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Comparative assessment of antimicrobial and antioxidant activity between whole plant and parts of *Sphaeranthus indicus* Linn. (Asteraceae)

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Abstract

Background: *Sphaeranthus indicus* is an important medicinal plant, which is used to cure various illnesses. The present study is the first investigation of the antimicrobial, antioxidant and phytochemical analysis of *Sphaeranthus indicus* from Chhattisgarh, India.

Methods: The whole plant and plant parts were extracted with polar and non-polar solvents such as water, methanol, ethyl acetate and hexane to assess various bioactivities. The antimicrobial and antioxidant activities were performed by ager well diffusion method and ferrous reducing capacity, respectively; However free radical scavenging activity was analyzed using DMPD and DPPH scavenging assay. The DMPD and DPPH assay were performed in a time dependent manner. Qualitative and quantitative analysis were performed for the total phytochemicals present in the plant extracts. The total content of phenols, flavonoids and terpenoids was analyzed by colorimetric methods.

Results: Ethyl acetate and hexane extract of plant inflorescence and stem exhibited significant antibacterial activity against tested bacterial pathogens. The clinically isolated gram positive pathogenic bacteria responded better as compared to clinically isolated gram negative bacteria as well as pathogenic gram positive and negative bacteria acquired from Microbial Type Culture Collection, India. The leaf and inflorescence exhibited potent antioxidant activity. The polar fraction of leaf methanol extract exhibited the highest reducing power capacity. The aqueous extract of inflorescence exhibited highest inhibition against DMPD and DPPH radicals. The whole plant aqueous extract showed maximum inhibition while aqueous extract of inflorescence exhibited high inhibition among different plant parts. Qualitative phytochemical analysis revealed the presence of terpenoids, phenols, flavonoids, tannins and cardiac glycosides in plant parts. Total terpenoid content was found to be highest in polar fraction of stem methanol extract and ethyl acetate extract of inflorescence. However, total phenol was found to be highest in polar fraction of leaf methanol extract, similarly highest flavonoid content was observed in aqueous extract of leaf.

Conclusion: The results suggest that biological activities of plant parts depend on content of active phytochemicals. The inflorescence could be a potential source of antimicrobial and antioxidant compound. Further, investigation pertaining isolation and characterization of active ingredient may provide an insight regarding its phytochemical activity.

Keywords: *Sphaeranthus indicus* L., Solvent extraction, Antibacterial activity, Antioxidant property

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Background

The plants produce several secondary metabolites during their physiological activities like, alkaloids, poly-phenols, phenols, flavonoids, flavonol, terpenoids, and carotenes etc. These secondary metabolites act as therapeutic and prophylactic agents for human health against several diseases and also help in improving the immunity [1]. Isolation, characterization and synthesis of these bioactive phytochemicals are an emerging area of research [2]. These secondary metabolites are potent source of anticancer [3], antioxidant [4], antiviral [5], anti-inflammatory [6] and antimicrobial [7] agents. The active biomolecules are not only distributed differentially on various parts of plant, but also show variation in different developmental stages of the plant. They also exhibit qualitative and quantitative variations provincially and geographically in their secondary metabolite content and are therefore worth investigating in various regions [8, 9].

Sphaeranthus indicus L. is a medicinal plant widely used in Indian traditional system of medicine to cure various illnesses [10]. Geographically, it is distributed throughout India, Sri Lanka, Africa and Australia. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India and survives up to 1200 m altitude [11]. The herb *S. indicus* is much branched, strongly scented, and erect with branched tapering roots [12]. It is used to treat fever, epilepsy, mental illness, hemicranias, jaundice, hepatopathy, gastropathy, hernia, diabetes, pectoralgia, cough, hemorrhoids, leprosy, helminthiasis, dyspepsia and skin diseases [10]. Several scientific reports are available on *S. indicus* exhibiting hypotensive, peripheral vasodilatory, cathartic [13], antimicrobial [14], nematocidal [15], larvicidal [16, 17], anti-inflammatory [18], immunomodulatory [19], anxiolytic [20], neuroleptic [21, 22], antioxidant [23, 24], antihyperglycemic [25, 26], analgesic, antipyretic [27], mast cell stabilizing action [28], renoprotective [29], hepatoprotective [24, 30], antiviral [25, 31], macrofilaricidal [32], sedative [22], bronchodilatory [33] and antihyperlipidemic activities [34].

In the present investigation efforts were made to evaluate antimicrobial and antioxidant properties of *S. indicus* of Chhattisgarh region and to assess the levels of such bioactive compounds *vis-á-vis* other reports from elsewhere. Chhattisgarh has rich biodiversity of medicinal plants. It is hypothesized that the percentage of bioactive compounds may be higher in the plants of Chhattisgarh region and may fluctuate according to geographic locations [35–38].

Materials and methods

Plant material

Sphaeranthus indicus was collected during flowering stage (January to mid February) from Raipur, Chhattisgarh. The plant was identified by a plant taxonomist, currently a consultant at National Center for Natural Resources (NCNR), Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India. The voucher specimen was deposited in herbarium of NCNR (No: 99777). They were washed with distilled water, sterilized with 0.1% mercuric chloride for 5 min, rinsed with water, wiped and air dried under shade condition at room temperature until getting constant weight. The dried parts viz., whole plant, leaf, inflorescence, stem and root were grinded by electric blender to make fine powder.

Preparation of extract

Extraction was carried out keeping in mind the nature of plant. It is an aromatic plant, so cold extraction was carried out as described earlier [39]. Dried powder of inflorescence, leaf, stem and root were sequentially macerated in 1:10 ratio with hexane, ethyl acetate and methanol for 48 h at room temperature. The solvent was evaporated in an oven at 40 °C. Methanol extract was further fractioned with chloroform and water in a separating funnel to isolate polar compounds from non-polar compounds. Non polar fraction primarily had chlorophyll and was not used for further analysis. The yield of dried extract was determined. A 10% stock solution of extracts was made in appropriate solvent after solubility standardization. Hexane extract was dissolved in 50% hexane with ethyl acetate; similarly ethyl acetate extract was dissolved in 30% DMSO with ethyl acetate also, while polar fraction of methanol extract was dissolved in 40% DMSO with methanol.

Antibacterial activity test

Bacterial cultures

The bacterial cultures for antibacterial assay were procured from IMTECH Chandigarh They included gram positive bacteria viz., *Bacillus cereus* MTCC-430, *Bacillus subtilis* MTCC-441, *Staphylococcus aureus* MTCC-96, *Staphylococcus epidermidis* MTCC-435, and gram negative bacteria, like, *Escherichia coli* MTCC-1687, *Klebsiella pneumoniae* MTCC-3384, *Pseudomonas aeruginosa*mtcc-741 and *Proteus vulgaris* MTCC-744. Their corresponding clinical isolates were obtained from Department of Microbiology, Pt. Jawaharlal Nehru Memorial Medical College, Raipur (Chhattisgarh), India.

Antibacterial activity

Antibacterial activity was carried out by agar well diffusion method [40] with some modification. Briefly, the 6 h old bacterial cultures were adjusted to 0.08 OD to achieve 1×10^8 CFU/ ml of bacteria at 620 nm using UV-VIS spectrophotometer (Model 108, Systronics, India). The inoculum was streaked on sterile Mueller-Hinton agar petri plates using sterile cotton swab. The plate was left for 10–15 min to allow excess surface

moisture to be absorbed. The well was bored using 6 mm cork borer. 50 μl (5 mg/ 50 μl) extract was poured into the test well. Streptomycin (10 μg/ ml) was used as positive control and the solvent as negative control. Petri plates, in triplicate, were incubated at 37 °C for 18 h. The antibacterial activity was recorded as zone of inhibition of bacterial growth around the well. The activity index of all the plant parts and their extracts in different solvent was determined using streptomycin as a standard. The activity index was expressed as zone of inhibition of test sample/ zone of inhibition with streptomycin.

In-vitro antioxidant activity assay

Reducing power assay

Antioxidant activity was carried out by Ferric reducing power assay as described earlier [41]. 1 ml of methanol polar extract of whole plant, leaf, inflorescence, stem and root (200 μg ml^{-1}) was mixed with 2.5 ml phosphate buffer (0.2 M; pH 6.6) and 2.5 ml 1% $K_3[Fe(CN)_6]$ and the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml 10% TCA was added and the reaction mixture was allowed to cool to room temperature. 2.5 ml of above mixture was mixed with 2.5 ml deionized water (Milli Q, Merk) and 0.5 ml of 0.1% $FeCl_3$. The absorbance was recorded at 700 nm against blank using a spectrophotometer. Ascorbic acid (30–70 μg ml^{-1}) was used as standard. The assay was performed in triplicate and the mean values were calculated. Results were expressed as μg ascorbic acid equivalent (AAE)/ ml plant extract.

