ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 4, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

FLUORESCENCE ANALYSIS, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES IN LEAVES AND STEM OF *EMBELIA RIBES* BURM. F

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Received: Received: 07 December 2018, Revised and Accepted: 13 March 2019

ABSTRACT

Objective: The objective of the present study is to carry out fluorescence analysis, phytochemical, and antioxidant activities using different solvent extract in leaves and stem of *Embelia ribes*.

Methods: In the present study, fluorescence analysis, phytochemical, and antioxidant activities of leaves and stem of *E. ribes* are carried out using standard procedures.

Result: The fluorescence analysis under visible and ultraviolet light for leaves and stem powder of *E. ribes* treated with various chemical reagents shown different fluorescence effect. In the phytochemical analysis, the methanolic extract of leaves has shown the highest total alkaloid content (19.05±0.42 mg CE/g DW), followed by the stem. The methanolic extract of stem exhibited highest total phenolic content (59.82±2.98 mg GAE/g DW), and ethanolic extract showed highest total flavonoid content (10.05±0.36 mg RE/g DW), followed by leaves. The ethanolic extract of stem possesses highest antioxidant activity toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) (84.86±0.11%), whereas methanolic extract of stem reported highest Ferric reducing antioxidant power (FRAP) activity (72.22±0.31 mg Fe+2E/g DW), followed by leaves. Antioxidant activities (DPPH and FRAP) were significantly correlated with TPC.

Conclusion: The results of the present study revealed that the fluorescence analysis of leaves and stem of *E. ribes* can be helpful for the standardization and quality control of indigenous drug. Both leaves and stem serve as the good source of secondary metabolite and antioxidant agents.

Keywords: *Embelia ribes*, fluorescence analysis, antioxidant, alkaloids, phenol, flavonoid.

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INTRODUCTION

Medicinal plants have been used as resourceful medium in traditional medicinal system from primitive period. The potentiality of plant depends on the presence of phytochemicals such as amino acids, proteins, alkaloids, phenols, flavonoids, and tannins. At present, about 75% of population depends on the traditional system of medicine for the treatment of various diseases [1]. Plant-based chemical substances show very minor side effect and serve as the best curative materials. Medicinal plants, during development, produce a variety of secondary metabolites, in which, phenolic compounds play a key role as antioxidants [2].

Alkaloids and their synthetic derivatives can be utilized for their analgesic and antispasmodic effects [3]. Natural antioxidants act as free-radical scavengers, chain breakers, complexes of pro-oxidant metal ions, and quenchers of singlet oxygen formation such as reactive oxygen species (ROS), which may damage cellular components of DNA, proteins, and lipids [4]. Flavonoid is reported to act as an antioxidants, which are scavengers of a wide range of ROS and also have inhibitors of lipid peroxidation [5].

Embelia ribes Burm F., belongs to family Primulaceae, is a woody liana found in the semi-evergreen to evergreen forest of India and is reported to be red-listed species [6]. The fruits are used as an anthelmintic, diuretic, carminative, contraceptive, antibacterial, anti-inflammatory, and anti-astringent as reported in various literatures [7]. Conventionally, the seeds are employed as a remedy for toothache, headache, and snakebite. The seeds are mainly used for maintaining healthy skin and to support the digestive function. It is also effective in the treatment of fevers and for the diseases of chest [8]. Embelin is the principle chemical compound present in the berries of *E. ribes*. The other chemical constituents isolated from the seeds are quercitol, tannin, christembine, vilangin, resinoid, and volatile oil [9,10]. Hence, taking into consideration the importance of *E. ribes*, the study was carried out to investigate the fluorescence analysis, along with phytochemicals and antioxidant potential of aqueous and various solvent extracts of leaves and stem using the spectrophotometric method.

MATERIALS AND METHODS

Materials

All the chemicals used in the present study including standards were purchased from the Sigma-Aldrich; Himedia.

Collection of the samples

The leaves and stem of *E. ribes* were collected from Nagavalli, Karnataka (14°02. 973'N, 074°43.201'E). The voucher specimen was deposited at the Herbaria, Shivaji University, Kolhapur (Accession No: VVK01).

Sample preparation for fluorescence analysis

Powdered drug of different parts of plant gave different fluorescence under ultraviolet (UV) radiation. Each fluorescence characteristic of the treated sample was observed under visible light and then under UV light of both long and short wavelengths [11]. Therefore, fluorescence evaluation is used for the identification of plant and powdered drug [12]. Some crude drugs are often assessed qualitatively in this way, and it is an important parameter of pharmacognostic evaluation [13,14].

