



ASSESSMENT OF PHYTOCHEMICAL, ANTIOXIDANT AND CYTOPROTECTIVE POTENTIAL OF METHANOLIC EXTRACT OF *ERYNGIUM FOETIDUM* L. (APIACEAE)- AN UNEXPLOITED MEDICINAL PLANT FROM THE KARADIMALA WILD, KERALA

Sruthi T¹, Dr. U. Danya²

¹M.Sc Student, Nirmala College For Women, Coimbatore

²Assistant professor, Nirmala College For Women, Coimbatore

Abstract

Eryngium foetidum commonly known as Culantro has been used in traditional medicine and also an edible food. It is cultivated worldwide being grown as an annual plant in temperate climates. In the present study phytochemical, antioxidant and cytoprotective studies of the whole plant of *E. foetidum* has been concentrated. Both qualitative and quantitative phytochemical analysis was evaluated for the plant. Antioxidant studies of the plant was carried out using DPPH and ABTS assays respectively. Cytotoxic activity of the plant was analysed against breast cancer cell line (MCF-7) using MTT assay. The qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, polyphenols and tannins. The quantitative analysis of the methanolic extract revealed the presence of tannins in high amount followed by polyphenols and glycosides. Antioxidant studies of methanolic extract of the plant revealed IC₅₀ values of 62.27mg/L and 53.33mg/L for DPPH and ABTS assays respectively. The results revealed that the plant has significant antioxidant activities. The study also revealed the cytotoxic activity of *E. foetidum* against the breast cancer cell line (MCF-7). Thus the present study reported that *E. foetidum* is rich in phytochemicals which is the reason for its antioxidants and its cytotoxic activity.

Keywords: *Eryngium foetidum*, phytochemical analysis, antioxidant, cytotoxic, MCF-7

INTRODUCTION

Medicinal plants have been used in traditional medicine since ancient times. *Eryngium foetidum* is an annually grown herb commonly known as Culantro. The plant is native to Mexico, Caribbean, Central and South America but it grown worldwide. It is an edible food which cure many human diseases. The phytochemicals like alkaloids, flavonoids, tannins, phenols, glycosides and antioxidants can be used for cancer prevention. Eryngial is a chemical compound isolated from the plant. The plant is used to cure burns, fevers, fits, asthma, malaria, diarrhea and stomach ache. The another name for the plant is *E. antihystericum*, in which the species name denotes that this plant is used to cure epilepsy (Paul *et al*, 2010). In the present study, methanolic extract of the plant is evaluated for phytochemical, antioxidant and cytotoxic activities.

MATERIALS AND METHODS

Plant material

The selected plant, *Eryngium foetidum* has been collected from Karadimala, a rural village of Palakkad district of Kerala. The healthy and fully grown plant was shade dried and finely powdered for further experiments. The sample was undergone cold extraction method for preparing the methanolic extract of the plant.

Phytochemical analysis

Qualitative analysis

The plant extract was tested for phytochemicals alkaloids, tannins, flavonoids, glycoside, phenol, saponins and steroids. They are determined by the following phytochemical tests by the method of Harborne 1984, Wagner *et al.* 1984 and Sthal *et al.*, 1965.

Qualitative Phytochemical Tests

SL. NO	PHYTOCHEMICALS	TEST	PROCEDURE
1	Alkaloids	Dragendorff's test	Few mL extract + 1-2 mL <i>Dragendorff's reagent</i>
2	Tannins	Braymer's test	1mL extract + 3mL distilled water + 3 drops 10% Ferric chloride solution
3	Flavanoids	Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution
4	Glycosides	Raymond's test	Extract solution + dinitrobenzene in hot methanolic alkali
5	Phenols	Iodine test	1mL extract + few drops of dil. Iodine sol.
6	Saponins	Foam test	5ml distilled water + plant extract + few drops of olive oil and mix well
7	Steroids	Chloroform test	2 mL chloroform + conc. H ₂ SO ₄ + 5mL plant extract

Quantitative analysis

Determination of Total Polyphenol Content (Folin-Ciocalteu, 1927)

20µl Sample is treated with 6.980ml distilled water, 2ml Sodium carbonate (Na₂CO₃) and 0.8ml Folin's reagent and is allowed for incubation at 2 hours. Then the optical density is measured at 765nm. The control contains 7ml distilled water, 2ml Na₂CO₃ and 0.8ml Folin's reagent.