DMPD cation free radical scavenging assay

The radical scavenging assay was carried out using DMPD free radical scavenging assay as described earlier [42]. Briefly, the DMPD free radical was generated by adding 0.1 ml of DMPD aqueous solution (100 mM) in 0.05 ml of aqueous $K_2S_2O_8$ (0.4 mM) and the final volume was made to 10 ml with sodium acetate buffer (pH 5.6). The solution was incubated in dark for 5 h before use, till it reaches 0.7–0.8 O.D. at 517.4 nm. The scavenging effect of extracts was measured by addition of 50–200 μl of extract into 3.5 ml DMPD radical and incubated for different time period. The radical scavenging activity was expressed as a percentage of DMPD discoloration using the equation:

$$DMPD.radical\ scavenging\ (\%) = [(Acontrol - Asample)/Acontrol] \times 100$$

Where A_{sample} is the absorption of extract/ reference compound, and $A_{control}$ is the absorbance of the DMPD·solution without addition of extract. Ascorbic acid was used as the reference compound in 40–80 μg/ ml concentration range. The assay was performed in triplicate and the mean values were calculated. Results were expressed as μg ascorbic acid equivalent (AAE)/ ml plant extract. The IC_{50} was calculated using regression analysis. This assay is end point measurement assay and radical absorbance increase after inhibition. Thus assay was carried out at 1 min time interval to check reaction completion time and the kinetics was also done.

DPPH scavenging assay

The DPPH scavenging assay was performed as described earlier [43]. Briefly, the 50 μM DPPH solution was prepared in methanol. The scavenging effect of extracts was measured by addition of 50–200 μl of extracts into 3 ml DPPH solution and incubated for different time period. The radical scavenging activity was expressed as a percentage of DPPH discoloration using the equation:

$$DPPH\ scavenging\ (\%) = [(Acontrol - Asample)/Acontrol] \times 100$$

Where A_{sample} is the absorption of extract/ reference compound, and $A_{control}$ is the absorbance of the DPPH solution without addition of extract. Ascorbic acid was used as the reference compound in 40–80 μg/ ml concentration range. The assay was performed in triplicate and the mean values were calculated. Results were expressed as μg ascorbic acid equivalent (AAE)/ ml plant extract. The IC_{50} was calculated using regression analysis. Assay was carried out at 1 min time interval to check reaction completion time and the kinetics was done.

Phytochemical analysis

Qualitative analysis of various phytoconstituents was performed as described earlier by Harborne (1998).

Total terpenoid assay

Total terpenoid assay was performed as described earlier [44] with some modification. 200 μl extract was taken as unknown sample. Linalool (40 to 100 mg) was used to prepare a standard curve. 1.5 ml chloroform was added to each tube, mixed thoroughly and allowed to rest for 3 min. 100 μl concentrated H_2SO_4 was added to each tube and further incubated for 10 min. The terpenoids settled down as dark brown precipitate. The supernatant was carefully decanted and the precipitate was dissolvedin 1.5 ml methanol. The Absorbance was recorded at 538 nm in a spectrophotometer against a methanol. The assay was performed in triplicate and concentration was expressed as equivalent to mg linalool/ g of extract.

Total phenol assay

Total phenolic contents of the extracts were analyzed using the Folin–Ciocalteu reagent as described earlier

[45] using gallic acid as standard. 0.1 ml of polar fraction of methanol extracts (2.5%) was mixed with 0.2 ml of two fold diluted Folin–Ciocalteu reagent. The mixture was incubated for 3 min at room temperature and 0.6 ml aqueous solution of 2% Na_2CO_3 was added. The total reaction mixture was made upto 6 ml with distilled water and further incubated in boiling water for 1 min. The tubes were cooled for 10 min and absorbance was read at 760 nm against blank. Gallic acid was used to prepare standard curve in different concentration (20–60 μg/ml). The assay was performed in triplicates. The concentration of phenol was expressed as mg gallic acid equivalent (GAE)/ g of extract.

Total flavonoid assay

The total flavonoid contents were determined as described earlier [46]. Briefly, 1 ml of extracts was mixed with 4 ml of deionized water and 0.3 ml of 5% (w/v) $NaNO_2$. The mixture was incubated for 5 min and 0.3 ml of (10% w/v) $AlCl_3$ was added. After 6 min, 2 ml of 1 M NaOH was added. Approximately, 2.4 ml of deionized water was immediately added to obtain a final volume of 10 ml. The mixture was shaken vigorously and the absorbance of the mixture was determined at 510 nm by using a UV–visible spectrophotometer. Quercitin was used to prepare a standard solution with different concentrations (20–100 μg/ml). Results were indicated as mg quercitin equivalents (QE)/ g on a dry weight of extract.

Statistical analysis

The results were analyzed by one way ANOVA. Tukey's test was used to identify significant differences among the mean (SPSS Statistics 20.0, IBM, Armonk, New York, USA). Difference among means at 5% level ($p < 0.05$) was considered statistically significant.

Results

Plant extracts

Among the different plant parts extracted through different solvents, the highest yield was recovered from aqueous extract of leaf (9.25%), while the lowest yield was recorded from root hexane extract (0.4%). The percentage yield is shown in Table 1.

Antibacterial activity

The antibacterial activity of extracts of plant parts was tested against MTCC and clinical isolates. Inflorescence and stem extracts inhibited maximum numbers of MTCC strains and clinically isolated bacteria than whole plant, leaf and root extracts. The antimicrobial activity against Gram positive and Gram negative pathogenic bacteria is summarized in Tables 2-3. Whole plant hexane extract exhibited potent activity against clinical *S. aureus*, *B. subtilis*, and *E. coli*, while moderate activity against *B. cereus* MTCC 430, *E. coli* MTCC 1687, *K. pneumonia* MTCC 3384, *P. aeuginosa* MTCC 741, *P. vulgaris* MTCC 744. Ethyl acetate extract inhibited all the clinical pathogens, while inhibited a limited numbers of MTCC bacterial strains. The high activity was demonstrated against clinical *S. epidermidis*, *S. aureus*, and *B. cereus*. The polar fraction of methanol had least activity against MTCC and clinical *B. cereus* and *P. aeruginosa*. The leaf hexane extract demonstrated good activity against *K. pneumonia* MTCC 3384, clinical *B. cereus* and *S. aureus.* Ethyl acetate extract and methanol polar fraction of leaf inhibited a limited numbers of bacterial isolates. Inflorescence hexane extract had highest activity against clinical *S. epidermidis* (31.33 mm) and lowest against clinical *E. coli* (9.83 mm); ethyl acetate extract inhibited all the bacterial pathogens with high to moderate activity. It was highest against clinical *S. epidermidis* (29.67 mm) and lowest against clinical *P. aeruginosa* (9.17 mm), while polar fraction of methanol exhibited least activity against a few numbers of clinical isolates. Stem hexane extract inhibited most of the pathogenic bacteria while ethyl acetate and polar fraction of methanol inhibited some of the bacterial strains. The polar fraction of methanol showed highest activity against clinical *S. aureus* (25.67 mm) and lowest against clinical *P. aeruginosa* (9.83 mm). Root extracts inhibited limited numbers of bacterial isolates with low activity. The antibacterial activity of the plant extracts was more prominent against clinical bacterial isolates *vis-à-vis* MTCC isolates. Aqueous extract of whole plant and their parts showed no activity against any of the tested organisms.

The activity index of extracts are shown in Fig.1 (a & b). Highest index was recorded in inflorescence hexane

Table 1 The yield of different part of *Sphaeranthus indicus* extracted in various solvents

Extracts	Whole plant	Leaf	Inflorescence	Stem	Root
Hexane	1.75%	3.94%	2.20%	0.45%	0.40%
Ethyl acetate	1.05%	4.55%	6.20%	1.20%	0.60%
Methanol-polar fraction	06.45%	9.25%	5.35%	3.65%	6.05%
Aqueous	10.23%	13.55%	9.66%	7.23%	10.11%

Table 2 The antibacterial activity (zone of inhibition in mm) of different parts of *Sphaeranthus indicus* extracted with various solvents against MTCC cultures