The leaves and stem of *E. ribes* were dried in the shade to prevent decomposition of active principle and make fine powder for the fluorescence study. A small quantity (1 gm) of dried and finely powdered leaves and stem of *E. ribes* was treated with freshly prepared acids, alkaline solutions, and different solvents. The drug powders were treated with acids (10% HCl, Conc. HCl, Conc. H_2SO_4, and Conc. HNO_3), alkaline solutions (1 N aqueous NaOH, 1 N alcoholic NaOH, and 5%

KOH), other chemicals (5% iodine, 5% $FeCl_3$, and acetone), and distilled water. They were subjected to study the fluorescence analysis in visible light and in short UV light (254 nm) and long UV light (365 nm).

Preparation of extract for phytochemical studies

Leaves and stem powder of *E. ribes* were extracted with 80% of methanol, ethanol, petroleum ether, and distilled water. Each sample (1 g) was extracted in 10 ml of each solvent separately by continuous shaking on an orbital shaker for 9 h at 110±2 rpm at controlled temperature (30±1°C). The extracts were then filtered through Whatman filter paper No. 1 and stored in air tight vials in a refrigerator.

Determination of total alkaloid content (TAC)

TAC of leaves and stem extracts of *E. ribes* was measured using 1, 10-phenanthroline method [15]. A volume of 100 µl of plant extract was mixed with 1 ml of 0.05 M of 1, 10-phenanthroline in ethanol and 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCL, and volume was adjusted to 10 ml by adding distilled water. The reaction mixture was incubated for 30 min in water bath maintained at 70°C. Above reaction mixture excluding plant extract, substituted by distilled water served as a blank. The absorbance was measured at 510 nm against reagent blank. The concentration of TAC was determined by milligrams colchicine equivalent per gram of dry weight by using calibration curve obtained from colchicine (mg/ml) standard solution [Fig. 1c]. All the samples measurements were performed in triplicates.

Determination of total phenolic content (TPC)

The determination of TPC of the various solvent extracts of leaves and stem of *E. ribes* was performed using Folin–Ciocalteu assay with some modification [16]. In brief, 125 µl of extract was mixed with 1.8 ml of Folin–Ciocalteu reagent and kept for 5 min at 25°C. After this, 1.2 ml of 15% Na_2CO_3 was added to the reaction mixture and kept for 90 min at room temperature. The absorbance of the reaction was measured at 765 nm. The concentration of the TPC was determined as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW) using an equation obtained from gallic acid calibration curve [Fig. 1a]. All the samples estimation was performed in triplicates.

Determination of total flavonoid content (TFC)

TFC of various plant extracts of leaves and stem of *E. ribes* was quantified using the aluminum chloride colorimetric method [17]. The each solvent extract (0.5 ml) was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The mixture was vortexed, and the reaction was kept at the room temperature for 30 min and absorbance of reaction mixture was measured at 415 nm. The concentration of the TFC was determined as milligram of rutin equivalent per gram dry weight (mg RE/g DW) using the calibration curve obtained from rutin (mg/ml) standard solution [Fig. 1b]. Estimation of all the samples was carried out in triplicate.

Determination of antioxidant activity

The antioxidant activity was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing ability (FRAP) systems.

Free-radical scavenging assay (DPPH)

The DPPH radical scavenging activity of the various extracts of leaves and stem of *E. ribes* was estimated using modified and stable DPPH assay [18]. Stock reagent solution was prepared by dissolving 2.5 mg of DPPH in 100 ml of chilled methanol. A volume of 100 µl extract was allowed to react with 3 ml of DPPH solution. The reaction mixture was vortex and kept in the dark at room temperature for 30 min, and absorbance was recorded at 517 nm. A control sample with no added extract was also analyzed, and the results were expressed as percent inhibition/radical scavenging activity (%RSA) and calculated using the following formula:

$$RSA\ (\%) = [A\ control - A\ sample/A\ control] \times 100$$

where, A = absorbance at 517 nm.

