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Evaluation of *in vitro* antioxidant, cytotoxicity and FTIR analysis of whole plant extract of *Elephantopus scaber* L. (Asteraceae)

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Abstract

Elephantopus scaber L. belongs to the family Asteraceae, is a common wild herb that forms undergrowth in shady places. Decoction of whole plant is used as an antioxidant, antiinflammatory, antipyretic, diuretic, emollient and tonic. The main aim of the proposed work is the evaluation of *in vitro* antioxidant, *in vitro* cytotoxicity and FTIR analysis of crude methanolic whole plant extract of *E. scaber* L. In the preliminary phytochemical analysis glycosides, flavonoids, alkaloids, terpenoids, phenols, tannins and saponins were found whereas steroids were absent. In antioxidant analysis, two important assays such a DPPH radical scavenging assay and Nitric oxide radical scavenging assay were selected. In DPPH radical scavenging assay, it was noticed that the ability of methanolic whole plant extract had highest scavenging effect on the DPPH radical which was increasing with an increase in the concentration of the sample whereas in Nitric oxide scavenging assay, compared to standard drug ascorbic acid methanolic whole plant extract showed moderate radical scavenging activity. In *in vitro* cytotoxic analysis, whole plant extract showed potential cytotoxic activity against different cell lines like MCF 7 (Human Breast Cancer Cell line) and HCT 116 (Human Colon Cancer Cell Line). The cytotoxic effect was expressed as IC_{50}. The IC_{50} value against MCF-7 cell line was 78.76 ± 1.23 µg/mL whereas 98.40 ± 1.56 µg/mL against HCT-116 cell lines, which indicated that the crude whole plant extract of *E. scaber* L. showed high cytotoxicity effect against selected cancer cells lines. FTIR spectroscopic analysis revealed that various characteristics frequencies with peak values. This study suggests that whole plant extract of *E. scaber* L. exhibit high antioxidant and cytotoxicity potentials and will be helpful for the sustainable utilization of the plant.

Keywords

cytotoxicity; *Elephantopus scaber* L.; FTIR analysis; *in vitro* antioxidant

Introduction

Medicinal plants have been emerging as a part of modern life of man with greatest demand due to its nutritional, pharmaceutical, cosmetic and medicinal application without much negative impact (1). Due to the pharmaceutical importance in international market, cultivation of medicinal plants replaces the traditional agricultural crops by many farmers. Medicinal plants can be used to produce safe and new herbal drugs because of their important contemporary relevance to millions of people, to provide health security as well as current trend in improved research on medicinal action of herbal drugs (2). Herbal plants considered as a good antioxidant,

anticancerous, antiinflammatory, antidiuretic, antipyretic since ancient times (3). One of such medicinally important herbal plant is E*lephantopus scaber* L.

Elephantopus scaber L. belongs to the family Asteraceae, is a common wild weed that forms undergrowth in shady places. The plant has been abundantly found throughout India. It is commonly known as prickly leaved elephant foot (4). It is a coarse, erect, rigid, hairy herb. Stem are forked and stiff while leaves are mostly in basal rosette and oblong ovate and often very much notched on the margins. Young leaves cooked and eaten like spinach. Decoction of whole plant is used as an antioxidant, antiinflammatory, antipyretic, diuretic, anti cough agent, antibiotics, emollient and tonic (4). In India it is used in case of cardiovascular diseases, bronchitis and smallpox. Roots are used in heart and liver trouble. The paste of roots is tied around the ear to cure headache (5). The entire plant or its decoction is used for quick delivery and expulsion of placenta and external application of emulsion of dried bark is used for wound healing. Its fresh roots are chewed to treat cough, cold and headache (6). In general, very little biological information on the medicinal properties of this valuable ethno medicinal plant is available. Some properties have been documented previously but still this is a small attempt to evaluate the *in vitro* antioxidant, *in vitro* cytotoxicity and FTIR analysis of whole plant extract of *E. scaber* L.

Materials and Methods

Preparation of whole methanol extract

Plant materials were collected just after the rainy season from various places of Kollam district. The botanical identification was carried out and the herbarium of the plant bearing the voucher number TKMCAS/KOL/XI 2024/ SA/01 deposited the same at TKM College of Arts & Science, Kollam for future reference. Collected materials (Whole plant) were cleaned, weighed and dried under shade followed by oven drying. The dried powdered material (50g) was defatted with petroleum ether (60 to 80^{0}C) by hot extraction method in a Soxhlet apparatus to remove waxy substances. The defatted powder material was further extracted with methanol for 72 h. Concentrated methanol extract was used for preliminary phytochemical studies, *in vitro* antioxidant, cytotoxicity and FTIR analysis.