Plant part	Extracts	Bc-430	Bs-441	Sa-96	Se-435	Ec-1687	Kp-3384	Pa-741	Pv- 744
Whole plant	Hexane	16.83 ± 0.44a	–	–	–	12.92 ± 0.19c	12.71 ± 0.12c	13.08 ± 0.19c	14.75 ± 0.22b
	Ethyl acetate	8.67 ± 0.28d, e	8.83 ± 0.21d,e	–	–	7.87 ± 0.21e	9.08 ± 0.08d,e	8.25 ± 0.28d,e	9.41 ± 0.96d
	Methanol polar fraction	8.25 ± 0.47	–	–	–	–	–	8.75 ± 0.48	–
	Aqueous	–	–	–	–	–	–	–	–
Leaf	Hexane	–	–	–	–	–	20.83 ±0.24[a]	–	–
	Ethyl acetate	–	–	–	–	8.67 ±0.14[b]	–	7.67 ±0.14[c]	–
	Methanol polar fraction	–	–	–	–	9.17 ±0.11b	–	–	9.08 ±0.34[b]
	Aqueous	–	–	–	–	–	–	–	–
Inflorescence	Hexane	12.17 ±0.17[c,d]	–	–	–	–	–	–	–
	Ethyl acetate	12.50 ±0.72[b,c]	14.17 ±0.60[a]	10.50 ±0.34[e]	13.67 ±0.21[a,b]	11.00 ±0.26[d,e]	10.83 ±0.40[d,e]	10.83 ±0.40[d,e]	12.17 ±0.40[c,d]
	Methanol polar fraction	–	–	–	–	–	–	–	–
	Aqueous	–	–	–	–	–	–	–	–
Stem	Hexane	8.33 ± 0.21[c,d,e]	12.00 ± 0.36[a]	8.50 ± 0.22[b,c,d]	9.33 ± 0.33[b]	7.83 ± 0.33[d,e]	ND	7.50 ± 0.22[e]	7.50 ± 0.22
	Ethyl acetate	9.00 ± 0.25[b,c]	8.83 ± 0.17[b,c]	–	8.33 ± 0.17[b,c]	–	–	8.33 ± 0.17[b,c]	–
	Methanol polar fraction	–	–	–	–	–	–	–	–
	Aqueous	–	–	–	–	–	–	–	–
Root	Hexane	8.83 ± 0.40[a]	–	–	9.50 ± 0.43[a]	–	8.33 ± 0.42[b]	–	–
	Ethyl acetate	–	–	–	8.33 ± 1.21[b]	–	–	9.33 ± 0.81[a]	–
	Methanol polar fraction	–	–	–	–	–	–	–	–
	Aqueous	–	–	–	–	–	–	–	–
Streptomycin		10.00 ±0.13	10.54 ±0.70	10.93 ±0.92	11.19 ±1.00	12.58 ±1.54	12.06 ±1.03	12.43 ±0.94	11.25 ±1.16

Bc- *B. cereus*, Bs- *B. subtilis*, Sa- *S. aureus*, Se - *S. epidermidis*, Ec – *E. coli*, Kp - *K. pneumonia*, Pa- *P. aeruginosa*, Pv – *P. vulgaris*

The values are expressed as Mean ± SE ($n = 3$). The different superscript letters denote the significant difference within row at $p < 0.05$ (Tukey's test)

Table 3 The antibacterial activity (zone of inhibition in mm) of different parts of *Sphaeranthus indicus* extracted with various solvents against clinical isolates

Plant part	Extracts	Bc	Bs	Sa	Se	Ec	Kp	Pa	Pv
Whole plant	Hexane	12.25 ± 0.13e	22.75 ± 0.28b	23.33 ± 0.14b	–	19.08 ± 0.14d	12.33 ± 0.22e	–	–
	Ethyl acetate	20.58 ± 0.15c	7.33 ± 0.14i	23.08 ± 0.15b	24.67 ± 0.14a	9.75 ± 0.13f	7.75 ± 0.13 h,i	9.58 ± 0.15f	10.17 ± 0.11f
	Methanol polar fraction	8.67 ± 0.28 g	–	–	–	–	–	8.33 ± 0.22 g, h	–
	Aqueous	–	–	–	–	–	–	–	–
Leaf	Hexane	16.92 ±0.24^{c}	–	24.83 ±0.36^{a}	–	–	–	–	–
	Ethyl acetate	17.08 ±0.31^{c}	–	16.33 ±0.38^{c}	18.67 ±0.14^{b}	–	–	–	–
	Methanol polar fraction	–	–	10.41 ±0.15^{d}	8.08 ±0.29^{e}	–	–	–	–
	Aqueous								
Inflorescence	Hexane	27.17 ±0.54^{b}	16.17 ±0.17^{b}	27.17 ±0.17^{b}	31.33 ±0.21^{a}	9.83 ±0.40^{f,g}	–	14.17 ±0.17^{d}	
	Ethyl acetate	26.33 ±0.33^{b}	13.17 ±0.40^{d,e}	26.17 ±0.48^{b}	29.67 ±0.42^{a}	12.17 ±0.17^{e}	10.17 ±0.17^{f,g}	9.17 ±0.17^{g}	14.67 ±0.33^{c,d}
	Methanol polar fraction	11.50 ±1.05^{e,f}	–	11.50 ± 0.50^{e,f}	14.50 ±0.43^{c,d}	–	–	–	–
	Aqueous								
Stem	Hexane	–	7.67 ± 0.21^{d,e}	7.50 ± 0.22^{e}	12.33 ± 0.88^{b}	9.33 ± 0.21^{c,d}	9.33 ± 0.21^{c,d}	13.17 ± 0.79^{b}	7.50 ± 0.22^{e}
	Ethyl acetate	9.50 ± 0.22^{c}	–	13.17 ± 0.17^{b}	9.67 ± 0.21^{c}	8.67 ± 0.21^{c,d,e}	9.17 ± 0.31^{c,d,e}	–	–
	Methanol polar fraction	–	–	25.67 ±1.21^{a}	12.50 ±0.55^{b}	–	–	9.83 ±0.31^{c}	10.17 ±0.40^{c}
	Aqueous								
Root	Hexane	7.67 ± 0.52^{b,c}	–	9.00 ± 0.83^{b,c}	–	7.83 ± 0.41^{b,c}	–	–	–
	Ethyl acetate	–	–	–	–	–	19.50 ± 1.64^{a}	–	–
	Methanol polar fraction	–	–	–	18.17 ± 1.33^{a}	–	–	9.83 ± 0.75^{b}	6.67 ± 5.24^{c}
	Aqueous								
Streptomycin		13.50 ±1.17	11.45 ±0.80	10.25 ±0.25	12.25 ±0.25	10.06 ±0.30	11.25 ±0.28	17.25 ±0.25	18.50 ±0.28

Bc- *B. cereus*, Bs- *B. subtilis*, Sa- *S. aureus*, Se - *S. epidermidis*, Ec – *E. coli*, Kp - *K. pneumonia*, Pa- *P. aeruginosa*, Pv – *P. vulgaris*

The values are expressed as Mean ± SE ($n = 3$). The different superscript letters denote the significant difference within row at $p < 0.05$ (Tukey's test)

