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Chemical profiling and antioxidant activity of *Equisetum ramosissimum* Desf. stem extract, a potential traditional medicinal plant for urinary tract infections

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Abstract

Background: *Equisetum ramosissimum* Desf. (*E. ramosissimum*) is a widely used traditional medicinal plant to treat urinary tract infections (UTIs) by ethnic people throughout the world. The utility of the plant in treating urinary-related disorders was evaluated against selected pathogenic bacteria which has major role in causing UTIs. Hence, the present study executed to extract phytochemicals like total phenolics and flavonoids, chemical profiling by GC–MS analysis and to test their antioxidant activity from stem extracts of *E. ramosissimum*. The extraction process was directed by petroleum ether, chloroform, ethyl acetate, methanol, and aqueous solvents.

Results: The GC–MS analysis yielded 24 phytoconstituents with linoleic acid, palmitic acid, nonacosane, hexahydrofarnesyl acetone, and octacosane as major compounds. Methanolic extract yielded maximum amount of phenolics (TPC) and flavonoids (TFC) with 600.02 ± 0.22 mg GAE/g and 631.38 ± 0.69 mg QE/g, respectively. Methanolic extract also exhibited notable free radical scavenging activity with an IC_{50} of 123.89 ± 0.73 , 150.10 ± 1.02 , 146.01 ± 0.54 , and 63.73 ± 6.12 μ g/mL for DPPH, FRAP, ABTS, and O_2^- assays, respectively. The minimum inhibitory concentration (MIC) required to inhibit the growth of tested pathogenic bacteria was observed in aqueous and methanolic extracts with the value being 31.25 μ g/mL against *R. equi* and *V. cholerae*. As like, methanolic and petroleum ether extracts efficiently inhibited the growth of *B. subtilis* with the MIC of 31.25 μ g/mL.

Conclusion: It was concluded that the notable effect of methanolic and aqueous extracts against the uropathogenic bacteria reported in this study supported the traditional uses of this plant in treating UTIs. The results acquired from this investigation revealed that *E. ramosissimum* stem extract might be considered as an interesting candidate in the development of antibacterial agent against UTIs coupled with antioxidant properties.

Keywords: Antioxidants, Equisetaceae, GC–MS analysis, Urinary tract infections

Background

Urinary tract infections (UTIs) are serious health problem with substantial clinical and financial burden affecting millions of people annually and especially women are more prone to be affected by the UTIs [1]. All UTIs are mostly caused by gram negative bacteria and *Escherichia coli* considered as most prevalent causative organism for UTIs with more than 90% of reported cases [2]. Various

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species of *Enterobacter*, *Enterococcus*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus* also cause significant amount of UTIs in human. The UTIs caused by bacteria establish resistance by regular intake of antibiotics that also sometime less efficacious and cause side effects like neurotoxicity, nephrotoxicity, and hepatotoxicity [3]. Severe infections in the urinary tract may lead to various skin diseases including rashes and wounds [1]. Oral administration of renowned antibiotics such as trimethoprim, losporins, fluoroquinolone, and nitrofurantoin showed promising results as short time cure for UTIs [4].

Medicinal plants have been used as an alternative in managing infectious diseases with little or no adverse side effects [3]. To overcome the prevalent condition of UTIs, medicinal plants are widely used by the people since ancient times. The mechanism of herbal medicines used in treating UTIs is not well explained. But it was stated that secondary metabolites present in various parts of plants act as immunomodulators, boost/regulate the body oxidant status by providing required amount of antioxidant compounds which in turn terminate the proliferation of microbes responsible for UTIs [5]. The major advantage in the use of medicinal plants and their bioactive constituents as potential herbal drugs is that the pathogenic bacteria have not developed resistance against them [6]. It may be due to the presence of rich amount of phytochemicals present in the herbal medicines.