Preliminary phytochemical studies

The preliminary phytochemical study of crude methanolic whole plant extract of *E. scaber* L. was done for the detection of phytoconstituents, using standard chemical tests (7).

In vitro Antioxidant Activity of E. scaber L.

DPPH radical scavenging Assay (2, 2-diphenyl -1-picryl hydrazyl Assay)

Radical scavenging activity of the whole plant extract against stable 2, 2- diphenyl 2- picrylhydrazyl hydrate (DPPH) reagent was determined according to the method of Rao *et al*. (8) with slight modification. In this assay, the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ mL). A 60 µM solution of DPPH in methanol was freshly prepared and a 200 µL of this solution was mixed with 50µl of sample at various concentrations (3.12, 6.25, 12.5, 25, 50, 100 µg/mL). The plates were kept in the dark for 15 min at room temperature and the decrease in absorbance was measured at 517 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95 % methanol was used as blank. DPPH radical scavenging activity was calculated by using the following formula:

% inhibition =

(O.D of control - O.D. of Test/O.D. of control) × 100 (Eqn.1)

The antioxidant activity of the extract was expressed as IC_{50}. IC_{50} value was defined as the concentration (in µg/ mL) of extracts that inhibits the formation of DPPH radicals by 50 % (9).

Nitric Oxide Scavenging Assay

Nitric oxide radical inhibition was estimated using Griess Illosvory reaction. In this investigation, Griess Illosvory reagent was generally modified by using naphthyl ethylene diamine di hydrochloride (0.1 %) instead of 1-napthylamine (5 %). The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitro prusside, 0.5 mL saline phosphate buffer and 0.5 mL of standard solution or samples (6.25-200 µg/mL) were incubated at 25°C for 150 min. After incubation, 1 mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for the completion of the reaction of diazotization. After this, a further 1 mL of the naphthyl ethylene diamine dihydro chloride was added, mixed and was allowed to stand for 30 min at 25°C. The concentration of nitrite was assayed at 540 nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but the same condition should be followed). Here buffer was used as blank solution and Ascorbic acid was taken as standard solution (10). The antioxidant activity of the extract was expressed as IC_{50}.

% inhibition =

(O.D of control - O.D. of Test/O.D. of control) ×100 (Eqn.2)

In vitro Cytotoxic Activity of *E. scaber* L.

MTT cell viability assay

MCF 7 -Human Breast Cancer Cell and HCT 116 -Human Colon Tumor Cell Line were purchased from National Centre for Cell Sciences (NCCS), Pune, India, were maintained in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10 % heat inactivated Fetal Bovine Serum (FBS) and 1 % antibiotic cocktail containing Penicillin (100 µg /mL), Streptomycin (100 µg/mL) and Amphotericin B (2.5 µg/mL). The cell containing TC flasks (25cm^2) were incubated at 37 ˚C at 5 % CO_2 environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany). The cells (2500 cells/ well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37 ˚C and 5% CO_2 environment in the incubator for 24 h. The test samples were prepared in DMEM media (10 mg/mL) and

filter sterilized using 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50 and 100 µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2h for the development of formazan crystals. The supernatant was removed and 100 µL DMSO were added per well. The absorbance at 570 nm was measured with micro plate reader. Two wells per plate without cells served as blank. All the experiments were done in triplicates (11).

The cell viability was expressed using the following formula:

Percentage viability= (OD of test/OD of control) × 100 (Eqn.3)

Fourier Transform Infrared Spectrophotometeric (FTIR) Analysis

FTIR analysis is an important powerful tool for identifying the types of functional groups (Chemical bonds) present in compounds. Dried powder of whole plant methanolic extract were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellets, to prepare translucent sample discs and was loaded in FTIR spectroscope, with a scan range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1}(12).

Statistical Analysis

All the above experiments were carried out in triplicate. Experimental assays results were expressed as Mean ± standard deviations were calculated by GraphPad InStat DTCG. Analysis of variance was done according to Tukey-Kramer Multiple Comparisons Test.

Results

Preliminary Phytochemical Evaluation of E. scaber L.