Ferric reducing antioxidant power (FRAP) activity

The FRAP assay of various solvent extract of leaves and stem of *E. ribes* was carried out according to the described method by Benzie and Strain, 1996 with some modifications [19]. FRAP reagent formed by assimilation of the acetate buffer (0.3M - pH-3.6), 2, 4, 6- tripyridyl-s-triazine (TPTZ, 10 mM)) in 40 mM HCL, and $FeCl_3$. $6H_2O$ (20 mM) in 10:1:1 ratio former to use and heated to 37°C in water bath for 10 min. The plant extracts of various concentrations were allowed to react with 2.7 ml of the FRAP reagent, and the final volume of the reaction was adjusted to 3 ml with distilled water; the reaction mixture was kept in dark for 30 min, and the absorbance was recorded at 593 nm. The results were expressed as $FeSO_4$ equivalent antioxidant capacity [Fig. 1d].

Statistical analysis

The experiments were done in triplicates. Results were expressed as mean±standard deviation using Excel.

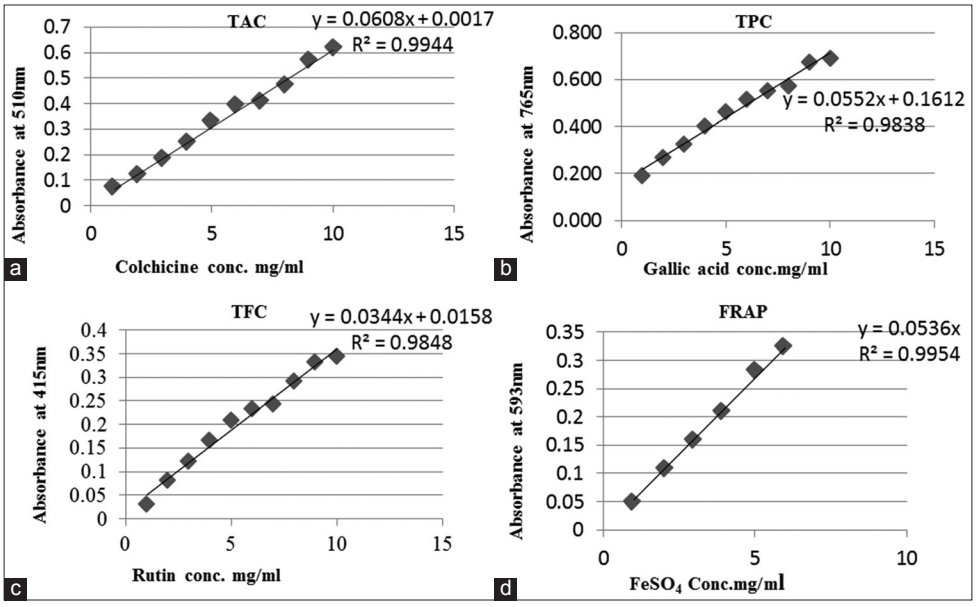

Fig. 1: Calibration curve of (a) Standard Colchicine, (b) Standard Gallic acid, (c) Standard Rutin, (d) Standard $FeSO_4$

RESULTS

Fluorescence analysis

The fluorescence study of leaves and stem powder of *E. ribes* was treated with different chemical reagents and was observed under visible light and UV light (254 nm and 366 nm), which shown predominantly fluorescence effect in both leaves as well as stem. Among the various chemical treatments, the leaves and stem powder of *E. ribes* showed the characteristic fluorescent green color when treated with 1 N NaOH, 10% HCL, Conc. HCl, Conc. HNO_3, Conc. H_2SO_4, 5% iodine, 5% KOH, 5% $FeCl_3$, acetone, and distilled water under short UV light [Tables 1 and 2].

Phytochemical analysis

Total alkaloid content (TAC)

The amount of TAC determined in different solvent extracts of leaves and stem of *E. ribes* [Table 3] showed the methanolic extract of leaves has highest TAC, that is, 19.05±0.42 mg CE/g DW, followed by ethanolic extract with 14.59±0.11 mg CE/g DW.

The petroleum ether extract of leaves has shown 13.38±1.10 mg CE/g DW of TAC, and water extract has shown 9.94±2.86 mg CE/g DW of TAC. The methanolic extract of stem has shown 11.60±2.86 mg CE/g DW of TAC, followed by 10.88±0.41 mg CE/g DW of TAC in ethanolic extract. The petroleum ether extract has shown 8.10±0.58 mg CE/g DW of TAC, and water extract has shown 7.37±4.97 mg CE/g DW of TAC. From the present study, it is found that the TAC is higher in methanolic extract of leaves as compared to the stem of *E. ribes*.

TPC

The amount of TPC determined in different solvent extracts of leaves and stem of *E. ribes* [Table 3] reveals that the methanolic extract of stem shown the highest TPC, that is, 59.82±2.98 mg GAE/g DW, followed by ethanolic extract with 44.65±3.35 mg GAE/g DW.