While conducting a survey among the Malayali tribals in the Kolli hills of Eastern Ghats, it was noticed that they are frequently using the dried aerial parts of *E. ramosissimum* in the treatment of urinary-related diseases including stone formation in kidney, stomachache, skin diseases, bone fracture, joint pains, and rheumatism [6]. Likewise, the plant is broadly used by different ethnic people throughout the world in treating UTIs and related diseases like stone formation and kidney troubles [7, 8]. Apart from its use in UTI infections, the plant has also used to treat inflammation, respiratory disorders, bone fracture, stomach problems, skin diseases (itching, scabies), gonorrhoea, rheumatism, female fertility, back pain, muscle pain, fever, sunstroke, and blood pressure [8].

The plant *E. ramosissimum* Desf. belongs to the family Equisetaceae and commonly referred as 'Horsetail'. The plant is widely distributed in Australia, USA, Africa, South Asia, India, Southeast China, Korea, and Japan. With the creeping subterranean rhizome, the plant is commonly distributed in sandy riverbanks of evergreen forests. In the study area, it is grown as a weed in cultivated lands of Kolli hills. The plant is reported to have monoterpenes, sesquiterpenes, sesquiterpene hydrocarbons, monoterpene hydrocarbons, α -bisabolol

oxide A, cumin aldehyde, carvacrol, α -terpinyl-acetate, β -caryophyllene, hexadecanoic acid, thymol [9], kaempferol 3-O-sophoroside, kaempferol 3-O-sophoroside-7-O-glucoside [10], cholest-5-enol, 24-methylcholest-5-enol, 24-ethylcholesta-5,22-dienol, and 24-ethylcholest-5-enol [11].

Though the plant has much importance in traditional medicine with huge medicinal properties, there is a lacuna in chemical profiling of different extracts of the plant. With this backdrop information, the present investigation was envisioned to evaluate the GC-MS analysis, total phenolics content, total flavonoids content, and antioxidant activity of the *E. ramosissimum* stem using different solvents. The utility of the plant in treating urinary-related disorders was also evaluated against selected pathogenic bacteria which have a major role in causing UTIs.

Methods

Chemicals

The chemicals for the preparation of reagents (Folin-Ciocalteu reagent and Nash reagent) and buffers (phosphate buffer and acetate buffer) were of commercial grade (Sigma-Aldrich, Mumbai, India) and purchased from the local supplier, Techno Scientific, Thanjavur, 613 002, India. Other chemicals and standard drugs used in the study were also purchased from the same supplier with the commercial brand of Merck Life Science Pvt. Ltd. Mumbai, India.

Collection and preparation of plant extracts

The stem of *E. ramosissimum* used in this study was collected during August 2019 from Kolli hills of Namakkal district, India, at an altitude of 901–1100 m. The provisional identification of this specimen was performed using the standard flora 'Pteridophytic flora of the Western Ghats in South India' [12] and further authenticated by the taxonomists at the 'Malabar Botanical Garden and Institute for Plant Sciences' Kozhikode, Kerala, by submitting a preserved herbarium specimen. The well-prepared voucher specimen (SPCH1070) was submitted in the herbarium of host institution for future references.

The extraction was done using petroleum ether, chloroform, ethyl acetate, methanol, and aqueous solvents which have a wide range of polarity. Briefly, the powdered stem material was macerated to aforesaid solvents in the ratio of 1:5 and kept under shaker at 125 rpm for 8 h at about 40 °C. Filtration was done through muslin cloth and Whatman No. 1 filter paper. The excess solvent was removed by vacuum distillation to yield crude extract. The crude extracts were evaporated by reduced pressure at 40 °C in an evaporator except for aqueous extract.

Then, concentrated extracts were stored in tightly sealed glass containers at -20°C for further studies.

Phytochemical analysis

Qualitative analysis of phytochemicals

The preliminary phytochemical screening of *E. ramosissimum* stem extract was done to find out the presence of different phytochemicals such as steroids (*Salkowski test*), triterpenoids (*Liebermann-Burchard reaction*), alkaloids (*Hager's test*), phenolic compounds (*Lead acetate test*), flavonoids (*Alkaline reagent test*), saponins (*Frothing test*), and tannins (*Potassium hydroxide test*) using standard methods [13].