The phytochemical screening aids in chemo profiling, which helps in determining the major therapeutically and pharmacologically useful constituents present in the plant extracts. The result of phytochemical screening of *E. scaber* L. were shown in the Table 1. Conventional protocols detected the presence of important secondary metabolites such as glycosides, flavonoids, alkaloids, terpenoids, phenol and tannins which could be the reason for highest antioxidant and cytotoxicity activity of the plant.

In vitro Antioxidant Activity

An antioxidant is a molecule capable of inhibiting the activity of free radicals. In this study, two important assays such as DPPH radical scavenging assay and Nitric oxide scavenging were used to evaluate free radical scavenging activity of whole plant extract of *E. scaber* L.

DPPH radical scavenging Assay (2, 2-diphenyl -1-picryl hydrazyl Assay)

In the present study, it was noticed that the ability of methanolic whole plant extract of *E. scaber* L. had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 3.12 to 100 µg/mL (Fig.1). Maximum scavenging activity (95.41±0.34 %) was observed at 100 µg/mL concentration and the IC_{50} value of the whole plant extract of *E. scaber* and its standard ascorbic acid were found to be 58.60±2.45 µg/mL and 38.176±1.78 µg/mL respectively.

Table 1. Preliminary phytochemical evaluation of *E. scaber* L

Phytochemicals	Standard chemical test	Whole plant extracts of *E. scaber* L.
Glycosides	Keller-Killani Test	++
Flavonoids	Shinoda Test	+
Alkaloids	Dragendorff's Method	++
Terpenoids	Libermann-Burchard Method	++
Steroids	Libermann-Burchard Method	-
Phenols	Ferric chloride test	++
Tannins	Lead Acetate test	++
Saponins	Frothing Test	++

Nitric Oxide Scavenging Assay

In the present study, methanolic whole plant extract of *E. scaber* L. showed significantly moderate capacity to compete with oxygen and reduce the production of nitrite ions (Fig.2). The IC_{50} value of whole plant extract and its standard drug ascorbic acid were found to be 105.57±2.12 µg/mL and 74.33±1.67 µg/mL respectively.

In vitro Cytotoxic Activity of *E. scaber* L.

In the present study, methanolic whole plant extract of *E. scaber* L. showed potential cytotoxic activity against different cell lines like MCF 7 (Human Breast Cancer Cell line)

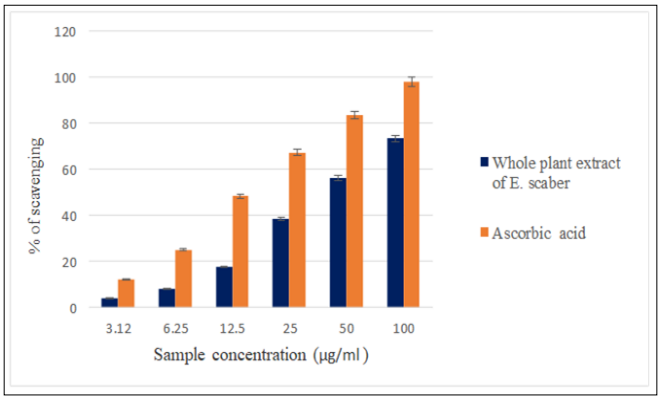

Fig. 1. Effect of methanolic whole plant extract of *E. scaber* in DPPH radical scavenging assay.

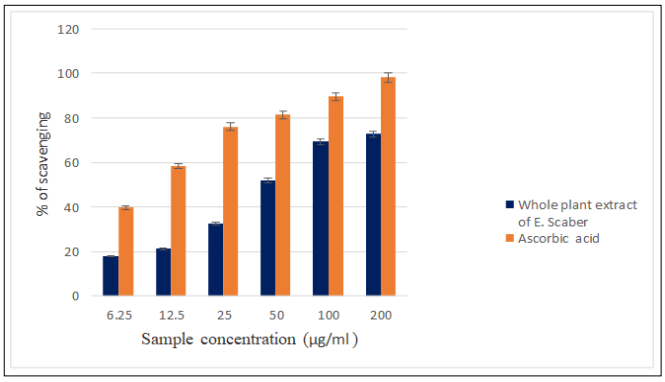

Fig. 2. Effect of methanolic whole plant extract of *E. scaber* in nitric oxide radical scavenging assay.

and HCT 116 (Human Colon Cancer Cell Line). Methotrexate was used as standard drug. Cells which were pre-treated with various concentrations of whole plant extracts (6.25, 12.5, 25, 50 and 100 µg/mL) showed high percentage of cancer cell death which was increased with increasing concentration of sample. The maximum cytotoxicity was observed with 100 µg/mL of the sample (Table 2, Fig.3 & 4). The cytotoxic effect was expressed as IC_{50}. To be a good drug, the IC_{50} values of such agent should be sufficiently low to avoid any possible unspecific effects. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product exerts an IC_{50} value < 30 µg/Ml (5). The IC_{50} value against MCF-7 cell line was 78.76 ± 1.23 µg/mL whereas 98.40 ± 1.56 µg/mL against HCT-116 cell lines which indicated that the crude whole plant extract of *E. scaber* L. showed significantly high cytotoxicity effect compared to standard drug methotrexate.