Determination Of Total Tannin Content (Folin-Ciocalteu, 1927)

20µl Sample is added with 980 µl of distilled water and 4.5ml Na₂CO₃. Then it is allowed to stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation. The optical density is measured at 725nm. The control is prepared by treating 1ml distilled water with 4.5ml Na₂CO₃. Then it is allowed to stand for 10 minutes. The add 0.5ml Folin's reagent and a 30 minutes incubation.

Determination Of Total Glycoside Content (Balget's test, 1981)

1ml of sample is treated with 1ml freshly prepared Balgets's reagent (95ml 1% Picric acid + 5ml 10% NaOH). Incubated for one hour. After incubation diluted with 10ml distilled water and the absorbance was read at 495nm.

Determination Of Total Alkaloid Content (Harborne, 1973)

5g sample is treated with 30ml 10% Glacial Acetic acid is covered and allow to stand for 5 hours. Sample is filtered, concentrate on water bath to get 1/4 of its original volume. Then add 10ml concentrated Ammonium hydroxide dropwise with continues stirring until the precipitate was complete. All the solution is allowed to settle. Collect the precipitate and washed with diluted Ammonium hydroxide (5ml ammonium hydroxide + 5ml water) and the filtered through a pre-weighed filter paper. The residue was dried and weighed.

Determination Of Total Flavanoid Content (Bohm and Kocipai-Abyazan, 1994)

5g sample is treated with 30ml 80% methanol. Cover and allowed to stand for 2 hours. Whole solution was filtered through the Whatman filter paper No:42. The filtrate was transferred into a crucible (pre-weighed) and evaporated into dryness and weighed to a constant weight.

Antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay (Blois, 1958)

A solution of free radical was prepared by dissolving DPPH in 100ml methanol. Then a test solution was added to the methanolic DPPH. The mixture prepared was shaken well and kept in room temperature in the dark. The absorbance of the mixture was measured at 515nm spectrometrically and also the absorbance of the DPPH radical without blank was measured. IC-50 value was calculated and a calibration curve was plotted.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Assay (Keesey,1987)

The ABTS radical was prepared by reaction with ABTS in water and potassium persulfate in 1:1 ratio and stored in room temperature. ABTS solution was diluted using methanol to obtain the absorbance at 734nm. After adding the plant extract to the prepared ABTS solution, the absorbance was measured. The percentage inhibition of absorbance at 734nm was calculated and a graph was plotted.

Cytotoxic Activity Against Breast Cancer Cell Line (MCF-7)**MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Mosmann, 1983)**

MCF-7 (Human Breast cancer) cell lines were cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico *et al.*, 2004).

RESULTS AND DISCUSSION**Phytochemical study**

The present study has been carried out to assess the phytochemical screening, antioxidant activity and cytotoxic activities of methanolic extract of *E. foetidum*. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, glycosides, polyphenols and tannin (Table-1). In the review of Aswathy and Oomen, there is only information about the qualitative analysis of *E. foetidum*. But the present study revealed the quantitative details about the study plant. The quantitative analysis of the methanolic extract reveals the presence of tannins in high amount followed by polyphenols and glycosides (Table-2).

The results are similar with the studies of Thi *et al.*, in 2020, they have explained the phytochemical studies of aqueous and ethanolic extracts of *E. foetidum*. The studies have revealed the presence of flavonoids, tannins, alkaloids and terpenoids in both the plant extracts. In another study, Wang *et al.*, 2012 also explained the phytochemical activities of *E. foetidum*. They revealed that presence of terpenoids, saponins, flavonoids, coumarine, polyacetylenes and steroids in the plant extract. In another study the methanolic extract of *E. foetidum* have alkaloids, phenols and flavanoids (Anata *et al.*, 2016). And also Malik *et al.*, in 2016 evaluated the phytochemical screening of *E. foetidum* plants from Manipur, they discovered the plant is rich in flavonoids, tannins, alkaloids and terpenoids. The results revealed the presence of alkaloids, flavonoids, tannins and glycosides. The present study revealed that the methanolic extract of *E. foetidum* was rich in secondary metabolites, may be it is a reason for its significant biological activity.

