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Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya

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The present study was carried out to determine the phytochemical constituents, physicochemical values and antioxidant activity of six traditionally used medicinal plants from Sikkim Himalaya according to the standard pharmacopoeial method. Antioxidant activity was determined in methanolic extracts by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ferrous chelating assay and ferric ion reducing assay for methanol extracts, which showed that methanolic extracts of all six plants particularly at higher concentration exhibited better antioxidant property. The phytochemical investigation revealed the presence of various secondary metabolites such as flavonoids, tannins, phenols, terpenoids, phlobatannins, saponins, glycosides and good content of four major phytochemicals (Phenol, flavonoid, flavonol and tannin), which could account for the high antioxidant activity. The results suggest the potential of selected medicinal plants as a source of new agents for the treatment of diseases related to oxidative stresses.

Keywords: Antioxidant activity, Reactive oxygen species, Secondary metabolites, Sikkim.

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Introduction

From the ancient times, people are using medicinal plants for treating their common ailments. Nowadays uses of herbal plants are growing all over the world for the treatment of various diseases, due to their potential antioxidant activities. It is well known that oxidative stress produces the number of reactive oxygen species (ROS)/free radicals, which play an important role in the pathogenesis of various diseases including inflammatory diseases. Oxidative stress is produced when there is an imbalance between the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the amount of cellular antioxidants that can lead to multiple reactions causing damage or death of cells[1]. Due to an imbalance between body's antioxidant mechanisms and oxidative stress production, results in the development of chronic disease as autoimmunity like Rheumatoid Arthritis (RA), cancer, etc.[2-4]. Antioxidants are the compounds of nature which either prevent the production or obstruct any that are produced and prevent the progression of chain reaction produced by various ROS[5]. In the case of active RA, it is found that oxidative stress increased while the level of endogenous antioxidants decreased[6]. Antioxidants constitute an important mechanism to block the action of free radicals which are implicated in the pathogenesis of many diseases and in the ageing process[7,8]. Antioxidants are our first line of defence against free radical damages and play an important role in maintaining optimum health and well-being[9]. Most of the antioxidant compounds are derived from plant sources belonging to various classes of secondary metabolites with a variety of physical and chemical properties[10]. Although our body has effective defence mechanisms that protect itself from the harmful oxidative reaction, the ability slowly reduces with ageing, creating a need for the constant supply of antioxidants in our daily food supplements. This has led to increased interest among the researchers globally to find and evaluate the plants having effective antioxidants in order to treat the diseases related to oxidative stress like RA. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay is one of the widely used methods to test the activity of compounds to act as free radical scavengers and to evaluate antioxidant activity. This test has been the most accepted model for evaluating the free radical scavenging activity of the new drug[11]. Many free radicals can be of ferrous iron (Fe^{2+}) due to its ability to transfer single electrons, starting

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relatively with non-reactive radicals[12]. Therefore, an iron chelating activity may be an effective method to evaluate the antioxidant activity of the plant extracts. Iron-related complications are reduced by chelation therapy and thereby it improves the overall quality of life[13]. In the present study, six medicinal plants viz. *Betula alnoides* Buch.-Ham.ex D.Don, *Equisetum diffusum* D. Don, *Litsea cubeba* (Lour.) Pers., *Quercus lamellosa* Sm., *Stephania glabra* (Roxb.) Miers and *Zingiber zerumbet* (L.) Sm. were selected since these plants were found to be used by the local healers in Sikkim Himalayan region for the treatment of Rheumatoid arthritis. These plants were used in local traditional system of medicine in Sikkim Himalaya from ancient period of time for curing their various ailments. *B. alnoides* is used to treat inflammation, dislocation of bones, postnatal pain and bleeding, wounds, joint pains, sprains, diabetes, indigestion, strength nourishing, fatigues, antimalaise and rheumatic ailments[14,15]. *E. diffusum* is traditionally used for the treatment of bone fracture, indigestion, sprain, bone dislocation, liver problems, mouth sores, kidney stone, chest complaints and dogbite[16,17]. *L. cubeba* is used to treat diabetes, stomachache, headache, dizziness, hysteria, memory loss, diarrhea, astringent and toothache[18-20]. *Q. lamellosa* is used as an astringent[21]. *S. g*labra is used to treat pain and swelling, psycho-disorders, tuberculosis, asthma, diabetes, dysentery, fever, intestinal troubles, cancer, sleep disturbances, malaria, pneumonia and typhoid[22-24]. *Z. zerumbet* is used against cough, stomachache, asthma, leprosy, skin diseases, diabetes, stomach troubles, fever, swelling, sores and loss of appetite[25,26]. Many authors have documented ethnomedicinal plants of Sikkim Himalaya based on local traditional knowledge and practices[21, 27-33]. However, very few studies have been conducted to scientifically validate the local healing system. Therefore, the objectives of the present study are to determine physicochemical analysis to study the phytochemical constituents (qualitatively as well as quantitatively) and to investigate the antioxidant activity of methanol extracts in order to support the knowledge of local healers about the use of these plants as an anti-arthritic agent.