Table 2. Cytotoxic effect of the standard drug (methotrexate) and methanolic whole plant extract of *E. scaber* in MTT assay

Sample concentration (µg/mL)	% of cell death			
	MCF 7 cell line		HCT 116 cell line	
	Whole plant extract of *E. scaber*	Standard drug (Methotrexate)	Whole plant extract of *E. scaber*	Standard drug (Methotrexate)
6.25	2.57±0.25	5.31±3.21	2.71±0.65	11.23±1.39
12.5	7.06±0.45	12.65±1.52	6.41±1.04	25.32±2.74
25	19.45±0.89	25.36±0.78	12.05±0.54	37.12±0.89
50	38.21±1.11	49.43±0.23	27.42±1.12	51.38±1.25
100	59.95±1.22	74.13±1.83	50.02±1.34	79.65±2.61
IC50	78.76±1.23 µg/mL	50.92±2.35 µg/mL	98.40 ±1.56 µg/mL	56.211±3.18 µg/mL

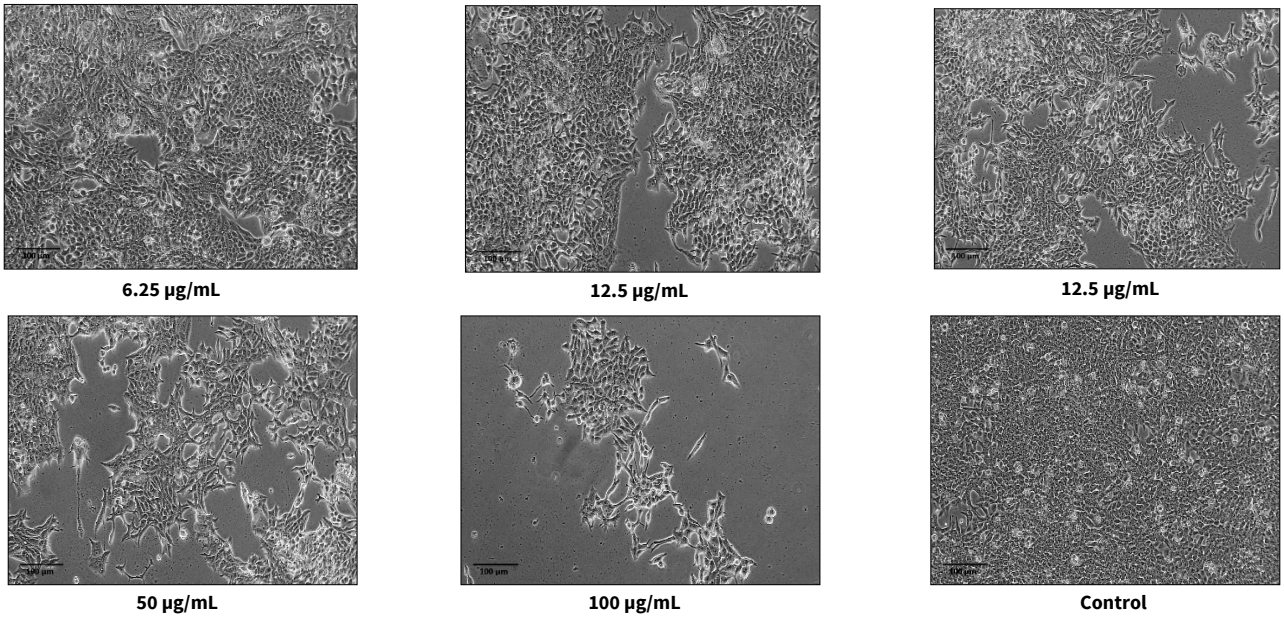

Fig. 3. Effect of crude methanolic whole plant extracts of *E. scaber* L. in MTT assay against MCF 7 cell line.