GC-MS analysis of stem extract

Gas chromatography-mass spectrometry analysis was carried out in Shimadzu (QP-2020) interfaced to a mass spectrometer for the petroleum ether, ethyl acetate, and methanol extracts of *E. ramosissimum* stem. The TD20 was used to obtain the chromatogram using the AB-Innowax column. The initial temperature was programmed with 50°C and extended up to 280°C at $40^{\circ}\text{C min}^{-1}$. Helium was used as carrier gas and the ion source temperature was maintained as 200°C . Identification of detected compounds in the stem extracts was measured by computing the usual peak area to total area and electronic libraries [14].

Determination of total phenolics (TPC) and flavonoids (TFC) content

The quantitative estimation of TPC in various solvent extracts of *E. ramosissimum* stem was done by the Folin-Ciocalteu method [15]. About 0.5 mL of 1 N Folin-Ciocalteu reagent was added with 2.5 mL of 20% sodium carbonate solution to each tube of stem extract at different concentrations. The absorbance was recorded at 725 nm against blank which possess water alone. Gallic acid was used as standard and the results were expressed in terms of milligrams gallic acid equivalents per gram of extracts. The TFC was estimated by AlCl_3 method [15]. Briefly, 300 μL of various concentration of stem extract was added with 2 mL of distilled water followed by 150 μL of NaNO_2 . Then, 150 μL of AlCl_3 was added and incubated for 6 min. To this, 2 mL of 4% NaOH was added, vortexed, and kept at 37°C . The absorbance was read at 510 nm against blank. Quercetin was used as standard and the results were expressed as milligrams quercetin equivalents per gram of extract.

In vitro antioxidant activity

The antioxidant potential of different solvent extracts of *E. ramosissimum* stem was determined by five in vitro assays. Absorbance was measured on 96 well microplate

reader (Robonik India Pvt. Ltd., Thane, India). The DPPH free radical scavenging, FRAP, ABTS, O_2^- , and OH^- free radical scavenging abilities of *E. ramosissimum* stem extracts were studied as defined in our earlier study [16]. Reference antioxidant used in all the assays was ascorbic acid. For each concentration, the percentage inhibition of standard and test samples was calculated and the graph was drawn between percentage inhibition and concentration. From the obtained graphs, the IC_{50} was computed as the concentration at which 50% of inhibition observed against the concentration of *E. ramosissimum* stem extract.

Antibacterial activity

Bacterial strains used

The stem extracts of *E. ramosissimum* were studied against four gram positive strains like *Staphylococcus epidermidis* (MTCC435), *Bacillus subtilis* (MTCC441), *Rhodococcus equi* (MTTC2558), and *Methicillin resistant Staphylococcus aureus* (B23), and gram negative strains such as *Escherichia coli* (MTCC40), *Vibrio cholerae* (MTTC3904), *Salmonella typhi* (MTCC3224), *Pseudomonas aeruginosa* (MTCC1748), *Klebsiella oxytoca* (B847), and *Citrobacter freundii* (B15) which are major causative agents of UTIs.

Broth microdilution method

The minimum inhibitory concentration required to inhibit the growth of pathogenic bacteria with *E. ramosissimum* stem extract was performed by microdilution method using 96 well plates [17]. For the experiment, 50 μL of stem extract at 500 to 0.488 $\mu\text{g/mL}$ concentrations was obtained using serial dilution method. Fifty μL of Mueller-Hinton broth was poured in each well. Then, 50 μL of bacterial inoculum was added to each well and incubated for 24 h at 37°C . Then, 20 μL of INT (p-iodonitro-tetrazolium chloride) was added and incubated for 30 min at 37°C . Ampicillin and Deflox were (30–0.029 $\mu\text{g/mL}$) used as positive controls. The MIC of stem extract was observed by change in colour after the addition of INT dye. The bacterial growth was predicted by red–pink colour in wells and no colour change indicated the inhibition of bacterial growth with stem extract.

Statistical analysis

All the experimental parts were done in triplicate and the results were calculated as mean $\pm$ standard deviation. All the statistical analysis was considered highly significant with more than 95% confidence interval. The graphical representations were done with GraphPad Prism version 8.0.2 (GraphPad Software, Inc).