Materials and Methods

Plant collection

Plant samples were collected from different regions of Sikkim Himalaya during the year 2016-2017. The collected specimens were identified and authenticated from Botanical Survey of India, Eastern Himalayan Circle (Sikkim). Herbarium of each plant was also prepared and deposited in the Department of Botany, Sikkim University. The plant specimens with family, local name, parts used, collection locality and voucher specimen number in brackets were: *Betula alnoides* Buch.-Ham. Ex D.Don (Betulaceae, *Saur*, bark, Rabongla-South Sikkim and BSHC/0207, SU/0096); *Equisetum diffusum* D.Don (Equisetaceae, *Salibesali*, whole plant, Tadong-East Sikkim and BSHC/0209, SU/0094); *Litsea cubeba* (Lours.) Pers. (Lauraceae, *Siltimur*, fruits, 9th Mile-East Sikkim and BSHC/0197, SU/0100); *Quercus lamellosa* Sm. (Fagaceae, *Bajranth*, bark, Soreng-West Sikkim and BSHC/0097, SU/0097); *Stephania glabra* (Roxb.) Miers (Menispermaceae, *Tamarkey*, tuber, Soreng-West Sikkim and BSHC/0216, SU/0098) and *Zingiber zerumbet* (L.) Sm. (Zingiberaceae, *Phachang*, rhizome, Namli-East Sikkim BSHC/0218, SU/0099). The selected plant samples were washed, cleaned and chopped into small pieces and dried at 40°C in a thermostatically controlled oven until they attained a constant weight. The samples were then crushed into fine powder, using the mechanical grinding machine.

Extraction

10 g powder of each selected plant was extracted in 100 mL of methanol by using Soxhlet apparatus for 24 hours at a temperature of 30 ^{o}C. After 24 hours, the obtained extracts were filtered and concentrated under vacuum using rotatory evaporator (Heidolph, Schwabach, Germany), the samples were further dried in vacuum desiccators. After complete dryness of extracts, the yield value was calculated and kept in the refrigerator at 4 ^{o}C until further study.

Physico-chemical analysis

The physicochemical analysis was done according to the standard method for the determination of extractive value, total ash content, acid insoluble ash, water-soluble ash and loss on drying percentage[34].

Phytochemical analysis

Qualitative phytochemical screening of selected medicinal plants was done according to standard procedures[35-37], in order to determine the presence or absence of various phytochemical constituents like alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, saponins, phlobatannins, anthraquinones, carbohydrates, glycosides and proteins.

Quantitative phytochemical estimation

The total phenolic content (TPC) was measured following the standard method with slight modification[38]. About 1 mL methanol extract of plant sample was mixed with 5mL of 10 times diluted Folin-Ciocalteu reagent and 4 mL of 7.5 % sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature and the absorbance was measured at 760 nm using UV spectrophotometer. A standard curve was prepared using gallic acid by serial dilution from 10-100 µg/ mL. Results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

The total flavonoid content (TFC) was measured by a modified colourimetric method with some modification[38]. 5 mL of methanol extract was mixed with 0.3 mL of 5 % sodium nitrite for 5 minutes in a test tube. Then 0.3 mL of 10 % aluminium chloride was added; after 6 minutes, 2 mL of sodium hydroxide was added to stop the reaction and mixture was further diluted with distilled water up to 10 mL. The absorbance was immediately measured at 510 nm using UV spectrophotometer. A standard curve was prepared using rutin concentration ranging from 10-100 µg/ mL. The total content of flavonoids was expressed as milligrams of rutin equivalent per gram of dry extract (mg of RtE/g).