The water extract of stem has shown the 30.44±1.43 mg GAE/g DW of TPC, and petroleum ether extract reported least 14.39±0.52 mg GAE/g DW of TPC. The methanolic extract of leaves has shown the 55.27±1.29 mg GAE/g DW of TPC, followed by ethanol with 32.23±1.29 mg GAE/g

Table 1: Fluorescence study with different chemical reagents in visible and UV light of *E. ribes* stem powder

S. No.	Stem powder+Reagent	Visible	Short wavelength	Long wavelength
1	Powder as such	Tawny brown	Peanut	Caramel brown
2	Powder+distilled water	Gingerbread brown	Pear green	Caramel brown
3	Powder+1 N NaOH in distilled water	Brunette brown	Pine green	Black
4	Powder+1 N NaOH in alcohol	Chocolate brown	Brunette brown	Black
5	Powder+10% HCl	Caramel brown	Pear green	Brunette brown
6	Powder+Conc. HCl	Syrup brown	Moss green	Black
7	Powder+Conc. HNO_3	Walnut brown	Moss green	Gingerbread brown
8	Powder+Conc. H_2So_4	Brown	Dark brown	Black
9	Powder+Acetone	Rust brown	Pickle green	Caramel brown
10	Powder+5% iodine	Amber brown	Juniper green	Black
11	Powder+5% KoH	Chocolate brown	Juniper green	Black
12	Powder+5% $FeCl_3$	Mocha brown	Juniper green	Brown

UV: Ultraviolet

Table 2: Fluorescence study with different chemical reagents in visible and UV light of *E. ribes* leaves powder

S. No.	Leaves powder+Reagent	Visible	Short wavelength	Long wavelength
1	Powder as such	Moss green	Olive green	Brown
2	Powder+distilled water	Coffee	Seaweed green	Brown
3	Powder+1 N NaOH in distilled water	Brown	Wood green	Black
4	Powder+1 N NaOH in alcohol	Brown	Chocolate brown	Black
5	Powder+10% HCl	Peanut brown	Pickle green	Caramel brown
6	Powder+Conc. HCl	Coffee	Hickory brown	Black
7	Powder+Conc. HNO_3	Caramel brown	Wood Brown	Pecan brown
8	Powder+Conc. H_2So_4	Chocolatebrown	Black	Black
9	Powder+Acetone	Coffee	Carob brown	Gingerbread brown
10	Powder+5% iodine	Walnut brown	Juniper green	Black
11	Powder+5% KoH	Caramel brown	Moss green	Chocolate brown
12	Powder+5% $FeCl_3$	Brunette	Moss green	Syrup brown

UV: Ultraviolet

Table 3: Averages of total alkaloid (mg CE g^{-1} DW), total phenolic (mg GAE g^{-1} DW), and flavonoid (mg RE g^{-1} DW) in different solvent extracts of *Embelia ribes* leaves and stem

S. No.	Plant part	Solvent	TAC mg CE/g DW	TPC mg GAE/g DW	TFC mg RE/g DW
1	Leaves	Methanol	19.05±0.42	55.27±1.29	7.23±2.47
2		Ethanol	14.59±0.11	32.23±1.29	9.33±2.94
3		Petroleum ether	13.38±1.10	9.69±3.02	4.78±1.32
4		Water	9.94±2.86	20.55±0.60	3.37±0.08
5	Stem	Methanol	11.60±2.86	59.82±2.98	8.15±0.12
6		Ethanol	10.88±0.41	44.65±3.35	10.05±0.35
7		Petroleum ether	8.10±0.58	14.39±0.52	5.84±0.24
8		Water	7.37±4.97	30.44±1.43	3.62±1.79

TAC: Total alkaloid content, TPC: Total phenolic content, TFC: Total flavonoid content

DW of TPC. The water extract of leaves has shown the 20.55±0.60 mg GAE/g DW of TPC, and petroleum ether extract has shown 9.69±3.02 mg GAE/g DW of TPC. From the present study, the TPC found to be higher in methanolic extract of stem as compared to leaves of *E. ribes.*