Results

Phytochemical profiling

The extract yield of *E. ramosissimum* stem was recorded as 3.91, 3.55, 5.86, 8.96, and 4.70% in petroleum ether, chloroform, ethyl acetate, methanol, and aqueous solvents, respectively. Qualitative phytochemical screening of *E. ramosissimum* stem showed the presence of tested secondary metabolites in most of the solvents used in the study (Table 1). Phenolic compounds were noticed in all the five extracts. Flavonoids, steroids, and tannins were found in petroleum ether, chloroform, methanol, and aqueous extracts. While triterpenoids were present in chloroform and methanolic extracts, alkaloids were found in petroleum ether, ethyl acetate, and methanolic extracts. Saponins were detected in ethyl acetate, methanol, and aqueous extracts.

The GC–MS analysis of *E. ramosissimum* stem extract yielded 24 compounds. The analysis enabled the identification of 10, 15, and 13 compounds amounting to 90.8, 96.1, and 98.4% for petroleum ether, ethyl acetate, and methanolic extracts, respectively (Table 2). Linoleic acid, palmitic acid, and octacosane are recorded as dominant compounds in the petroleum ether extract of *E. ramosissimum* stem with 41.4, 19.4, and 6.9%, respectively (Fig. 1a). Campesterol is one of the sterol groups of compound present in petroleum ether extract. In ethyl acetate extract, palmitic acid (34.4%), nonacosane (11.5%), and hexahydrofarnesyl acetone (9.4%) were identified as major constituents (Fig. 1b). As like, methanolic extract also contributes palmitic acid (44.3%) and hexahydrofarnesyl acetone (9.8%) as major constituents followed by nonacosane (7.8%) and *n*-tetracosane (6.9%) (Fig. 1c).

The measured levels of TPC and TFC are presented in Fig. 2. The gallic acid equivalent was computed from linear regression of standard calibration curve ($y=0.0113+0.204x$; $R^2=0.9931$) for TPC. The TPC was ranged from 58.67 ± 2.57 to 600.02 ± 0.22 mg GAE/g. The maximum amount of TPC was recorded in methanolic extract with 600.02 ± 0.22 mg GAE/g followed by petroleum ether and aqueous extracts with 393.48 ± 14.36

and 303.77 ± 3.84 mg GAE/g, respectively. The TFC of *E. ramosissimum* extract varied in the ranges of 47.93 ± 1.55 to 631.38 ± 0.69 mg QE/g. For quantification of TFC, quercetin equivalent was calculated from linear regression of standard calibration curve ($y=0.0117+0.1018x$; $R^2=0.9907$). The TFC was also higher in methanolic extract (631.38 ± 0.69 mg QE/g) followed by aqueous and petroleum ether extracts with 493.77 ± 0.96 and 405.14 ± 9.43 mg QE/g, respectively.

Antioxidant activity

In vitro antioxidant activity of *E. ramosissimum* stem extract was studied by DPPH, ABTS, O_2^- , OH^- , and FRAP methods (Fig. 3). The lower the IC_{50} values, higher the antioxidant activity of plant extract. The *E. ramosissimum* stem had comparable IC_{50} values for DPPH free radical scavenging capacity in tested concentrations (2.5, 5, 10, 20, 40, 80, and 160 μ g/mL). Methanolic extract at a concentration of 160 μ g/mL showed highest free radical scavenging ability with an IC_{50} of 123.89 ± 0.73 μ g/mL followed by ethyl acetate and aqueous extracts (146.46 ± 0.54 and 154.93 ± 0.54 μ g/mL, respectively). It was observed that significant difference was found in petroleum ether ($p \leq 0.0001$) and chloroform ($p = 0.005$) extracts. However, methanol and ethyl acetate extracts were showed no significance difference when compared to the standard. Similar to DPPH assay, methanolic extract of *E. ramosissimum* stem at 160 μ g/mL concentration showed higher reducing power in FRAP method with an IC_{50} of 150.10 ± 1.02 μ g/mL and least activity was observed in petroleum ether extract (IC_{50} of 248.12 ± 2.50 μ g/mL). However, petroleum ether, chloroform, and ethyl acetate extracts are statistically different from the reference compound.