The total flavonols content was measured by the standard method with some modification[39]. Exaclty 2 mL of 2 % aluminium trichloride and 3 mL sodium acetate solution were added in 2 mL of methanol extract. The solutions were kept for 2.5 hours at 20 ºC and the absorbance was measured at 440 nm using UV spectrophotometer. Results were mentioned as milligrams of rutin equivalent per gram of dry extract (mg RtE/g).

Tannin content was measured by following the standard method with some modification[40]. 0.1 mL of each extract was taken in a test tube containing 7.5 mL distilled water followed by the addition of 0.5 mL Folin-Ciocalteu reagent and 1 mL of 35 % Na_2CO_3 and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. Absorbance was measured at 725 nm using a UV spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/G).

Evaluation of antioxidant activities

DPPH free radical scavenging assay

The free radical scavenging activities of methanolic extract of selected medicinal plants were determined on the basis of their scavenging activity of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical as per the standard method with slight modifications[41]. About 1 mL of each solution of different concentrations (100, 200, 300, 400 and 500 µg/ mL) of methanol extract was added to 3 mL of 0.04 % methanolic DPPH free radical solution. After 30 minutes the absorbance of the solution was taken at 517 nm by using a UV spectrophotometer which was compared with the absorbance of standard ascorbic acid concentrations (100-500 µg/ mL). Then the % inhibition was measured by using the following formula:

$$\% \text{ inhibition } = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{(\text{Absorbance of blank})} \text{ x } 100$$

From the calibration curves obtained from different concentrations of extract, the IC_{50} (inhibitory concentration 50 %) was determined. IC_{50} value denotes the concentration of plant sample that inhibits the DPPH free radical by 50 %[42].

Ferrous chelating assay

The chelating activity of the extract for ferrous ions Fe^{2+} was measured according to the standard method with slight modifications[43]. To 0.4 mL of extract of different concentrations (200, 400, 600, 800 and 1000 µg/mL), 1.6 mL of methanol was diluted and mixed with 0.04 mL of $FeCl_3$ (2 mM) followed by addition of 0.8 mL of ferrozine (5 mM). The mixture was shaken well and incubated at room temperature for 10 minutes. The absorbance of the mixture was measured at 562 nm against a blank. The EDTA was used as positive control. The ability of chelating activity was calculated using the above formula. From the calibration curve obtained, the IC_{50} (µg/mL) value was determined.

Ferric ion reducing assay

The reducing activity of selected medicinal plants for ferric ion Fe^{3+} was measured according to the standard method with slight modifications[44]. To 1 mL of different concentrations of samples (200, 400, 600, 800, 1000 µg/mL) was taken and then mixed with 2.5 mL of PBS solution (pH 6.6) and 2.5 mL of potassium ferricyanide solution, followed by incubation at 50ºC in water bath for 20 minutes, the reaction was stopped by addition of 2.5 mL of 10 % trichloroacetic acid and allowed to stand at room temperature for 10 minutes. The upper portion of solution 2.5 mL was taken, added with the same volume of distilled water and 0.5 mL of 0.1 % $FeCl_3$ and left for

20 minutes at room temperature and absorbance was measured at 700 nm. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used for standard control.

Statistical analysis

All the measurements were made in triplicate and the results obtained were expressed as the mean±standard deviation (SD). To calculate the IC_{50} values, linear regression analysis was carried out using Microsoft Office Excel 2008 (Microsoft Corporation, USA). The IC_{50} values were compared by paired t-tests, $p < 0.05$ was considered as significant and $p > 0.05$ was considered as non-significant.

Results

Physico-chemical analysis

The physicochemical parameters of the selected medicinal plants including total ash content, acid insoluble ash, water-soluble ash, loss on drying and their extractive value in methanol are presented in Table 1.

Phytochemical analysis

The preliminary phytochemical screening was carried out on the methanolic extract of selected medicinal plants that revealed the presence of a range of phytoconstituents (viz. flavonoids, carbohydrates, terpenoids, phenols, tannins, saponins, anthraquinones, alkaloids, glycosides, protein and phlobatannins) are presented in Table 2.

Quantitative phytochemical estimation

On the basis of results obtained from the preliminary phytochemical test, quantitative estimation was carried out for some major phytoconstituents such as phenol, flavonoid, flavonol and tannin. A comparative study of total phenol content was expressed as mg gallic acid equivalent (GAE) per gram dry extract weight is presented in Fig. 1. Among the samples analyzed, methanolic extract of *L. cubeba* contained a maximum (62.6±0.056 mg GAE/g dry extract wt.) and methanolic extract of *E. diffusum* contained the minimum (17.4±0.060 mg GAE/g dry extract wt.) phenolics.