TABLE 1: Preliminary qualitative phytochemical analysis of methanolic extract of *Eryngium foetidum*

SL.NO.	PHYTOCHEMICALS	METHONOLIC EXTRACT
1	Alkaloids	+
2	Flavanoids	+
3	Tannins	+
4	Glycosides	+
5	Saponins	-
6	Polyphenols	+
7	Steroids	+

+: Present -: Absent

TABLE 2: Preliminary quantitative phytochemical analysis of methanolic extract of *Eryngium foetidum*

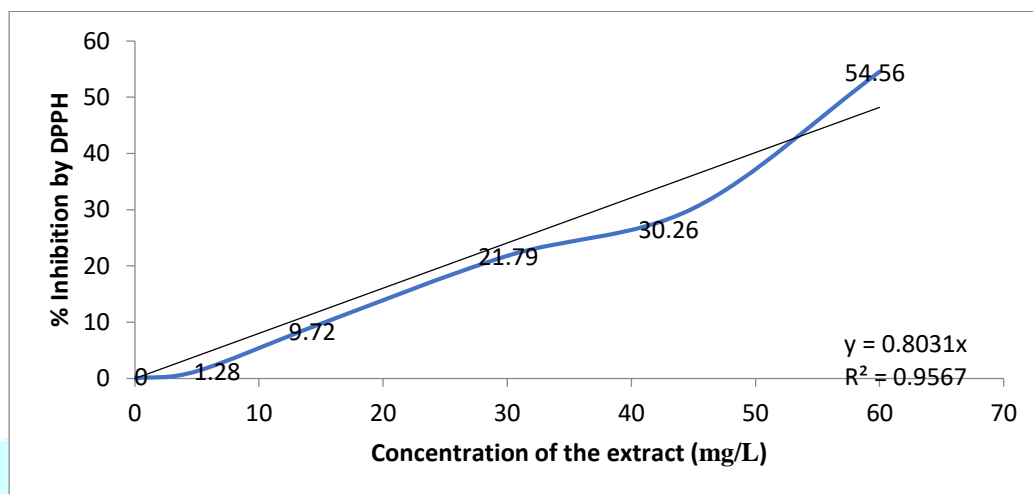
SL.NO.	PHYTOCHEMICALS	VALUES (mg/g)
1	Alkaloids	11.14
2	Flavanoids	14.3
3	Glycosides	15.5
4	Tannins	29.5
5	Polyphenols	19.16

Antioxidant activity

The antioxidant study reveals the free radical scavenging property of the methanolic extract of *E. foetidum*. The DPPH and ABTS assays have been widely used to determine the free radical-scavenging activity of various plants and pure compounds. Among numerous methods for antioxidant activity estimation, DPPH and ABTS are the most popular and commonly used ones due to their ease, speed, sensitivity and the usage of stable radicals. Six varying concentrations (0, 5, 15, 30, 45 and 60 mg/L) of methanol solvent extract of *E. foetidum* demonstrated different percentage of inhibition.

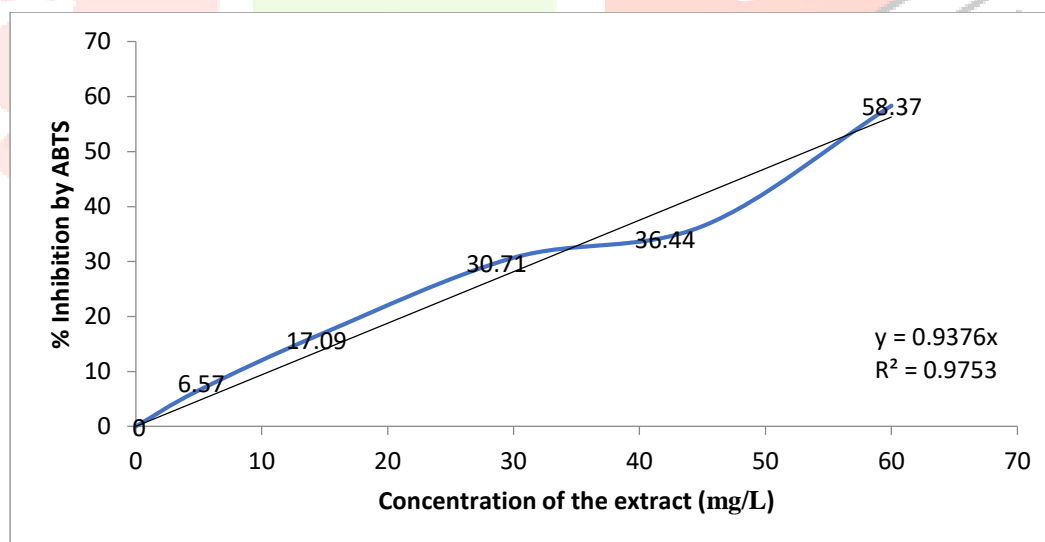
The methanolic plant extracts were most active and fully scavenged DPPH. The 60 mg/L extract showed 54.56% of inhibition in methanolic extract. The 5 mg/L extract showed lowest antioxidant activity and the scavenging activity of extract was increased with increase in concentration. Linear regression equation is used for calculating the IC₅₀ value of the free radical scavenging method, and the IC₅₀ value was 62.27mg/L (Figure-1).