For the ABTS assay, highest free radical scavenging activity was recorded in methanolic and aqueous extracts at 160 μ g/mL concentration with an IC_{50} of 146.01 ± 0.54 and 162.42 ± 0.49 μ g/mL, respectively. These extracts were observed with no significant difference to the standard. On the other hand, petroleum ether and chloroform

Table 1 Qualitative phytochemical screening of *E. ramosissimum* stem extracts

Phytochemicals tested	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Steroids	+	+	–	++	++
Triterpenoids	–	++	–	+	–
Alkaloids	+	–	+	+++	–
Phenolic compounds	++	+	+	+++	++
Flavonoids	+	+	–	+++	++
Saponins	–	–	+	+	+
Tannins	+	+	–	++	+++

The symbols +++, ++, +, and – refer to appreciable, moderate, trace amounts, and absence of secondary metabolites, respectively, in the tested solvent extracts

Table 2 GC–MS analysis of petroleum ether, ethyl acetate, and methanolic extracts of *Equisetum ramosissimum* stem

S. no	Retention time	Compounds	Area %		
			PE	EA	ME
1	17.279	Methyl palmitate	4.9	–	–
2	17.330	<i>n</i> -Tridecane	–	3.5	–
3	17.669	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	–	–	3.8
4	17.725	Neophytadiene	–	2.7	–
5	17.875	Hexahydrofarnesyl acetone	–	9.4	9.8
6	17.904	Ethyl palmitate	4.7	–	–
7	18.855	Methyl linoleate	3.8	–	–
8	18.930	Methyl 9,12,15-octadecatrienoate	4.3	–	–
9	19.315	Palmitic acid	19.4	34.4	44.3
10	19.765	Linoleic acid	41.4	–	–
11	21.196	<i>n</i> -Eicosane	–	2.9	–
12	21.266	Cyclopentanecarboxylic acid, 4-methylpentyl ester	–	–	2.1
13	21.381	Sulphurous acid, cyclohexylmethyl hexyl ester	–	3.1	–
14	24.172	1-Docosene	–	1.8	1.6
15	24.702	<i>n</i> -Docosane	–	2.2	3.1
16	25.503	<i>n</i> -Tricosane	–	2.5	3.7
17	26.053	<i>n</i> -Tetracosane	–	4.2	6.9
18	26.228	Hexacosane	2.6	3.5	3.5
19	27.638	Heptacosane	–	7.8	8.1
20	27.733	Campesterol	2.3	–	–
21	27.808	Squalene	–	–	1.5
22	27.933	Octacosane	6.9	3.7	2.2
23	30.100	Nonacosane	–	11.5	7.8
24	31.070	Solanesol	–	2.9	–
		Total	90.8	96.1	98.4

PE petroleum ether, EA ethyl acetate, ME methanol

extracts were positively significant ($p \leq 0.0001$) among the tested extracts. Likewise, aqueous and methanolic extracts at 160 $\mu\text{g/mL}$ showed highest superoxide scavenging activity with an IC_{50} of 63.73 ± 6.12 and 82.18 ± 2.39 $\mu\text{g/mL}$, respectively. The higher hydroxyl free radical scavenging activity was recorded at the concentration of 160 $\mu\text{g/mL}$ in methanolic, aqueous, and chloroform extracts with an IC_{50} of 122.91 ± 3.32 , 144.87 ± 1.30 , and 169.31 ± 1.76 $\mu\text{g/mL}$, respectively. In which, highest significance in difference was observed in petroleum ether ($p \leq 0.0001$) and ethyl acetate extracts ($p = 0.005$).