Total flavonoid contents expressed as rutin equivalent (RE) per gram dry extract weight, are presented in Fig.1. The flavonoid content varied among the studied plants ranging from *B. alnoides* (56.6±0.12 mg RE/g dry extract wt.) to *L. cubeba* (3.8±0.042 mg RE/g dry extract wt.).

Total flavonol contents expressed as rutin equivalent (RE) per gram dry extract weight, are

Table 1 — Physico-chemical parameters and their extractive values in methanol (%W/W)

Plant sample	Total ash	Acid insoluble ash	Water soluble ash	Loss on drying	Extractive value
Betula alnoides Buch. Ham ex D.Don	6.3	9	5.78	13.74	15.1
Equisetum diffusum D.Don	12.6	26.7	9.61	8	15.9
Litsea cubeba (Lours.) Pers.	10.8	4.72	9.5	9.26	51.5
Quercus lamellosa Sm.	6.5	3.98	2	13.95	14.4
Stephania glabra (Roxb.) Miers	7.7	4.53	5.8	6.74	21.1
Zingiber zerumbet (L.) Sm.	13.2	5.1	11.98	13.94	8.35

Table 2 — Preliminary phytochemical screening of methanolic extract of selected medicinal plants

Phytochemicals	*Betula alnoides*	*Equisetum diffusum*	*Litsea cubeba*	*Quercus lamellosa*	*Stephania glabra*	*Zingiber zerumbet*
Alkaloids	+	+	+	-	+	+
Flavonoids	+	+	+	+	+	+
Tannins		+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	+	+	-	-	+	-
Phlobatannins	+	+	-	+	-	+
Terpenoids	+	-	+	+	+	+
Saponins	+	+	+	+	-	-
Anthraquinones	-	-	-	+	-	-
Proteins	-	-	+	-	+	-
Glycosides	-	+	+	-	+	-
Carbohydrates	-	+	-	+	+	+

Note: + (presence); - (Absence)

represented in Fig. 1. Among the samples analyzed *B. alnoides* contains maximum (83.5±0.13 mg RE/g dry extract wt.) and methanolic extract of *Q. lamellosa* contains the minimum (37.53±0.073 mg RE/g dry extract wt.) flavonol content.

Total tannin contents, expressed as gallic acid equivalent (GAE) per gram dry extract weight, is presented in Fig. 1. Tannin content was found to be maximum in *B. alnoides* (62±0.034 mg GAE/g dry extract wt.) and minimum in *E. diffusum* (37.8±0.051 mg GAE/g dry extract wt.).

Evaluation of antioxidant activities

Antioxidant activities of the methanol extract of each selected medicinal plant were examined by using DPPH radical scavenging assay, ferrous chelating activity and ferric ion reducing assay in order to support the medicinal values of selected plant species.

The 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging assay

The doses dependent curve of DPPH radical scavenging activity of methanolic extract of each selected medicinal plant is given in Fig. 2. It shows that the methanolic extract of all plants on higher concentration possesses the better antioxidant property when compared to reference standard ascorbic acid. Antioxidant activity in terms of IC_{50} value was also calculated and presented in Table 3.

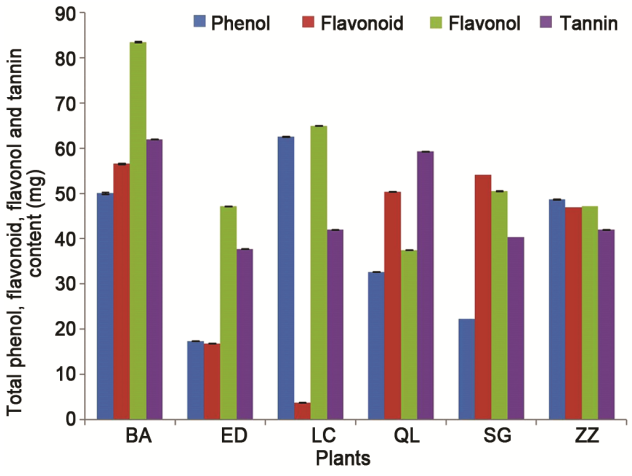

Fig. 1 — Quantitative estimation of *selected* medicinal plants in the methanolic extract, total phenol (mg GAE/g dry extract wt), total flavonoid (mg RE/g dry extract wt), total flavonol (mgRE/g dry extract wt), total tannin content (mg GAE/g dry extract wt). Values are mean±SDM of three replicates. BA- *Betula alnoides*; ED- *Equisetum diffusum*; LC- *Litsea cubeba*; QL- *Quercus lamellosa*; SG-*Stephania glabra*; ZZ- *Zingiber zerumbet*; GAE-Gallic acid equivalents; RE- Rutin equivalents; SDM-Standard Deviation of Mean.