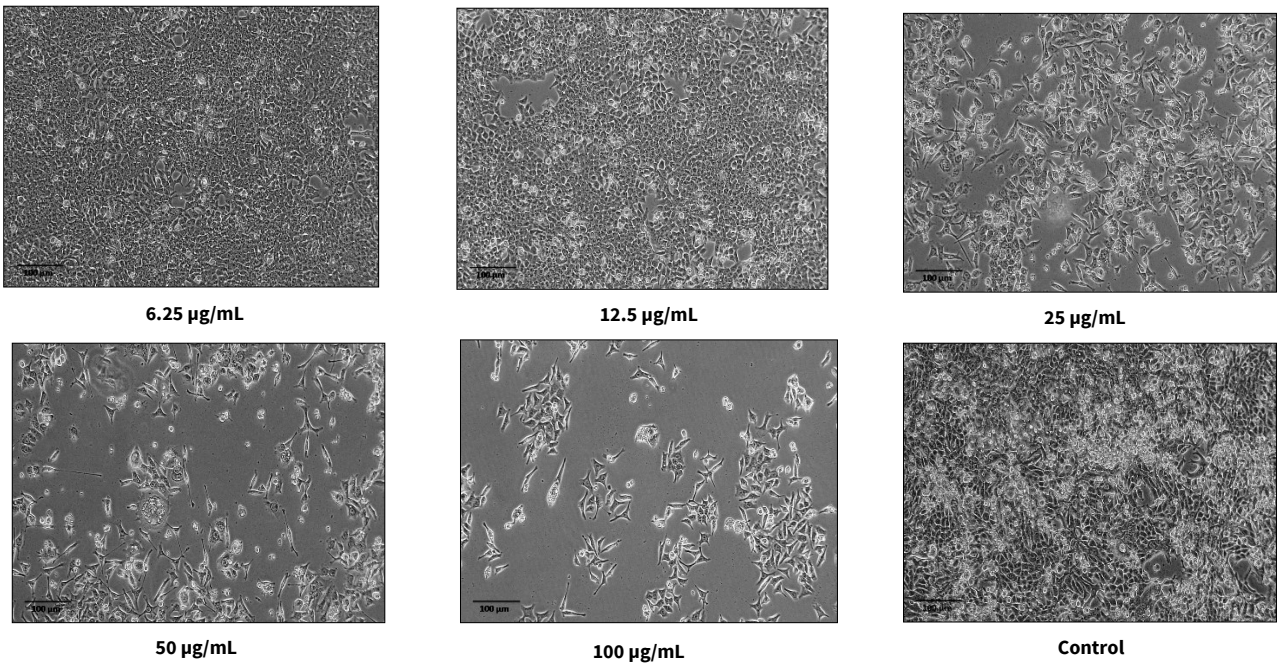

Fig. 4. Effect of crude methanolic whole plant extracts of *E. scaber* L. in MTT assay against HCT116 cell line.

Fourier Transform Infrared Spectrophotometeric (FTIR) Analysis

The functional groups or chemical bonds present in the whole plant extract of *E. scaber* were predicted using FTIR spectral analysis which revealed the presence of various functional groups such as phenolic O-H stretch (3755cm^{-1}), methylene C-H stretch (2937 cm^{-1}), conjugated ketones (1699 cm^{-1}), amine C-N stretch (1278,1050 cm^{-1}) and aryl disulfides. (436 cm^{-1}) (Table 3 and Fig. 5).

Table 3. FTIR spectra of the whole plant extract of *E. scaber* L

Sl No.	Peak values (Frequency,	Wave number (cm^{-1})	Functional group assignment
1	3755	3750-3645	O-H stretching Phenols
2	2937	2935-2915	Methylene C-H stretch
3	2336	2700-2250	N-H stretching
4	1699	1580-1699	C=C conjugated with C=O
5	1398	1410-1310	Phenols or tertiary alcohol, O-H bend
6	1278	1340-1250	Aromatic primary amine, C-N stretch
7	1050	1090-1020	Primary amine, C-N stretch
8	436	500-430	Aryl disulfides (S-S stretch)