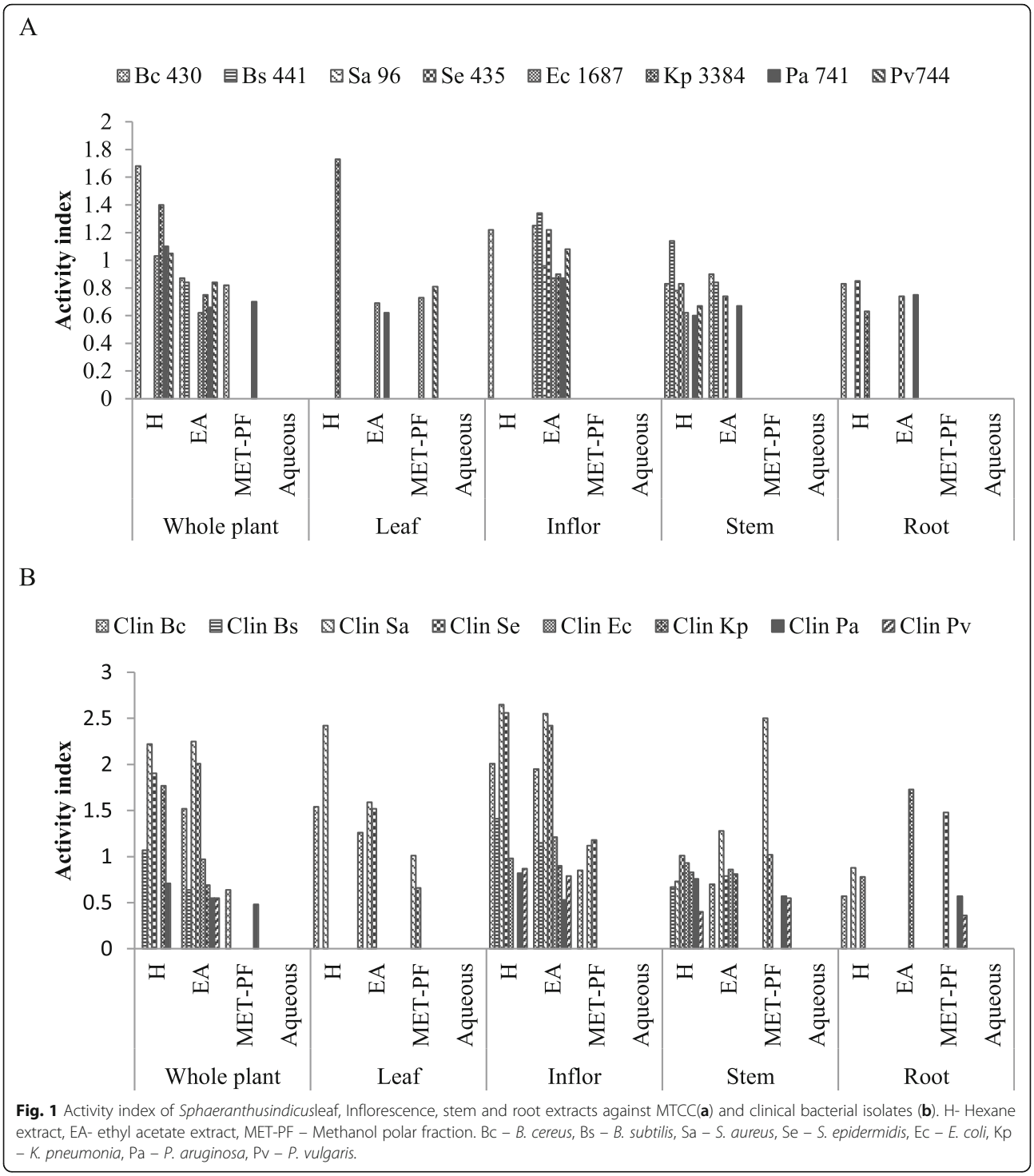

Fig. 1 Activity index of *Sphaeranthusindicus*leaf, Inflorescence, stem and root extracts against MTCC(**a**) and clinical bacterial isolates (**b**). H- Hexane extract, EA- ethyl acetate extract, MET-PF – Methanol polar fraction. Bc – *B. cereus*, Bs – *B. subtilis*, Sa – *S. aureus*, Se – *S. epidermidis*, Ec – *E. coli*, Kp – *K. pneumonia*, Pa – *P. aruginosa*, Pv – *P. vulgaris*.

extract against clinically isolated Gram-positive *S. aureus* (2.65) and leaf hexane extract against MTCC *K. pneumonia* (1.73). The lowest index was noticed in methanol polar fraction of root against clinical *P. vulgaris* (0.36).

Antioxidant activity

The reducing power was measured in polar fraction of methanol extracts and aqueous extracts due to the limitation of reaction with hydrophilic phyto-constituents. The highest reducing ability was observed in aqueous

Table 4 Antioxidant activity of different parts of their extracts of *S. indicus*

Parts	Extracts	Reducing power (μg AAE/ ml of extract)	DMPD (IC50 in μg AAE/ ml of extract)	DPPH (IC50 in μg AAE/ ml of extract)
Whole plant	Hexane	–	–	17.97 ± 0.18^{i}
	Ethyl acetate	–	–	$21.57 \pm 0.06^{g,h}$
	Methanol-polar fraction	52.87 ± 2.51^{e}	$23.62 \pm 0.58^{b,c}$	$35.40 \pm 0.02^{b,c}$
	Aqueous	49 ± 0.11^{e}	$19.24 \pm 0.26^{e,f}$	38.12 ± 2.13^{a}
Leaf	Hexane	–	–	22.37 ± 0.37^{g}
	Ethyl acetate	–	–	26.66 ± 0.04^{f}
	Methanol-polar fraction	60.33 ± 0.07^{d}	28.29 ± 0.05^{a}	$37.69 \pm 0.27^{a,b}$
	Aqueous	328.93 ± 0.24^{a}	25.53 ± 0.24^{b}	$35.82 \pm 0.05^{a,b,c}$
Inflorescence	Hexane	–	–	18.20 ± 0.07^{i}
	Ethyl acetate	–	–	31.26 ± 0.08^{e}
	Methanol-polar fraction	43.4 ± 0.23^{f}	28.65 ± 0.19^{a}	$34.61 \pm 0.08^{c,d}$
	Aqueous	109.53 ± 0.18^{c}	29.69 ± 0.19^{a}	$35.91 \pm 0.31^{a,b,c}$
Stem	Hexane	–	–	$19.68 \pm 0.03^{h,i}$
	Ethyl acetate	–	–	$35.33 \pm 0.08^{b,c}$
	Methanol-polar fraction	36.73 ± 0.07^{g}	$22.49 \pm 0.20^{c,d}$	31.29 ± 0.07^{e}
	Aqueous	60.6 ± 1.62^{d}	$20.82 \pm 0.43^{d,e}$	$32.40 \pm 0.06^{d,e}$
Root	Hexane	–	–	9.47 ± 0.08^{j}
	Ethyl acetate	–	–	17.44 ± 0.24^{i}
	Methanol-polar fraction	36.27 ± 0.07^{g}	$24.09 \pm 0.76^{b,c}$	26.54 ± 0.08^{f}
	Aqueous	134.47 ± 0.18^{b}	17.87 ± 0.40^{f}	26.79 ± 0.06^{f}

The results are mean ± SE ($n = 3$). The different superscript letters denote the significant at $p < 0.05$ (Tukey's test)

extract of leaf (328.93 μg/ml), while polar fraction of root methanol extract (36.27 μg/ml) possessed lowest activity as shown in Table 4.

The DMPD scavenging capacity was also measured in polar fraction of methanol extracts and aqueous extracts for the same reason as in reducing power assay, while DPPH scavenging activity was carried out for all the extracts of *S. indicus* because of solubility of organic solvent and aqueous extracts in methanol. The scavenging activity of *S. indicus* was determined over time against DMPD and DPPH radicals at 1 min time interval to understand the behavior of active compounds (Figs. 2 & 3). The inhibitions with respect to time were different according to parts, extracts and phytochemical composition of *S. indicus* at the lowest volume of extracts.

When polar fraction of methanol extracted whole plant, leaf, inflorescence, stem and root vis-a-vis their aqueous extracts were used to inhibit DMPD radical, the completion of inhibition varied according to the parts and the extraction (Fig. 2 a & b). The polar fraction of methanol extract showed the inhibition of 20.28% with the whole plant extract. Among the parts highest inhibition was achieved by inflorescence (42.01%) followed by root, stem and leaf. The whole plant aqueous extract showed 16% inhibition in 29 min. However, among the parts inflorescence (44.87%) inhibited DMPD radical efficiently in 25 min. The leaf aqueous extract also exhibited impressive inhibitory activity followed by stem and root aqueous extracts.

IC_{50} values for DMPD radical scavenging capacity (Table 4) revealed that the aqueous extract of inflorescence possessed higher scavenging capacity (29.69 μg/ml) against DMPD radical, while aqueous extract of root exhibited comparatively weak scavenging capacity (17.87 μg/ml).

Inhibition of DPPH radical also varied in different parts and their extracts (Fig: 3). The whole plant extracts showed 27.55% - 87.25% inhibition. The leaf extracts inhibited the radical in the range of 34.41% - 81.81%. The inhibition with inflorescence extracts was recorded between 31.93% – 85.76%. The root extracts exhibited the inhibition potential between 14.94% – 64.07%. The aqueous extract of these parts inhibited DPPH radical more efficiently as compared from other extracts. Stem extracts showed 26.47% – 72.27% inhibition. The ethyl acetate extract possessed more scavenging potential.

The highest DPPH scavenging ability was recorded for polar fraction of leaf methanol extract (37.69 μg/ml) while lowest was recorded for root hexane extract (9.47 μg/ml). Thus all the parts and their extracts possessed antioxidant activity. The polar extracts of *S. indicus* exhibited more antioxidant activity.