Maximum antioxidant activity was shown by *Q. lamellosa* at the concentration of 143.3 µg/mL among all the selected plants which is found to be same as the standard ascorbic acid (143.3 µg/mL), followed by *S. glabra* (149.2 µg/mL), *B. alnoides* (200.1 µg/mL), *E. diffusum* (253.2 µg/mL), *L. cubeba* (288.18 µg/mL) and *Z. zerumbet* (305.2 µg/mL).

Ferrous chelating assay

The ferrous chelating assay also served as a significant method for evaluation of antioxidant activity. The reducing ability of the methanolic extract of all the plants by ferrous ion binding ability is given in Fig. 3. It shows that the reducing ability of all plants increased with the increase in their concentration. Reducing activity in terms of IC_{50} value was also determined and presented in Table 3. Maximum reducing activity was shown by *E. diffusum* (439.74 µg/mL), which is found to be close to standard EDTA (386.8 5 µg/mL) and minimum by *Q. lamellosa* (727.5 µg/mL).

Ferric ion reducing assay

In this assay, there is a conversion of complex ferricyanide to ferrous form due to the reducing ability of the plant sample. The formation of Pearl's Prussian blue colour was measured at 700 nm, in order to determine the concentration of ferrous ions. Increased absorbance of the solution indicated the increased reducing activity of the plant extracts. The dose-dependent curves of all plants are given in Fig. 4. It shows that the reducing ability of all

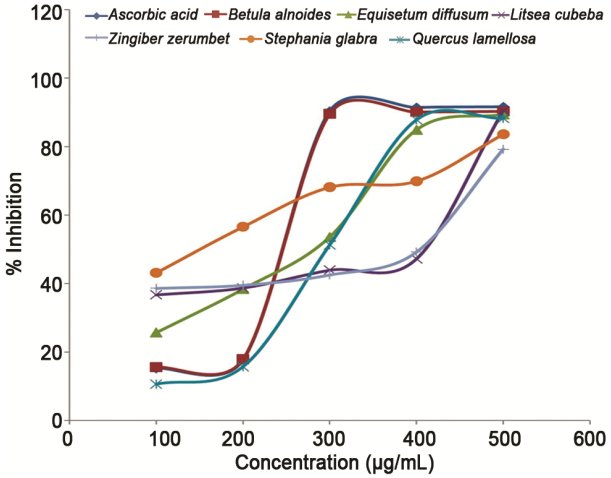

Fig. 2 — DPPH free radical scavenging activity of selected medicinal plants in comparison with standard Ascorbic acid. Values are expressed as the mean value±standard deviation (n= 3) and the concentration of the extract is provided in terms of µg/mL.

Table 3 — DPPH scavenging activity (IC_{50} values) of selected plants (ascorbic acid as a reference compound) and Ferrous chelating activity (IC_{50} values) of selected plants (EDTA as reference compound)

Extract/Reference	DPPH IC_{50} Value (ug/mL)	Ferrous chelating IC_{50} Value (ug/mL)
Betula alnoides	200.1±0.754 (5)*	470±0.737 (5)**
Equisetum diffusum	253.1±0.07 (5)*	439.74±0.519 (5)*
Litsea cubeba	288.18±0.32 (5)*	670.2±0.394 (5)*
Quercus lamellosa	143.3±0.314 (5)*	727.5±0.392 (5)*
Stephania glabra	149.15±0.324 (5)**	653.4±0.763 (5)**
Zingiber zerumbet	305.2±0.243 (5)**	456.7±0.493 (5)*

IC_{50} values are expressed as mean±S.D. Data in parenthesis indicate a number of DPPH assay and number of the Ferrous chelating assay. The IC_{50} value of the reference compound (Ascorbic acid) for DPPH is 143.3±0.392. and the IC_{50} value of the reference compound (EDTA, Ethylenediaminetetraacetic acid) is 386.8±0.382.
* p >0.05, Non significant (NS)
**p <0.05, significant (S)