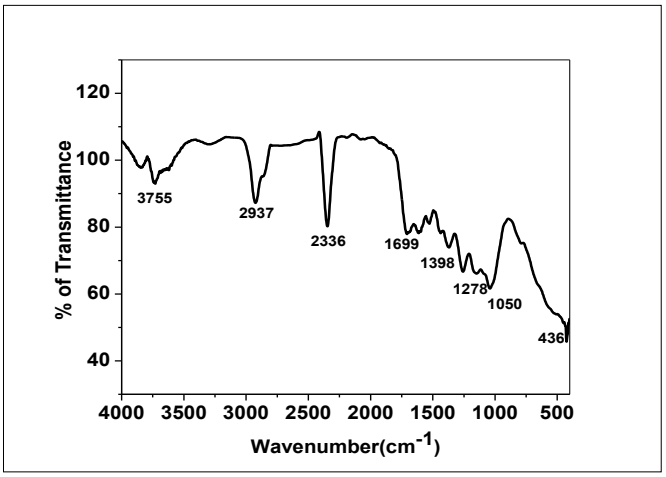

Fig. 5. FTIR spectra of the whole plant extract of *E. scaber* L.

Discussion

DPPH (1,1-diphenyl-2-picryl hydrazyl) is a stable free radical with purple colour which turns yellow when scavenged. The DPPH assay used this character to show free radical scavenging activity. Antioxidants reacted with DPPH and reduced it, therefore absorbency decreased. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (14). In the present study, it was noticed that the ability of whole plant extract of *E. scaber* had significant scavenging effect on the DPPH radical. Highest scavenging activity (95.41 ± 0.34 %) was observed at 100 µg/mL concentration. Similarly highest DPPH scavenging activity of ethanolic leaf extract was reported where the maximum scavenging activity (78.73 ± 0.89 %) at 100 µg/mL concentration of sample (14). Another study reported that the maximum DPPH radical scavenging activity of the methanol extract of *E. scaber* L. root and leaves was reported to be 37.83 % and 45.23 %, respectively, at a concentration of 200 µg/mL (15). Compared to these, the result of whole plant extract of *E. scaber* showed highest DPPH scavenging activity than that of root and leaves extracts.

In Nitric oxide scavenging assay, sodium nitro prusside in aqueous solution at physiological pH, spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of nitric oxide may lead to tissue damage (16). Moderate level of nitric oxide scavenging activity was noticed in methanolic extract of *Doronicum hookeri* Hook f. (16) which was low as compared to *E. scaber* L.

Cancer is a class of diseases in which a cell or a group of cells represents uncontrolled growth, invasion and metastasis. For the treatment of cancer, limited number of effective anticancer drugs is currently in use, even though they have higher cases of nausea, vomiting, diarrhea, skin rashes and headache, etc (5). So that there is real need for new, side effect safe, cheap and effective anticancer drugs to combat this dreaded disease. Natural products continue to be a major source of pharmaceuticals and for the discovery of new bioactive molecules (17). The cytotoxic activity of the ethanol extract of *E. scaber* L. on MCF-7-derived multicellular tumor spheroids has been reported (11). Additionally, the cytotoxic effect of *E. scaber* extract against breast cancer (T4&D) cells has also been documented (17). In the present study evaluated the cytotoxic activity against MCF 7 and HCT 116 cancer Cell Lines. The IC_{50} value against MCF-7 cell line was 78.76 ± 1.23 µg/mL whereas 98.40 ± 1.56 µg/mL against HCT-116 cell lines which indicated that the crude whole plant extract of *E. scaber* L. showed significantly high cytotoxicity effect compared to standard drug methotrexate.

The FTIR analysis was mainly used for the identification of functional group of the active compounds in the samples based on the peak value in the region of infrared radiation. In the present study, FTIR analysis was performed for the identification of functional group in the whole plant extract of *E. scaber*. The FTIR analysis of methanolic leaf and root extracts of *E. scaber* has been reported to indicate the presence of characteristic functional groups such as carboxylic acids, amines, amides, sulfur derivatives, nitrates and carbohydrates (15). In the present study indicated the presence of phenolic O-H group (3755cm^{-1}), methylene C-H group (2937 cm^{-1}), conjugated ketones (1699 cm^{-1}), amine group (1278,1050 cm^{-1}) and aryl disulfides. (436 cm^{-1}) Phenolic and conjugated ketones might be the reason for antioxidant and anticancer properties of the plant.

Conclusion

The present study concluded that the methanolic whole plant extract of *E. scaber* L. act as a promising antioxidant and anticancer agent. FTIR analysis revealed the presence of various secondary metabolites which might be contributing for the medicinal properties of the plant. This will help the maximum utilization of the plant to isolate their active constituents to develop novel antioxidant and cytotoxic drugs.

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Authors' contributions

GS conceived and designed the experiments, analysed and interpreted the data, MSJ wrote the original draft, review and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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