Antibacterial activity

The results of the MIC value of the *E. ramosissimum* stem extracts are represented in Table 3, and inhibition images are provided as Additional files 1 and 2. Aqueous and methanolic extracts showed excellent MIC which requires just 31.25 $\mu\text{g/mL}$ of plant extract to inhibit the growth of *R. equi* and *V. cholerae*. Methanolic and petroleum ether extracts efficiently inhibited the growth of

B. subtilis at 31.25 $\mu\text{g/mL}$ concentration. The growth of *B. subtilis* is commendably controlled by all the tested solvent extracts at lower concentration of plant extracts (31.25–125 $\mu\text{g/mL}$). However, *C. freundii* is susceptible for most of the tested solvent extracts at even higher concentrations except aqueous (62.5 $\mu\text{g/mL}$) and ethyl acetate (125 $\mu\text{g/mL}$) extracts.

Discussion

The presence or absence of different phytochemicals in plant extract plays a major role in antioxidant and antibacterial activities of a specific plant part [18]. In the present study, qualitative phytochemical screening of *E. ramosissimum* revealed the presence of significant amount of secondary metabolites like phenolics, flavonoids, steroids, and tannins (Table 1). Previous studies also confirmed the presence of various phytochemicals in different parts of *E. ramosissimum* as reported in the present work [10, 11]. To support our study, Chiu et al. [10] also identified sterol compounds from aerial parts of *E. ramosissimum* with chloroform–methanolic extract.

Fig. 1 GC–MS analysis of the petroleum ether (a), ethyl acetate (b), and methanol (c) extracts of *E. ramosissimum* Desf. stem

The phytochemical analysis of *E. ramosissimum* stem extract using GC–MS showed the presence of 24 compounds with palmitic acid (44.3, 34.4, and 19.4% in methanolic, ethyl acetate, and petroleum ether extracts, respectively) and linoleic acid (41.4% in petroleum ether extract) as major constituents (Table 2). Palmitic

acid is a saturated fatty acid (16-C long chain molecule), an essential component for human body and could be supplemented in diet or made endogenously from the other fatty acids, amino acids, and carbohydrates [19]. It has a major pathological role in metabolic syndromes, cancer, inflammation, cardiovascular, and neurodegenerative diseases and major proportion

Fig. 2 Total phenolics and flavonoids content in *E. ramosissimum* stem extracts

Fig. 3 In vitro antioxidant activity of *E. ramosissimum* Desf. stem extracts by DPPH, ABTS, superoxide, hydroxyl radical scavenging, and FRAP assays. Different letters in the graph indicate significant differences ($a p \leq 0.05$; $b p \leq 0.01$; $c p \leq 0.001$; n , no significance) in the tested extracts with the reference standard, ascorbic acid

Table 3 Minimum inhibitory concentration of *E. ramosissimum* leaf extracts against the selected pathogenic bacteria

Extracts	Minimum inhibitory concentration (µg/mL)									
	<i>E. coli</i>	<i>MRSA</i>	<i>S. epidermis</i>	<i>B. subtilis</i>	<i>R. equi</i>	<i>V. cholerae</i>	<i>S. typhi</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>C. freundii</i>
Petroleum ether	> 500	> 500	> 500	31.25	> 500	> 500	> 500	> 500	> 500	> 500
Chloroform	500	> 500	> 500	125	> 500	> 500	500	125	250	> 500
Ethyl acetate	250	> 500	> 500	125	250	15.625	250	500	125	125
Methanol	250	125	62.5	31.25	125	250	62.5	62.5	62.5	500
Aqueous	250	125	125	62.5	31.25	31.25	125	125	62.5	62.5
Ampicillin (Std.)	0.937	3.75	7.5	1.875	0.937	7.5	15	15	1.875	0.234
Deflox (Std.)	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029	< 0.029

of saturated fatty acids found in human body cells and serum are comprising of this palmitic acid [20].

As reported in our study, Alebous et al. [11] also revealed the presence of linoleic acid (4.6%) in essential oil extracted from aerial parts of *E. ramosissimum*. Linoleic acid is one of the important polyunsaturated fatty acids which is necessary energy per day (at least 1–2%) for normal human growth and development process and considered as a precursor in the synthesis of arachidonic acid and eicosanoids [21]. The compound is most commonly found in the seeds, nuts, oils, and cereals of various plants and major vegetable oils like corn, olive, cotton seed, palm, sunflower, and coconut comprising about 1% of linoleic acid [22]. Linoleic acid is also involved in various health promoting properties like reducing the catabolic effects of immune stimulation, facilitates growth regulation and promotion, reduces body fat, anticarcinogenic and antiatherogenic activities [23].