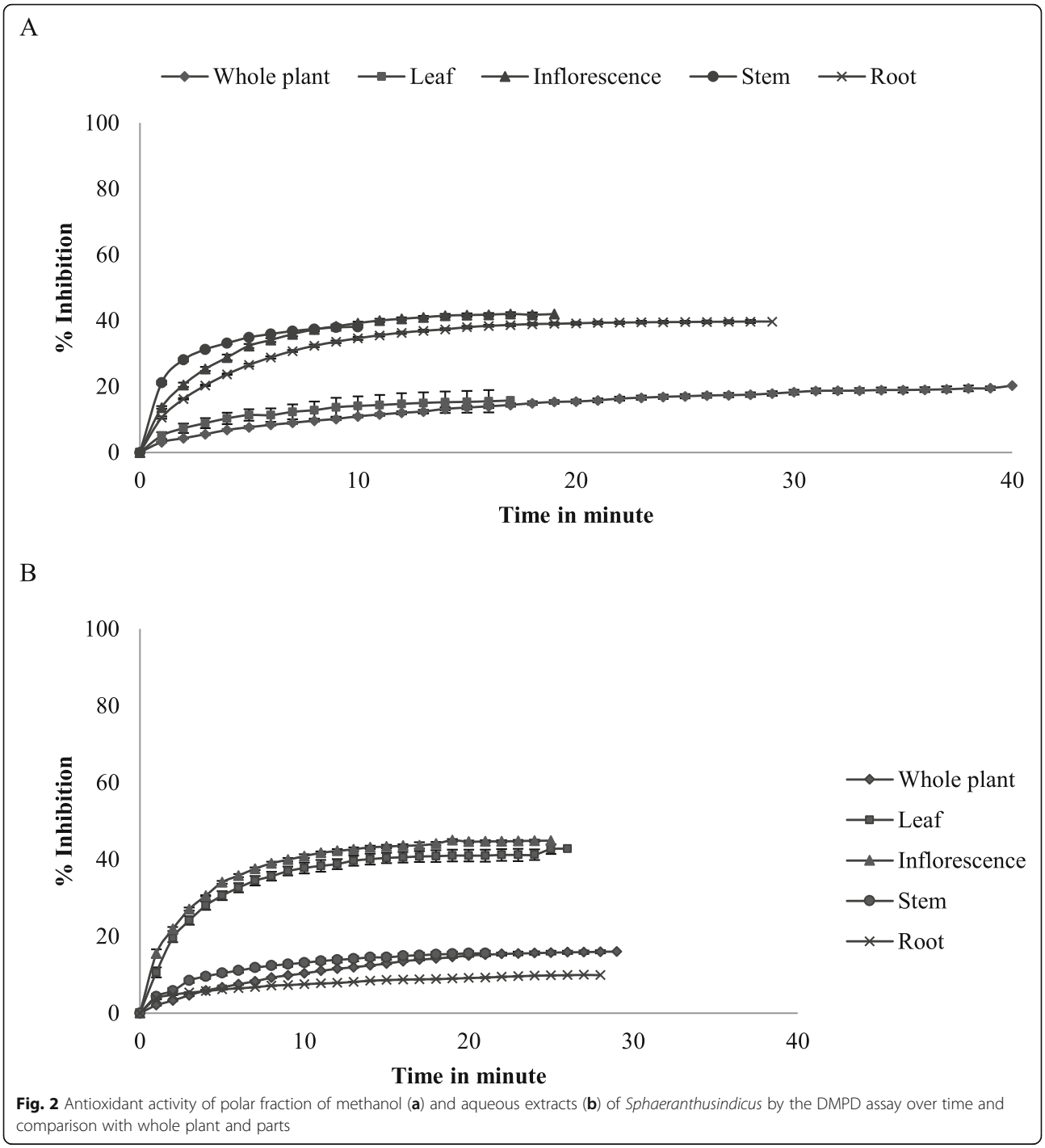

Fig. 2 Antioxidant activity of polar fraction of methanol (**a**) and aqueous extracts (**b**) of *Sphaeranthusindicus* by the DMPD assay over time and comparison with whole plant and parts

Preliminary phytochemical analysis

The phytochemical screening was done based on antibacterial activity (Table 5). Total Terpenoid was detected in all the extracts. Phenol, flavonoids, tannin and cardiac glycosides were detected in methanol polar fraction of all the parts. Alkaloid were absent in the extracts.

Quantitative estimation of phytochemicals

The total terpenoid content of *S. indicus* extract was determined using sulfuric acid and expressed as mg Linalool equivalent (LE)/ g dry extract. The difference between plant parts and their extracts was statistically significant ($p < 0.05$, Table 6). The terpenoids were highest in stem methanol polar fraction (551.48 mg LE/ g dry

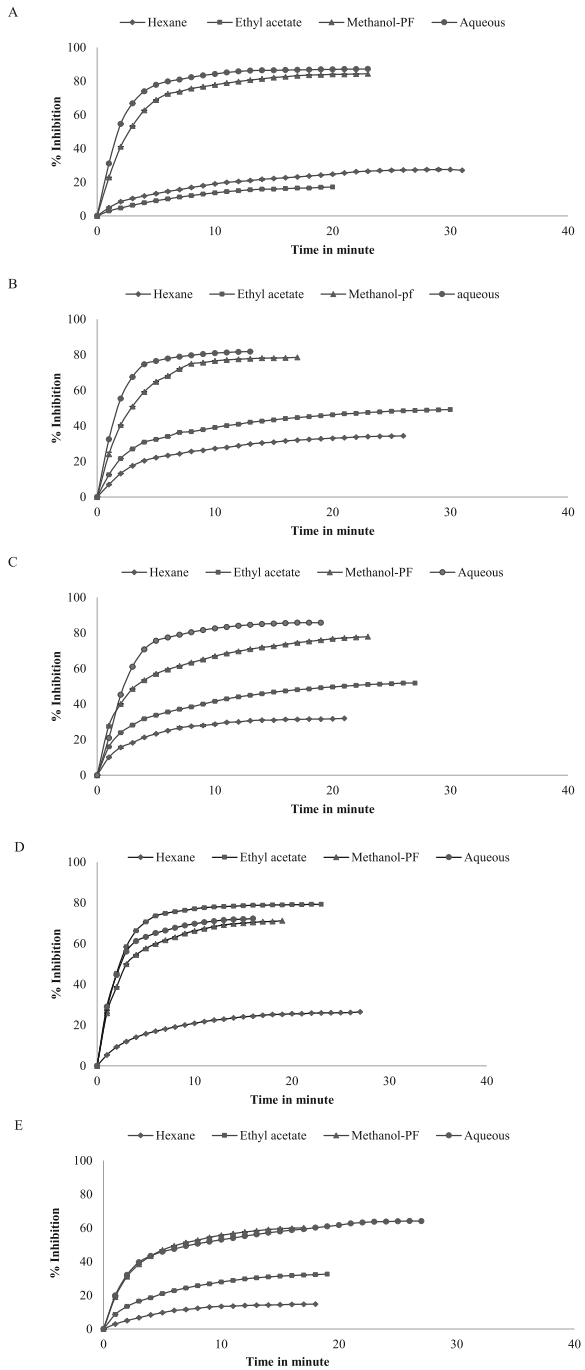

Fig. 3 Antioxidant activity of **a** whole plant, **b** leaf, **c** inflorescence, **d** stem and **e** root extract of *Sphaeranthusindicus*using DPPH assay over timeand comparison between parts and extracts

weight) and lowest in root hexane extract (551.48 mg LE/ g dry weight). Difference in total phenolic content among whole plant and parts of polar fraction of methanol extract was statistically significant ($p < 0.05$). Polar fraction of leaf methanol extract showed highest phenolic content followed by stem, inflorescence and root. However, aqueous extract of leaf contained high amount of phenolics. The flavonoid content of *S. indicus* was statistically significant ($p < 0.05$). The flavonoids were more abundant in leaf aqueous extract (273.82 mg QE/ g of extract) and less in polar fraction of root methanol extracts (62.04 mg QE/ g of extract).

Discussion

The whole plant and various parts of *Sphaeranthusindicusviz,* leaf, inflorescence, stem and root were used for phytochemical extraction, antimicrobial and antioxidant activity. The four solvent systems hexane, ethyl acetate, methanol and water were used in increasing order of polarity. The non-polar material was removed from methanol extract and polar fraction was used. The highest yield was recovered from leaf and lowest from root when extracted via hexane, as well as highest yield of ethyl acetate extract was from inflorescence and lowest was from root. However, highest yield was recovered from methanol-polar fraction which was maximum in leaf and minimum in stem. The highest yield of aqueous extract was recovered from leaf and lowest from root. The yield of leaf methanol extract was higher than finding of [47] which was 5.28%, extracted for 48 h in shaking condition. The antimicrobial activity of whole plant, leaf, inflorescence, stem and root extracts of *S. indicus* was assessed in the present paper. Except aqueous extract, all the extracts inhibited gram positive and gram negative pathogenic bacteria in differential manner. Hexane and ethyl acetate extract of whole plant possessed moderate to weak antimicrobial activity against most of the pathogens, while leaf & root extracts from these solvent and methanol polar fraction of the whole plant and all parts exhibited low potentiality while hexane extract of stem inhibited most of the bacteria except *K. pneumonia* MTCC 3384 and clinical *B. cereus*. Ethyl acetate extract of inflorescence of *S. indicus* inhibited all the bacterial pathogens.

The clinical isolates were more susceptible than MTCC pathogenic bacteria. The clinical Gram-positive pathogenic bacteria isolates were more susceptible than Gram- negative one. The presence of lipopolysaccharide in outer membrane on gram negative bacteria inhibits or limits the drugs access [48–50]. However, hexane extracts of stem and ethyl acetate extract of inflorescence were able to inhibit growth of all clinically isolated gram negative bacteria, although with weak activity. The extraction of bioactive compound(s) from natural sources depends on solvent system [51]. It might be polar or non-polar. These studies revealed that bioactive constituents of *S. indicus* were more accessible in organic solvents.

