fig. 10. Diagrammatic representation of comparative FRAP of all three ethanolic and aqueous extracts samples of *S. indicus* (SI-1, SI-2, SI-3)

On the basis of result obtained, the highest antioxidant activity has been observed in Rajgarh region (SI-1). Despite having the same plant species, variation in phytochemicals has been found in plants of different regions, which may be due to environmental factors and soil elements composition. A thorough phytochemical analysis should be performed to identify the active phenolic and flavonoid components and there is also a need to examine the effects of soil properties.

Conclusion

The present study showed that *Sphaeranthus indicus* is a promising source of bioactive phenol and flavonoids which has a good antioxidant activity. This study demonstrated that the total phenolic content and flavonoid content of the plants is responsible for antioxidant potential of *S. indicus* (SI-1) plant collected from Rajgarh region which makes them more potent antioxidant scavenger, whereas the other plant SI-2 and SI-3 from Nagda and Rewa regions as per their phytochemical composition showed different levels of antioxidant potential. Further studies are needed to explore the potential phenolics compounds of *S. indicus* collected from particular site and *in vivo* studies are needed for better understanding their mechanism of action.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R.A. Roberts, D.L. Laskin, C.V. Smith, F.M. Robertson, E.M.G. Allen, J.A. Doorn and W. Slikker, *Toxicol. Sci.*, **112**, 4 (2009); https://doi.org/10.1093/toxsci/kfp179
2. U.S. Ramjith, B. Roopitha and C.M. Jacob, *Asian J. Pharm. Clin. Res.*, **6**, 228 (2013).
3. O.A. Adesanoye, A.E. Adekunle, O.B. Adewale, A.E. Mbagwu, A.A. Delima, S.A. Adefegha, O.R. Molehin and E.O. Farombi, *Toxicol. Ind. Health*, **32**, 47 (2016); https://doi.org/10.1177/0748233713498436
4. A. Adeyanju, O.R. Molehin, E. Ige, L. Adeleye and O. Omoniyi, *J. Appl. Pharm. Sci.*, **8**, 106 (2018); https://doi.org/10.7324/JAPS.2018.81014
5. D. Krishnaiah, R. Sarbatly and R. Nithyanandam, *Food Bioprod. Process.*, **89**, 217 (2011); https://doi.org/10.1016/j.fbp.2010.04.008
6. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, National Institute of Science Communication, New Delhi, Ed.1, p. 232 (1996).
7. S.L. Bodhankar, S.D. Ambavade, N.A. Mhetre and V.D. Tate, *Indian J. Pharm.*, **38**, 254 (2006); https://doi.org/10.4103/0253-7613.27021
8. S.K. Singh, K.M. Saroj, V.H. Tripathi, A.K. Singh and R.H. Singh, *Int. J. Crude Drug Res.*, **26**, 235 (1988); https://doi.org/10.3109/13880208809053925
9. K.S. Dubey, A.H. Ansari and M. Hardaha, *Asian J. Chem.*, **12**, 577 (2000).
10. A.R. Bafna and S.H. Mishra, *ARS Pharmaceutica*, **45**, 281 (2004).
11. S.C. Garg and H.L. Kasera, *Fitoterapia*, **54**, 37 (1983).
12. R.T. Mahajan, G.S. Chaudhari and M.Z. Chopda, *Environ. Bulletin*, **15**, 61 (1999).
13. F. Sadaf, R. Saleem, M. Ahmed, S.I. Ahmad and Navaid-ul-Zafar, *J. Ethnopharmacol.*, **107**, 161 (2006); https://doi.org/10.1016/j.jep.2006.02.022
14. R.N. Yadava and S. Kumar, *Fitoterapia*, **70**, 127 (1999); https://doi.org/10.1016/S0367-326X(98)00018-5
15. P.P. Pujar, D.D. Sawaikar, S.R. Rojatkar and B.A. Nagasampagi, *Fitoterapia*, **71**, 264 (2000); https://doi.org/10.1016/S0367-326X(99)00157-4
16. S.R. Rojatkar and B.A. Nagasampagi, *Phytochemistry*, **31**, 3270 (1992); https://doi.org/10.1016/0031-9422(92)83492-H
17. V. Lodha, *Indian Perfumer*, **47**, 29 (2003).
18. M.S. Shekhani, P.M. Shah, K.M. Khan and Atta-ur-Rahman, *J. Nat. Prod.*, **54**, 882 (1991); https://doi.org/10.1021/np50075a025
19. S.K. Singh, V.J. Tripathi and R.H. Sing, *Indian Drugs*, **26**, 317 (1988).
20. Ayurvedic Pharmacopoeia of India. Annony, Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy, Ed.: 1, vol. 3, Part 1, New Delhi, p. 21 (1998).
21. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Vitic.*, **16**, 144 (1965).
22. T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identification of Flavonoids. Springer-Verlag: New York, p. 354 (1970).
23. G.S. Kartashova and L.V. Sudos, *Pharm. Chem. J.*, **31**, 488 (1997); https://doi.org/10.1007/BF02464310
24. A. Hagerman, M.I. Harvey and H.P.S. Makker, Quantification of Tannins in Tree Foliage-A Laboratory Manual, FAO/IAEA, Vienna, pp. 4-7 (2000).
25. W. Brand Williams, M.E. Cuvelier and C. Berset, *Lebensm. Wiss. Technol.*, **28**, 25 (1995); https://doi.org/10.1016/S0023-6438(95)80008-5
26. M.S. Blios, *Nature*, **181**, 1199 (1958); https://doi.org/10.1038/1811199a0
27. T.C.P. Dinis, V.M.C. Madeira and L.M. Almeida, *Arch. Biochem. Biophys.*, **315**, 161 (1994); https://doi.org/10.1006/abbi.1994.1485
28. D.C. Garrat, The Quantitative Analysis of Drugs, Chapman & Hall Ltd., vol. 3, pp. 456-848 (1964).
29. M. Nishikimi, N. Appaji Rao and K. Yagi, *Biochem. Biophys. Res. Commun.*, **46**, 849 (1972); https://doi.org/10.1016/S0006-291X(72)80218-3
30. M. Oyaizu, *Jpn. J. Nutr.*, **44**, 307 (1986); https://doi.org/10.5264/eiyogakuzashi.44.307
31. P. Prieto, M. Pineda and M. Aguilar, *Anal. Biochem.*, **269**, 337 (1999); https://doi.org/10.1006/abio.1999.4019
32. I.F.F. Benzie and J.J. Strain, *Anal. Biochem.*, **239**, 70 (1996); https://doi.org/10.1006/abio.1996.0292