Among the 24 compounds recorded in our study, only palmitic acid, hexacosane, and octacosane are noticed in all the tested extracts (Table 2 and Fig. 1). The compounds like, *n*-tridecane, methyl palmitate, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, ethyl palmitate, methyl linoleate, linoleic acid, *n*-eicosane, methyl 9,12,15-octadecatrienoate, sulphurous acid, cyclohexylmethyl hexyl ester, cyclopentane carboxylic acid, bis(2-ethylhexyl) phthalate, 4-methylpentyl ester, campesterol, squalene, and solanesol were recorded as least represented phytochemicals in any one of the solvent extract.

Flavonoids are one of the major types of polyphenols and most of them have been reported to have antioxidant, anti-inflammatory, and antidiabetic activities [24]. The amount of TPC observed in the present study was noticeably higher than the earlier reports. Previously, methanolic extract of the stem of *Equisetum telmateia* (*E. telmateia*) was recorded with 262.7 ± 1.0 mg RU/g pf TPC [25]. Stajner et al. [26] reported 1.75 ± 0.09 mg/g of TFC in aerial parts of *E. ramosissimum* phosphate buffer

extract. The results of this study revealed that the extracts of high polar solvents like methanol and aqueous showed highest amount of TPC and TFC in *E. ramosissimum* stem which showed highest extraction efficiency (Fig. 2). The polarity of solvent has a significant role in quantity of TPC and the efficiency of extraction for TPC and TFC is reduced with the decrease in polarity of solvents. It was well known that hydrophilic and hydrophobic features of phytochemicals recorded a high influence on their solubility, and hence, polarity of solvents used has a potential role in extracting efficiency of these phytochemicals [27].

Antioxidants can diminish the adverse effects of free radicals, viz., reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated during cellular metabolism. The ROS exert supportive effects on cellular redox signalling and immune functioning even at low concentrations. At higher concentrations, ROS and RNS deregulate several cellular functions and often lead to diverse pathological illnesses [28]. The use of plant-based natural antioxidants like flavonoids, phenolics, and tocopherols are biologically act at molecular level and reported to inhibit/reduce the formation of free radicals [29]. Antioxidant capacity of plant extracts could be due to the existence of polyphenolic compounds which have the ability to donate hydrogen atoms to their hydroxyl groups [17].

The DPPH was used as a source of free radicals, since it simulates ROS and RNS by in vitro that affect biological systems and involved in inhibiting the oxidative stress-induced cellular damage and lipid peroxidation [30]. To support our study, significant free radical scavenging effect with the DPPH method was reported by Paulsamy et al. [31] in methanolic extracts of *E. ramosissimum* whole plant with an IC_{50} of 78.58 µg/mL. According to Li et al. [32], ethyl acetate extract of *E. ramosissimum* at 200 µg/mL showed comparable DPPH activity with an IC_{50} of 43.41 ± 7.68 µg/mL. Previously, Stajner et al. [26] reported comparable antioxidant effect of aerial parts of

E. ramosissimum, *Equisetum arvense* (*E. arvense*), and *E. telmateia* with phosphate buffer extracts using FRAP methods with an effective IC_{50} of 5.44 ± 0.72 , 2.85 ± 0.45 , and 44.1 ± 2.11 $\mu\text{g/mL}$, respectively.

Methanolic extract of whole plant parts of *E. ramosissimum* showed ABTS scavenging activity of 1946.36 ± 2.12 μm of TE/g DW [31] as like our study. Also, ethanolic extract of *E. arvense* exhibited higher scavenging activity in ABTS assay with an IC_{50} of 98.13 ± 3.84 [33]. Our results of O_2^- radical scavenging activities are in accordance with studies of Nagai et al. [34] who reported significant O_2^- radical scavenging activities of aqueous and ethanolic extracts of *E. arvense* aerial parts with 7.33 and 54.2% of inhibition, respectively. They also reported 12.8 and 80.6% of inhibition by the stem of *E. arvense* with aqueous and ethanol extracts. Likewise, Canadanovic-Brunet et al. [35] reported the OH^- radical scavenging effect of the ethyl acetate and aqueous extracts of *E. arvense* aerial parts with the EC_{50} of 2.29 ± 0.11 and 3.29 ± 0.16 mg mL^{-1} , respectively. The values for antioxidant activity of methanolic and aqueous extracts of *E. ramosissimum* stem exhibited no significant changes in ABTS, hydroxyl radical, superoxide, and FRAP assays (Fig. 3).