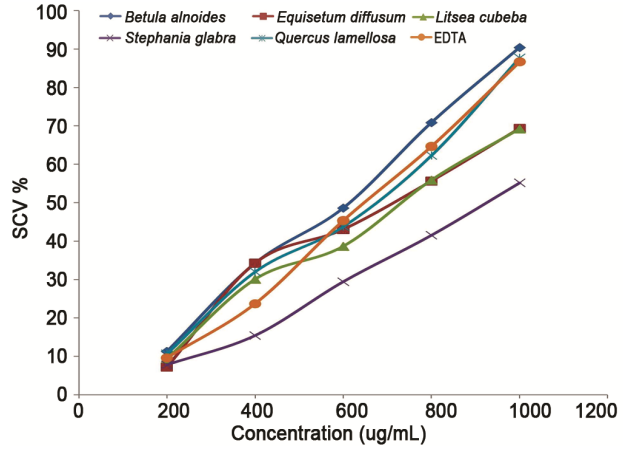

Fig. 3 — Ferrous chelating free radical scavenging activity of selected medicinal plants in comparison with standard EDTA. Values are expressed as the mean value±standard deviation (n= 3) and the concentration of the extract is provided in terms of µg/mL.

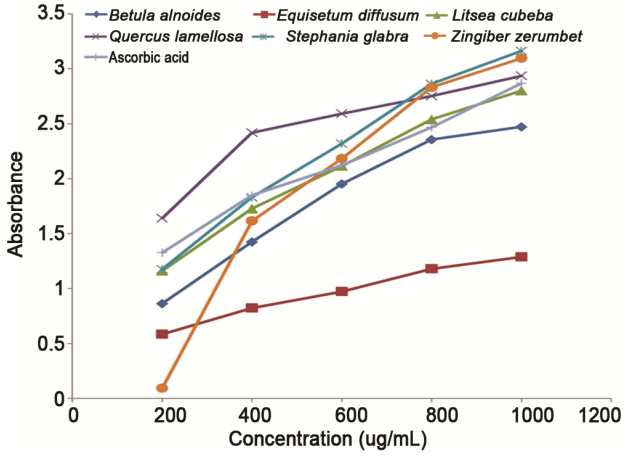

Fig. 4 — Ferric ion reducing the ability of selected medicinal plants in comparison with standard Ascorbic acid. Values are expressed as the mean value±standard deviation (n= 3) and the concentration of the extract is provided in terms of µg/mL.

the plants increased with the increase in their concentration. At concentration of 1000 µg/mL the standard ascorbic acid absorbance was 2.869 and at the same concentration *S. glabra* was found to have the absorbance value 3.162, followed by *Z. zerumbet* (3.097), *Q. lamellosa* (2.936), *L. cubeba* (2.8), *B. alnoides* (2.472) and *E. diffusum* (1.288). Absorbance value shows that the plant samples possess the high reducing ability in comparison with standard ascorbic acid.

Discussion

Plants are one of the major sources of antioxidants, anti-arthritic and anti-inflammatory agents[45,46]. Free radicals are important mediators that provoke inflammatory processes and their neutralization by antioxidants and radical scavengers reduce the effects of inflammation[47]. Antioxidant property is based on the reaction mechanism and closely related to the complex nature of phytochemicals[48]. Pharmacological activities of plant extracts are attributed to the presence of phenols, flavonoids, tannins, flavonols, proanthocyanidins, nitrogenous compounds, vitamins and terpenoids[49,50]. Phenols are one of the most important phytoconstituents in plants with a number of health benefits. Phenolic compounds are also found to be effective hydrogen donors and contain strong antioxidant property[51]. So, it is reasonable to determine the total phenolic content in selected plant species. Several studies were done to evaluate the relationship between antioxidant activity and phenolic content of the plant products. Some authors reported the correlation between the phenolic content and the antioxidant activity, while others found no relationship. One such study reported a high correspondence between total phenolic content and