Comparable results were also reported for antimicrobial activity of *Sphaeranthus indicus.* The hexane,

Table 5 Phytochemical analysis of different parts of *Sphaeranthusindicus*extracted in various solvents

Phytochemical	Whole plant				Leaf				Inflorescence				Stem				Root			
Alkaloid	H	EA	Met	Aq	H	EA	Met	Aq	H	EA	Met	Aq	H	EA	Met	Aq	H	EA	Met	Aq
Mayer's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hager's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Wagner's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tannic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Marquis test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Phenol																				
Ferric chloride test	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
Lead acetate test	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
Gelatin test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Flavonoids																				
Alkaline reagent test	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
Lead acetate test	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
Terpenoids																				
Salkowski test	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	–
Tannin																				
Ferric chloride test	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
Gelatin test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycoside																				
Killer killiyani test	+	+	+	–	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
Salkowski test	+	+	+	–	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–

H- Hexane, EA – Ethyl acetate, M-PF – Methanol polar fraction, Aq- Aqueous

benzene, chloroform, ethyl acetate, petroleum ether and methanol extracts of *S. indicus* were effective against pathogenic bacteria [47, 52–54]. In this study, we have also found the low activity of polar fraction of methanol extract and no activity of aqueous extracts, which is in agreement with the result of Tambekar & Khante [55], while in another study it showed good activity against various pathogens [56, 57].

Another study on antimicrobial activity of plant crude extract against mentioned bacteria has been reported with respect to different parts. The methanol extract of root *Rheum ribes* inhibited *E. coli*, *K. pneumonia*, *Proteus*, *P. aeruginosa* and *N. gonorrhoeae* significantly more than leaves and stalk. The antimicrobial activity of stalk was weak [58]. Rahman et al [59] reported the antimicrobial activity of *Achyranthusferruginea* chloroform extract against different Gram positive and Gram negative bacteria including *B. subtilis*, *S. aureus*, *E. coli*, *Klebsiella species* and *P. aeruginosa*. The extract showed moderate activity against these bacteria.In another study, methanol extract of *Leucus aspera* root exhibited highest antibacterial activity than leaf, flower and stem against tested bacteria [60]. The ethyl acetate and ethanol extract of *Lawsonia inemis* L. fruit, flower and leaf have shown higher antibacterial activity than dichloromethane extract against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. Additionally, the ethyl acetate extract of flower and fruits was more effective than leaf extract against all tested bacteria [61]. In the present work, result of antibacterial activity of ethyl acetate extract of *S. indicus* inflorescence against clinical *S. aureus* was similar to the result of ethyl acetate extract of *L. inemis* flower.

This study also aimed to determine antioxidant activity of the *S. indicus* using ferric reducing power capacity, including DMPD & DPPH assay. The reducing power assay was based on reduction potential of antioxidants. The antioxidant reacts with potassium ferricynide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which further reacts with ferric chloride to form ferric ferrous complex and form green to Persian-blue colour that has an absorption maximum of 700 nm. The aqueous extracts of leaf exhibited highest capacity to reduce ferric to ferrous.

The antioxidant activity was measured using DMPD and DPPH assay to study the scavenging property of plant extract. It was based on reduction of absorbance which corresponds to the activity. The DMPD assay work on the theory that oxidation potential of radical cation of this amine is compatible with the reduction potential of the alkoxyl and peroxyl radicals. DMPD is

Table 6 Total terpenoids, total phenols and total flavonoids in different parts and extracts of *Sphaeranthus indicus*

Parts	Extract	Total terpenoids (mg linalool equivalent/ 1 g dry weight)	Total phenol (mg GAE/ 1 g of dry weight)	Total flavonoid (mg QE/ 1 g of dry weight)
Whole plant	Hexane	80.94 ± 0.53	0.00	0.00
	Ethyl acetate	165.30 ± 0.40	0.00	0.00
	Polar fraction of methanol	278.80 ± 0.20	18.23 ± 0.16^{e}	164.10 ± 0.52^{e}
	Aqueous	0.00	19.30 ± 0.004^{e}	195.45 ± 0.57^{d}
Leaf	Hexane	$176.25 \pm 2.40^{d,e}$	0.00	0.00
	Ethylacetate	196.49 ± 1.32^{d}	0.00	0.00
	Polar fraction of methanol	276.23 ± 14.83^{c}	43.12 ± 1.41^{b}	238.93 ± 0.47^{b}
	Aqueous	0.00	58.54 ± 0.07^{a}	273.82 ± 3.53^{a}
Inflorescence	Hexane	124.77 ± 0.34^{e}	0.00	0.00
	Ethylacetate	387.97 ± 0.46^{b}	0.00	0.00
	Polar fraction of methanol	120.13 ± 2.98^{e}	10.65 ± 0.06^{f}	208.83 ± 0.47^{c}
	Aqueous	0.00	30.40 ± 0.03^{c}	239.34 ± 0.46^{b}
Stem	Hexane	31.27 ± 0.11^{f}	0.00	0.00
	Ethylacetate	48.20 ± 0.19^{f}	0.00	0.00
	Polar fraction of methanol	551.48 ± 41.41^{a}	22.35 ± 0.23^{d}	134.61 ± 0.47^{f}
	Aqueous	0.00	22.79 ± 0.02^{d}	165.53 ± 0.46^{e}
Root	Hexane	11.47 ± 0.03^{f}	0.00	0.00
	Ethylacetate	39.43 ± 0.10^{f}	0.00	0.00
	Polar fraction of methanol	$179.38 \pm 7.67^{d,e}$	7.90 ± 0.03^{g}	62.04 ± 0.47^{h}
	Aqueous	0.00	6.34 ± 0.004^{g}	92.46 ± 0.47^{g}

The results are mean ± SE ($n = 3$). The different superscript letters denote the significant at $p < 0.05$ (Tukey's test)

based on decolourization assay; DMPD at an acidic pH forms a purple coloured radical cation by reacting with potassium persulphate (an oxidizing agent) and show a maximum absorbance at 517.4 nm. Antioxidants that are able to transfer a hydrogen atom to DMPD radical cation, scavenge the colour of the solution proportionate to their amount. The reduction of DMPD radical cation is rapid and shows a stable end point. Since after reaching at stable end point, the absorbance of DMPD radical again increases which misleads the measurement, hence required time profiling. Both the methanol and aqueous inflorescence extract scavenged more DMPD radical in less time when compared with other parts of the plant.

The antioxidant with hydrogen donating potential has the ability to scavenge DPPH radical. After reacting with antioxidant, purple coloured DPPH radical converts into yellow, non radical reduced form, viz, diphenylpicrylhydrazine [62]. DPPH scavenging effect of extracts of different parts of *S.indicus* was assessed at 1 min interval for accurate measurement. In the parts, aqueous extract of inflorescence scavenged DPPH radical in minimum time and much better than others. The results are in agreement with DPPH scavenging and reducing power activity of *Allium sativum* bulb, where aqueous extract showed more activity than methanol extract [63]. The different behavior of the parts and extracts showed inconsistency due to the different mechanism, molecular structure, and numbers of active groups and hydro/ lipophilicity of the antioxidants [64].

The reaction was completed with steady state against both the radicals and formed a plateau. This mechanism can be understood by the equation proposed by Mishra et al [65]:

$$[R^{\cdot}] + [AH] \leftrightarrow R\text{–}H + [A^{\cdot}] \quad (1)$$

$$[R^{\cdot}][A^{\cdot}] \rightarrow R\text{–}A \quad (2)$$

$$[A^{\cdot}] + [A^{\cdot}] \rightarrow A\text{–}A \quad (3)$$

The equation elucidates transfer of hydrogen ions from antioxidants to scavenge the radicals in faster rate (Eq. 1), which leads to conversion of antioxidants in radical form [$A^{\cdot}$] (Eq. 2), this further reacted with available radicals forming radical-antioxidant complex. At this

point, there was a slow reaction leading to steady state condition. The remaining [A] reacted with themselves and ultimately the reaction was stopped (Eq. 3). The DMPD and DPPH scavenging assays come under hydrogen atom transfer (HAT) ability of antioxidant based methods. These are kinetic based assays and involve a competitive reaction scheme in which antioxidant and substrate compete for radicals as described in above equation. Whereas reducing power and Folin-Ciocalteu assay come under electron transfer (ET) based methods which measure the reduction potential of antioxidant through changes in colour. These assays use chromogenic redox reagent. The present study revealed that all the extracts possessed both the mechanism. HAT ability was highest in aqueous extract of inflorescence while ET ability was more in leaf aqueous extract.