The stem extracts of *E. ramosissimum* were effective against the growth of pathogenic bacteria (done by microdilution method) like *S. epidermidis*, *B. subtilis*, *R. equi*, *MRSA*, *E. coli*, *V. cholerae*, *S. typhi*, *P. aeruginosa*, *K. oxytoca*, and *C. freundii* which are recognized as well-known causative agents of UTIs (Table 3 and supplementary file). Sarkar et al. [36] reported a high degree of antibacterial activity in ethyl acetate extract of *E. ramosissimum* whole plant against *E. coli* and *S. aureus* with the zone of inhibition of 7–8 mm. Ethyl acetate extracts of the aerial parts of *Equisetum hyemale* showed good range of antibacterial activity (13.1–52.4 mg/mL) against the bacterial strains *S. aureus*, *E. coli*, and *P. aeruginosa* [37].

Conclusions

In the present study, we perceived for the first time that all the tested extracts derived from the *E. ramosissimum* stem possessed antioxidant and antibacterial properties. The GC–MS analysis of stem extract yielded 24 compounds with palmitic acid, linoleic acid, nonacosane, hexahydrofarnesyl acetone, and octacosane as prominent compounds. Our data suggest that the potency of tested biological activities is higher in polar solvents with considerable result in methanolic extract. The extracts recorded with significant amount of total phenolics and flavonoids exhibited significant antioxidant properties and antibacterial activities against the pathogenic bacteria. The notable effect of methanolic

and aqueous extracts against the uropathogenic bacteria supported the traditional uses of this plant in treating UTIs. The isolation of pure bioactive compounds may have the potential usage to improve and manage novel drugs in treating UTIs by investigating in vivo methods. Further investigations still need to be conducted on toxicity of stem extracts to effectively maximize human health benefits. This study draws attention to methanolic extracts of the stem of *E. ramosissimum* as a potent source of active principles that can be further utilized as a prominent bioresources in drug discovery efforts.

Abbreviations

ABTS: 2,2'-Azino-bis (3-ethylbenzo-thiozoline-6-sulfonic acid); $AlCl_3$: Aluminium chloride; DMSO: Dimethyl sulphoxide; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; *E. arvense*: *Equisetum arvense*; *E. ramosissimum*: *Equisetum ramosissimum*; *E. telmateia*: *Equisetum telmateia*; FRAP: Ferric reducing antioxidant power; GC–MS: Gas chromatography–mass spectrometry; MHA: Mueller-Hinton Ager; MIC: Minimum inhibitory concentration; O_2^- : Superoxide radical scavenging; OH^- : Hydroxyl free radical scavenging; TFC: Total flavonoid content; TPC: Total phenolic content; UTI: Urinary tract infection.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-021-00339-8>.

Additional file 1. Results of the minimum inhibitory concentrations of *E. ramosissimum* stem extracts.

Additional file 2. Raw data for the results of quantitative phytochemical investigation, antioxidant and antibacterial activities of *E. ramosissimum* stem extracts.

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Authors' contributions

JS, SA, JK, and AT evaluated the experimental data of antioxidant, phytochemical screening, and antibacterial activity. They also carried out the bioassay parts and drafted the manuscript. RM contributed to the characterization of GC–MS analysis. SSG and RM were accompanied in supervision of experimental part along with MA. VS contributed and helped in statistical analysis of the obtained results. MA revised the manuscript and supervised overall experimental and writing process. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The data obtained in the results of the study will be produced on request from the readers of manuscript as well as publishers, when required.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare that there are no conflicts of interest.

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