antioxidant activity in selected fruits, vegetables and grain products[52]. The present study did not show any relationship between phenolic content and antioxidant activity which is in agreement with some of the earlier studies[53-57]. Among the selected plant samples, there is variation in total phenol content ranging from *L. cubeba* (62.6±0.056 mg GAE/g dry extract) to *E. diffusum* (17.4±0.060 mg GAE/g dry extract). Flavonoids also contained the scavenging activity and act as scavengers of various oxidizing species i.e. superoxide anion, hydroxyl radicals or peroxy radicals and quenchers of singlet oxygen [58]. Flavonoids are polyphenolic compounds and are categorized into different classes based on their chemical structures (flavonols, flavones, flavonones, catechins, anthocyanidins and chalcones) and its pharmacological activities were also related to their functional groups. Flavonoid may exert their cell structure protection through a number of mechanisms and one of their potent effects may be through their ability to produce high-level glutathione, a powerful antioxidant as suggested by researchers[59]. Among the studied plant samples, there is variation in total flavonoid content ranging from *B. alnoides* (56.6±0.12 mg RE/g dry extract) to *L. cubeba* (3.8±0.042 mg RE/g dry extract). *E. diffusum*, *Q. lamellosa*, *S. glabra* and *Z. zerumbet* also showed a good amount of flavonoid content. Some studies have shown that many flavonoids and related polyphenols contributed to the antioxidant and anti-inflammatory activity of many plants[60,61]. Tannin-rich foodstuffs also have a number of health benefits which include immunomodulatory, anticancer, antioxidant, radical scavenging functions, anti-inflammatory, cardioprotective, vaso-dilating, antithrombotic effects and UV-protective functions[62,63]. Among the studied plant samples, total tannin content varies among the selected plant samples ranging from *B. alnoides* (62±0.034 mg GAE/g dry extract wt.) to *E. diffusum* (37.8±0.051 mg GAE/g dry extract wt.). A study which was done on chemical composition and some antioxidant indices of *Alstonia. boonei* stem bark extract indicated the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides along with important vitamin, ascorbic acid having DPPH radical scavenging activity (41.58 %), reducing power (0.32) and total phenolic content (2 mg/ g gallic acid equivalent). Results indicated the medicinal value of this plant was due to the presence of various phytochemicals[64]. Another study was done on phytochemical analysis of 13 medicinally important plants of Margalla hills and surroundings and evaluated the qualitative and quantitative analysis of the major bioactive constituents and found the presence of alkaloids, saponins, tannins, anthraquinones, flavonoids, flavones, flavonols, chalcones, terpenoids, phlobatannins, coumarins, steroids and cardiac glycosides were analyzed qualitatively and total alkaloids, total flavonoids, total tannins, total phenols, and saponins were also analyzed quantitatively[65]. Pharmacognostical and phytochemical evaluation of *Cinnamomum wightii* Meissn was done and recorded to contain various phytoconstituents including total tannins (156.5 mg/g), total phenolics (146.40 mg/g), total flavonoids (30 mg/g) and total flavonols (3.6 mg/g) and it may serve as diagnostic tools for identification of crude drug[66]. In another study done for the determination of antioxidant activity by reducing assay recorded that the reducing power increases with the increase in the concentration of the crude extract[67], which is found to be similar in our study as well. In this study, the antioxidant activities of *B. alnoides, E. diffusum, L. cubeba, Q. lamellosa, S. glabra and Z. zerumbet* were found to increase in a concentration-dependent manner. The present study indicates that the selected medicinal plant species contain the effective antioxidant activity in three different assays (DPPH radical scavenging assay, ferrous chelating assay and ferric ion reducing assay). So, these findings are in accordance with previous reports and confirm the medicinal value of selected plant species.

Conclusion

Phytochemical screening of methanolic extract of six medicinal plants (*Betula alnoides, Equisetum diffusum, Litsea cubeba, Quercus lamellosa, Stephania glabra and Zingiber zerumbet*) has revealed the presence of various phytoconstituents like phenols, flavonoids, alkaloids, tannins, terpenoids, saponins by positive reaction with their respective test reagents and contain a good amount of four major phytocompounds i.e. phenol, flavonoid, flavonol and tannin. Antioxidant activity assays indicate that the plant extracts contain good antioxidant activity and could be the significant sources of natural antioxidant, which is relevant for preventing the progress of various oxidative stresses and its related diseases including Rheumatoid arthritis. The plants which were used for this study showed good effects in

antioxidant activity but there have been limited scientific studies. Hence, further study is necessary to prove the uses of these plants including identification, isolation, and purification of biologically active compounds having potent medicinal values in order to validate the uses of these plants.

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