TFC

The amount of TFC was determined in different solvent extracts of leaves and stem of *E. ribes* [Table 3] reveals that the ethanolic extract of stem has shown the highest TFC with 10.05±0.35 mg RE/g DW, followed by methanolic extract with 8.15±0.12 mg RE/g DW. The petroleum ether extract of stem has shown TFC with 5.84±0.24 mg RE/g DW, followed by water with 3.62±1.7924 mg RE/g DW. The ethanolic extract of leaves has shown 9.33±2.94 mg RE/g DW of TFC, followed by methanolic extract with 7.23±2.47 mg RE/g DW. The petroleum ether extract of leaves has shown 4.78±1.32 mg RE/g DW of TFC, followed by water extract with 3.37±0.08 mg RE/g DW. From the present study, the TFC found to be higher in ethanolic extract of stem as compared to the leaves of *E. ribes.*

ANTIOXIDANT ACTIVITY

Free-radical scavenging assay (DPPH)

DPPH free-radical scavenging activities of different solvent extracts of *E. ribes* leaves and stems [Table 4] reveal that ethanolic extract of stem exhibited higher DPPH radical scavenging activity, that is, 84.86±0.11%, followed by methanolic extract with 77.19±0.13% inhibition. The petroleum ether extract of stem exhibited 35.55±1.78% inhibition, and the lowest DPPH radical scavenging activity 21. 80±0.55% is shown by aqueous extract. The ethanolic extract of leaves has shown 79.81±0.15% DPPH radical scavenging activity, followed by methanolic extract with 71.15±0.14% inhibition. The petroleum ether extract of leaves exhibited 19.82±1.58% inhibition, and the lowest DPPH radical scavenging activity 18.43±0.98% is shown by aqueous extract. From the present study, it is found that the DPPH radical scavenging activity is higher in ethanolic extract of stem as compared to the leaves of *E. ribes*.

Ferric reducing antioxidant power activity (FRAP)

FRAP free-radical scavenging activities of different solvent extracts of *E. ribes* leaves and stem [Table 4] reported highest amount of FRAP activity in the methanolic extract of stem with 72.22±0.31 mg Fe^{+2}/g, followed by ethanolic extract with 65.05±0.26 mg Fe^{+2}/g activity. The aqueous extract of stem has shown 36.89±0.62 mg Fe^{+2}/g FRAP activity and the lowest FRAP activity, that is, 17.50±0.10 mg Fe^{+2}/g is exhibited by petroleum ether extract of stem. The methanolic extract of leaves has shown 66.66±0.27 mg Fe^{+2}/g of FRAP activity, followed by ethanolic extract with 64.91±0.36 mg Fe^{+2}/g. The aqueous extract of leaves has shown 39.27±1.26 mg Fe^{+2}/g of FRAP activity. The lowest FRAP activity with 14.95±0.69 mg Fe^{+2}/g is exhibited by petroleum ether extract of leaves. From the present study, it is found that the FRAP free-radical scavenging activity is higher in methanolic extract of stem as compared to the leaves of *E. ribes*.

DISCUSSION

Fluorescence analysis is an efficient, responsive, and precise method for the determination of various components in short period of time compared to several time-consuming dilutions steps involved in the analysis of pharmaceutical samples [20,21]. The fluorescence color shows specificity for each compound. The various plant materials show different coloration when subjected to different chemical reagents. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. At varied wavelength, crude powder of plants shows diverse fluorescence due to the presence of different chemical constituents in the drug [14,22].

Table 4: Averages of antioxidant assay DPPH (% inhibition) and FRAP (mgFe+g^{-1} DW) in different solvent extracts of *Embelia ribes* leaves and stem

S. No.	Plant part	Solvent	DPPH % inhibition	FRAP mgFe+2/g
1	Leaves	Methanol	71.15±0.14	66.66±0.27
2		Ethanol	79.81±0.15	64.91±0.36
3		Petroleum ether	19.82±1.58	14.95±0.69
4		Water	18.43±0.98	39.27±1.26
5	Stem	Methanol	77.19±0.13	72.22±0.31
6		Ethanol	84.86±0.11	65.05±0.26
7		Petroleum ether	35.55±1.78	17.50±0.10
8		Water	21. 80±0.55	36.89±0.62

A correlation is found between a compound present in the drugs and their fluorescent behavior under different conditions. Under short UV radiation coumarin, especially hydroxyl amino acid derivative such as o-Coumaric acid appears yellowish green in alkaline condition. Flavonones which appear to be light yellow in aqueous condition, but under UV light, it appears to be bright yellow under alkaline conditions. Phytosterols when treated with 50% H_2SO_4 appear to be green under UV light. Sapogenin and terpenoids show yellow green fluorescence under short UV light [23]. Quinine, aconitin, berberin, and emetin show specific colors of fluorescence. Least fluorescence is shown by fixed oils and fats [11]. In the present study, the major bioactive compounds present in the crude drug of *E. ribes* leaves and stem were found to be phenols, flavonoids, tannins, alkaloids, steroids, and quinine.