Comparable results have been reported on antioxidant activity of *S. indicus*. The ethanol hot extract of root exhibited highest radical scavenging property against ABTS followed by DPPH and nitric oxide radical at the same concentration while exhibited moderate iron chelating capacity [23]. In another study, methanol extract of root and leaf had highest DPPH radical scavenging activity; hydroxyl radical scavenging capacity was highest in root and stem extracts; nitric oxide scavenging activity was highest in stem and leaf extracts and superoxide radical scavenging activity was highest in fruit and stem extracts [66].

Similarly, other authors have compared the antioxidant activity between extracts of different parts. The methanol extract of *Leucus aspera* root have significant DPPH scavenging capacity than flower, leaves and stem [60]. The methanolic extract of *Morus alba* L. stem bark [67] and *Tabebuia pallida* leaf [68] exhibited highest total antioxidant activity, reducing power capacity, DPPH radical scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibition activity than other parts.

Terpenoids, cardiac glycosides, tannins, phenols, flavonoids were observed in *S. indicus* in the present study corroborated with earlier reports [19, 54]. Quantitatively total terpenoids were highest in polar fraction of stem methanol extract and ethyl acetate extract of inflorescence. Total phenols and flavonoids were highest in aqueous extract of leaf and inflorescence. Since the phenol and flavonoid possessed good antioxidants and antimicrobial property and had less side effects [69, 70] therefore, it was relevant to assess the contents of these metabolites.

The methanol has been reported as suitable solvent for extracting phenol and flavonoid [71]. Although aqueous extract of all parts of *S. indicus* contained more phenol and flavonoids than polar fraction of methanol in the present case, they were unable to inhibit pathogens and did not exhibit antimicrobial potential, whereas the antioxidant activity was fairly high. However, the methanol polar fraction exhibited both antimicrobial as well as antioxidant ability. The activity of phenols and flavonoids depends upon their structure which is referred as structure activity relationship (SAR) [72]. Since the hydroxyl group play a key role in antioxidant activity, more hydroxylation in the phenyl ring of phenol exhibit more antioxidant activity [73]. The terpenoids from all parts of *S. indicus* showed both antimicrobial and antioxidant activities which signify the importance of terpenoids in drug discovery. The magnitude of the activity may differ due to the different structure and active group present in the compound [62].

The study revealed that the selection of plant parts and solvents are an important factor for isolation of bioactive compounds. The study further revealed that leaf, inflorescence and stem of *S. indicus* were the potent source of antimicrobial and antioxidant activity and can be exploited in herbal drug research.

Conclusion

The results suggest that *S. indicus* contain potent bioactive chemicals which have ability to inhibit bacterial pathogens as well as to scavenge free radicals. Among the parts and their different extracts, ethyl acetate extract of inflorescence and hexane extract of stem contain potent antimicrobial compounds. The whole plant extracts showed better activity than parts against some bacteria. This may be due to the synergistic effect between phytochemicals. The polar part of leaf methanol extract and inflorescence aqueous extract has strong antioxidant activity than other parts as they contain more phenol and flavonoids which are known as potent antioxidants. The phenol and flavonoids are also known for their antimicrobial activity but the polar fraction of methanol extracts and aqueous extracts of parts which contain both the chemicals had no significant antimicrobial activity. Although the terpenoid containing inflorescence ethyl acetate extract and stem hexane extract possessed the activities. So these parts and their corresponding extracts could be a potential source of antimicrobial and antioxidant agents. Therefore, further research is needed for the isolation and identification of individual bioactive compound(s) from these parts and their extracts.

Abbreviations

$K_3[Fe(CN)_6]$: Potassium ferricyanide; TCA: Trichloroacetic acid; $FeCl_3$: Ferric chloride; DMPD: N,N Dimethyl-p-phenylenediamine oxalate; $K_2S_2O_8$: Potassium persulfate; DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; H_2SO_4: Sulfuric acid; Na_2CO_3: Sodium carbonate; $NaNO_2$: Sodium nitrite; $AlCl_3$: Aluminum chloride; NaOH: Sodium hydroxide

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Authors' contributions
Dhananjay Tandon contributed to the work, prepared and submitted the manuscript. A. K. Gupta reviewed and gave critical analysis of the manuscript. Both authors read and approved the final manuscript.

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References

1. Kang S, Min H. Ginseng, the 'Immunity Boost': The Effects of *Panaxginsengon* immune System. J Ginseng Res. 2012;36:354–68.
2. Mohammadi A, Nazari H, Imani S, Amrollahi H. Antifungal activities and chemical composition of some medicinal plants. J MycoMed. 2014;24:e1–8.
3. Cragg GM, Newman DJ. Plants as source of anti-cancer agents. J Ethnopharmacol. 2005;100:72–9.
4. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnol. Mol. Biol. Rev. 2007;1(4):97–104.
5. Orhan DD, Özçelik B, Özgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res. 2010;165:496–504.
6. Bazylko A, Granica S, Filipek A, Piwowarski J, Stefańska J, Osińska E, Kiss AK. Comparison of antioxidant, anti-inflammatory, antimicrobial activity and chemical composition of aqueous and hydroethanolic extracts of the herb of Tropaeolummajus L. Ind Crops Prod. 2013;50:88–94.
7. Saranraj P, Sivasakthi S. Medicinal Plants and its Antimicrobial Properties: A Review. Global J Pharmacology. 2014;8(3):316–27.
8. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. Food ChemToxicol. 2008;46:409–20.
9. Capasso A. Antioxidant action and therapeutic efficacy of *Allium sativum* L. Molecules. 2013;18:690–700.
10. Kirtikar KR, Basu BD. ICS. Indian medicinal plants. In: Blatter E, Caius JF, Mhaskar KS, editors. vol. 2. 2nd ed. Allahabad India: Lalit Mohan Basu; 1981. p. 1346–8.
11. Chatterjee A, Pakrashi SC. The treatise on Indian medicinal plants 1st ed. Vol5. NISCAIR, New Delhi; 2003. p. 177.
12. Vikani KV, Dangar RD, Kapadia NS. Shah MB.A pharmacognostic study on *Sphaeranthus indicus*. J Nat Rem. 2008;8:61–7.
13. Srivastav SC, Khan MS, Vohra SB. Pharmacological and haemostatic investigation on *Sphaeranthus indicus* Linn. Indian J PhysiolPharmacol. 1971; 15:27–33.
14. Shaikh D, Naqui BS, Shaikh R. The antimicrobial principles of *Sphaeranthus indicus*: Isolation, purification and antimicrobial action. Pak J Scientific Ind Res. 1986;29:366–71.
15. Sharma M, Saxena RC. *Sphaeranthus indicus* as mosquito larvicide. J ApplZool Res. 1996;7:87–8.
16. Hameed SVS, Shah DS. Effect of aqueous extracts of *S. indicus* against *Calex fatigans*, weid (Diptera; calicidae). J Exp Zool. 2003;6:279–84.
17. Tiwari A, Saxena RC. Repellent and feeding deterant activity of *Sphaeranthusindicus*agaistTriboliumcastanum. Bioresearch Bull. 2003;1:179–284.
18. Jain A, Basal E. Inhibition of *Propionibacterium acnes* induced mediators of inflammation by Indian herbs. Phytomedicine. 2003;10:34–8.
19. Bafna AR, Mishra SH. Immunomodulatory activity of methanol extract of flower heads of *Sphaeranthusindicus*Linn. ArsPharmaceutica. 2004;45:281–91.
20. Ambavade SD. MhetreNA, Tate VD, Bodhankar SL. Pharmacological evaluation of the extracts of *Sphaeranthusindicus*flowers on anxiolytic activity in mice. Indian J Pharmacol. 2006;38:254–9.
21. Mhetre NA, Ambavade SD, Bodhankar SL. Neuroleptic activity of extract of *Sphaeranthus indicus* in mice.Indian. J Nat Prod. 2006;22:24–7.
22. Galani VJ, Patel BG. Psychotropic activity of *Sphaeranthusindicus*Linn.in experimental animals. Pharmacognosy Resources. 2009;1:307–13.
23. Shirwaikar A, Prabhu KS, Punitha IS. In vitro antioxidant studies of *Sphaeranthus indicus* (Linn). Indian J Exp Bio. 2006;4:993–6.
24. Bk T, Khosa RL. Hepatoprotective and antioxidant effects of *Sphaeranthus indicus* against acetaminophen induced hepatotoxicity in rats. J PharmaSci Res. 2009;1:26–30.
25. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity. Indian J Exp Biol. 1968;6:232–47.
26. Prabhu KS, Lobo R, Shirwaikar A. Antidiabetic properties of the alcoholic extract of *Sphaeranthus indicus* in streptozotocin nicotinamide diabetic rats. J Pharm Pharmacol. 2008;60:909–16.
27. Nanda BK, Jena J, Rath B, Behera BR. Analgesic and Antipyretic activity of whole parts of *Sphaeranthus indicus*Linn. J ChemPharma Res. 2009;1:207–12.
28. Mathew JE, Srinivasan KK, Dinakaran V, Joseph A. Mast cell stabilizing effects of *Sphaeranthus indicus*. J Ethnopharmacol. 2009;122:394–6.
29. Srinivasan VM, Jessy KK, Anand Alex EM. Effect of *Sphaeranthus indicus* Linn on gentamicin induced acute renal failure in rats. Indian J Pharmacol. 2008; 40:71–2.
30. Nayak SS, Maity TK, Maiti BC. Hepatoprotective activity of *Sphaeranthus indicus*Linn. Int J Green Pharm. 2007;1:32–6.
31. Vimalanathan S, Ignacimuthu S, Hudson JB. Medicinal plants of Tamil Nadu (Southern India) are a rich source of antiviral activities. Pharma Biol. 2009;47: 422–229.
32. Nisha M, Kalyanasundaram M, Paily KP, Abidha VP, Balaraman K. *In vitro* screening of medicinal plant extracts for macrofilaricidal activity. Parasitol Res. 2007;100:575–9.
33. Sarpate RV, Deore TK, Tupkari SV. Bronchodilatory effect of *Sphaeranthus indicus* Linn against allergen induced bronchospasm in guinea pigs. Pharmacog Mag. 2009;5:74–7.
34. Pande VV, Dubey S. Antihyperlipidemic activity of *Sphaeranthus indicus* on atherogenic diet induced hyperlipidemia in rats. Int J Green Pharm. 2009;3: 159–61.
35. Vuuren SFV. ViljoenAM, Őzek T, Demirci B, Başer KHC.Seasonal and geographical variation of Heteropyxisnatalensis essential oil and the effect thereof on the antimicrobial activity. South African Journal of Botany. 2007; 73:441–8.
36. Alimuddin S, Hemlata R, Patel NM. Evaluation of antimicrobial activity of stem bark of *Ficus bengalensis* Linn. collected from different geographical regions. Pharmacognosy J. 2010;2(7):178–80.
37. Rahimmalek M, Mirzakhani M, Pirbalouti AG. Essential oil variation among 21 wild myrtle (*Myrtus communis* L.) populations collected from different geographical regions in Iran. Ind Crop Prod. 2013;51:328–33.
38. Maurya S, Kushwaha AK, Singh S, Singh G. An overview on antioxidative potential of honey from different flora and geographical origins. Indian J Nat Prod Resour. 2014;5(1):9–19.
39. Harborne JB. *Phytochemical* method: A guide to modern techniques of plant analysis. 3rd ed. New Delhi: Springer; 1998.
40. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; Approved Standard 11thed; M02-A11. 2012;32(01):9-13.
41. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutrition. 1986;44:307–15.
42. Asghar MN, Khan IU, Arshad MN, Sherin L. Evaluation of antioxidant activity using an improved DMPD radical cation decolorization assay. Acta Chim. Slov. 2007;54:295–300.
43. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol. 1995;28:25–30.
44. Ghorai N, Chakraborty S, Gucchait S, Saha SK, Biswas S. Estimation of total terpenoids concentration in plant tissues using a monoterpenes, Linoalool as standard reagent. Nature Protoc Exch. 2012. https://doi.org/10.1038/protex.2012.055.

45. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16:144–58.
46. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effect on superoxide radicals. Food Chem. 1999;64:555–9.
47. Thakur R, Singh R, Jain N. Evaluation of antibacterial activity of *Sphaeranthus indicus* L. leaves. J Pharm Res. 2012;5:4382–8.
48. Hancock REW, Speert DP. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. Drug Resist Updat. 2000;3:247–55.
49. Poole K. Mechanisms of bacterial biocide and antibiotic resistance. J Appl Microbiol. 2002;92:55S–64S.
50. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nature Reviews. 2015;13:42–51.
51. Kalidindi N, Thimmaiah NV, Jagadeesh NV, Nandeep R, Swetha S, Kalidindi B. Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn. leaves. J Food Drug Anal. 2015;23(4):795–802.
52. Duraipandiyan V. Kannan P. Ignacimuthu S (2009) Antimicrobial activity of *Sphaeranthus indicus* L. ethanobotanical Leaflets. 2009;13:320–32.
53. Zachariah SM, Pappachen LK, Aneesh TP, Alex L, Sumith G, John MS, Praseetha MC. Nagalekshmi R (2010) Phytochemical and pharmaceutical screening of *Sphaeranthus indicus* Linn for antimicrobial activity. Int J Pharm Sci Res. 2010;1:169–73.
54. Kavitha KS, Satish S. In vitro antioxidant and antimicrobial activities of *Sphaeranthus indicus* L. Journal of Biologically Active Products from Nature. 2015;5:163–71.
55. Tambekar DH, Khante BS. Antibacterial evaluation of medicinal plants used by korkus in melghat forest against gastrointestinal infections. Int J Pharmaceutical Sci Res. 2011;2(3):577–83.
56. Doss A, Doss PA, Dhanabalan R. In-Vitro antimicrobial activity of extracts of *Passifloraedulis*(Passilfloraceae) and *Sphaeranthus indicus* (Asteraceae). Ethnobotanical leaflets. 2008;12:728–33.
57. Malik F, Mirza T, Riaz H, Hameed A, Hussain S. Biological screening of seventeen medicinal plants used in the traditional systems of medicine in Pakistan for antimicrobial activities. Afr J Pharm Pharmaco. 2010;4:335–40.
58. Bazzaz BSF, Khajehkaramadin M, Shokooheizadeh HR. In Vitro antibacterial activity of *Rheum ribes*extract obtained from various plant parts against clinical isolates of gram negative pathogens. Iran J Pharm Res. 2005;2:87–91.
59. Rahman MM, Alam AHMK, Sadik G, Islam MR, Khondkar P, Hossain MA, Rashid M. Antimicrobial and cytotoxic activities of *Achyranthes ferruginea*. Fitoterapia. 2007:260–2.
60. Chew AL, Jessica JJA, Sasidharan S. Antioxidant and antibacterial activity of different part of *Leucus aspera*. Asian Pac J Trop Biomed. 2012:176–80.
61. Jeyaseelan EC, Jenothiny S, Pathmanathan MK, Jeyadevan JP. Antibacterial activity of sequentially extracted organic solvent extract of fruits, flowers and leave of *Lawsonia inermis* L. from Jaffna. Asian Pac J Trop Biomed. 2012;2:798–802.
62. Demirci B, Koşar M, Demirci F. Dinç, BaşarKHC. Antimicrobial and antioxidant activities of the essential oil of *Chaerophyllum libanoticum* Boiss.*et* Kotschy. Food Chem. 2007;105:1512–7.
63. Meriga M, Mopuri R, Muralikrishna T. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. Asian Pac J Trop Med. 2012;5(5):391–5.
64. Bruni R, Medici A, Andreotti E, Fantin C, Muzzoli M, Dehesa M, Romagnoli C, Sacchetti G. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocoteaquixos* (Lam.) Kosterm. (Lauraceae) flower calices. Food Chem. 2004;85:415–21.
65. Mishra K, Ojha H, Chaudhary NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chem. 2012;130:1036–43.
66. Krishna MT, Thota SP, Jadhav M, Kumar K, Venuganti A, Devi M, Vadiari S, Mittapelli G. Studies on in vitro antioxidant and antimicrobial activities of *Sphaeranthus indicus* (Linn). Int J Pharmaceutical Res Biomedical Analysis. 2013;2:01–9.
67. Khan MA, Rahman AA, Islam S, Khandokhar P, Parvin S, Islam MB, Hossain M, Rashid M, Sadik G, Nasrin S, Mollah MNH, Alam AHMK. A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morusalba* L. (Moraceae). BMC Res Notes. 2013;6:24.
68. Rahman MM, Islam MB, Biswas M, Alam AHMK. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallid* growing in Bangladesh. BMC Res Notes. 2015;8:621.
69. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1999;2(4):152–9.
70. Proestos C, Boziaris IS, Nychas GJE, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006;95:664–71.
71. Akgul C, Saglikoglu G. Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemis tinctoria*. Indian J BiochemBiophys. 2005;42:395–7.
72. Saravanan S, Parimelazhagan T. In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis* Juss. fruit pulp. Food Sci Human Wellness. 2014;3:56–64.
73. Kurćubić VS, Mašković PZ, Vujić JM, Vranić DV, Vesković-Moračanin SM, Okanović DG, Lilić SV. Antioxidant and antimicrobial activity of *Kitaibelia vitifolia* extract as alternative to the added nitrite in fermented dry sausage. Meat Sci. 2014;97:459–67.

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