Alkaloids are utilized as stimulants, narcotics, pharmaceuticals, and poisons. At present, they are used in clinical treatments such as antimalarial quinine, the anesthetic cocaine, the stimulant caffeine and nicotine, the analgesics morphine and codeine, the gout suppressant colchicine, the antibiotic sanguinarine, the antiarrhythmic ajmaline, the anticancer vinblastine, taxol, and certain sedative [24,25]. Here, we are reporting the TAC from leaves and stem of *E. ribes* using different solvent extracts. Similar result for TAC in different plant parts of *Chinchona officinalis* has been reported [26].

Phenolic compound enhances the quality of plant by helping in plant growth and reproduction; they are produced in the plant as a response to environmental factors such as pollution, light, and irradiation [27]. From the leaves exact of *E. ribes,* 57.89±2.94 mg GAE/g dry basis TPC was reported [28], whereas bark reported 26.59 mg GAE/100g of TPC [29].

In the present study, it was noticed that TPC content was higher in stem as compared to leaves in *E. ribes.* Flavonoid is a polyphenolic secondary metabolite which helps in pollination, as a repellent to pest, responsible for pigmentation of various parts of plant and in nodule formation [30-32]. It has potential antioxidant property [33]. From the bark extract of *E. ribes,* 01.35 mg QE/100g was reported [29]. Since no data are available for TFC in leaves and stem of *E. ribes*, from the present study, we are reporting the TPC and TFC contents in stem are found to be higher than leaves. Similar kind of work done using different solvent extraction is carried out in *P. minima Linn* and *Eclipta prostrata* (L.) L [34,35]

Antioxidant, molecules act as protector against free-radical damage. They have shown a major contribution to the prevention of many chronic diseases such as cancer, cardiovascular disease, hepatitis, asthma, atherosclerosis, arthritis, and diabetes [36-38]. Since some plant extracts show complication, use of different methods is recommended for the evaluation of antioxidant activity [32]. In the present study, we have used DPPH and FRAP antioxidant assays for leaves and stem of *E. ribes*. FRAP activity of 60.75±0.16 mg Asc AE/g dry basis was reported in the leaves of *E. ribes* [28]. It seems no data are available for the DPPH and FRAP free-radical scavenging activities on the stem of *E. ribes*. It was observed that antioxidant activity in stem is higher than that of leaves. There is influence of different solvents on the extraction of plant material. Similar kind of results are reported from wheat and *Salacia chinensis* L [38,39]. Antioxidant activity was observed in the phenolic extract from *Russula laurocerasi* with the similar kind of result [40]. DPPH free-radical scavenging assay was used to observed antioxidant activity in various solvent extract of sweet orange peel and makrut lime [41,42].

CONCLUSION

From the present study, it can be concluded that leaves and stem of *E. ribes* show the presence of different bioactive compounds in the fluorescence analysis, which might be ideal for various pharmaceutical applications. It is clearly indicated from the present study that polarity of solvent plays a major role in extraction of phytoconstituents. Among the four solvents used for extraction, methanolic extract of stem was found to be the superior compared to ethanolic, water, and petroleum ether extracts with respect to TAC, TPC, and FRAP. Ethanolic extract of stem found to be superior for TFC and DPPH activities. The leaves and stem of *E. ribes* are good source of antioxidants and secondary metabolite, which can be used for the various pharmaceutical applications.

ACKNOWLEDGMENT

The authors are thankful to the Head, Department of Botany, Shivaji University, Kolhapur, for providing the necessary facilities. VVK is thankful to UGC, New Delhi, for financial support under UGC-BSR Fellowship in Sciences for Students (No.F.25-1/2013-14 (BSR)/7-163/2007(BSR)).

AUTHORS' CONTRIBUTIONS

Vidya Kamble has performed all the experiments in the laboratory of Cytogenetics and Plant Breeding, Department of Botany, Shivaji University, Kolhapur. Dr. Nikhil Gaikwad has given the experimental design, intellectual content for the selection of plant and is the mentor of the present work.

CONFLICTS OF INTEREST

The authors are declaring that there are no conflicts of interest regarding the publication of this research article.

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