Figure 1: Shows the radical scavenging activity of *Eryngium foetidum* represented by percentage of inhibition by DPPH



The methanolic plant extracts were most active and fully scavenged ABTS. The scavenging activity of extract was increased with increase in concentration. The plant extract showed high amount of ABTS radical scavenging activity in 53.33 mg/L concentration. The 5 mg/L extract showed lowest antioxidant activity. The 60 mg/L extract showed 58.37% of inhibition in methanolic extract. The results were expressed as trolox equivalence in µg/L extract (Figure-2).

Figure 2: Shows the radical scavenging activity of *Eryngium foetidum* represented by percentage of inhibition of by ABTS



The IC₅₀ value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC₅₀ value, the higher the antioxidant activity of samples. Thus, DPPH assay showed the highest antioxidant activity of the sample. The results obtained for the antioxidant screening of plant showed that they had appreciable amount of bioactive components. The plant contained appreciable amount of metabolites like alkaloids, saponin, tannin, phenols and flavonoids. It also contained free radical scavenging property which could have resulted to the inhibitory activity exhibited by plant the extract.

The results are similar with the studies of Anata *et al.*, 2016 and Mallik *et al.*, 2016 have studied the antioxidant activity of *E. foetidum* from lower Assam and Manipur respectively. The antioxidant activity of the plant in both the studies was determined by DPPH assay which revealed that the plant has high antioxidant property. Thi *et al.*, 2020, explained the antioxidant activity of aqueous and ethanolic extracts of *E. foetidum* using DPPH and ABTS scavenging assay. DPPH assay exhibited stronger scavenging activity than ABTS assay. In another study, antioxidant study of *E. foetidum* was evaluated by Shashi *et al.*, in 2014. The methanolic extracts of *E. foetidum* by linear regression analysis were expressed as IC₅₀ value found to be

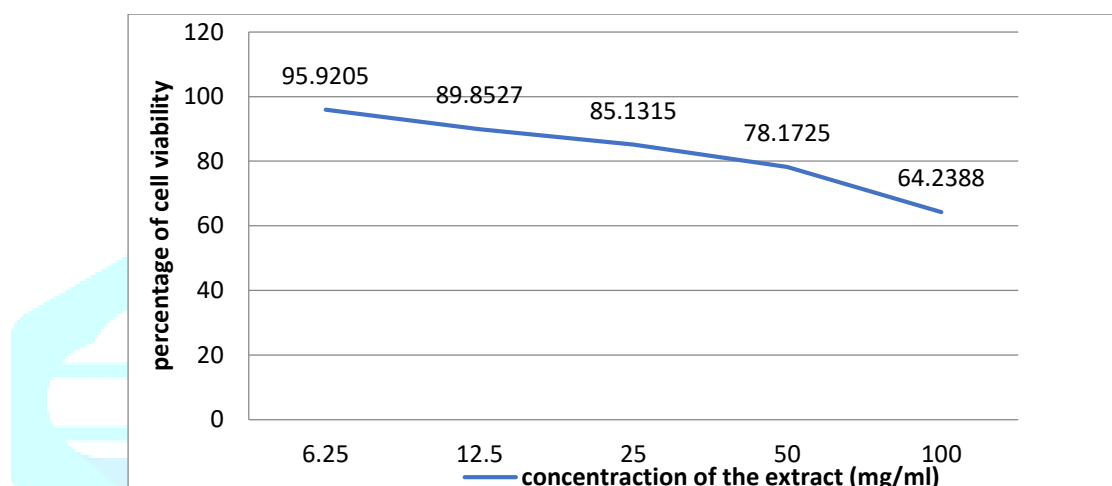
131.94mg/L for DPPH assay. The present study revealed the significant antioxidant activities of *E. foetidum*. It was evaluated by ABTS and DPPH assays to confirm the free radical scavenging activity of the plant.

Cytotoxic study

The methanolic extract of *E. foetidum* has selectively cytotoxic to the Human breast cancer cell line (MCF-7).

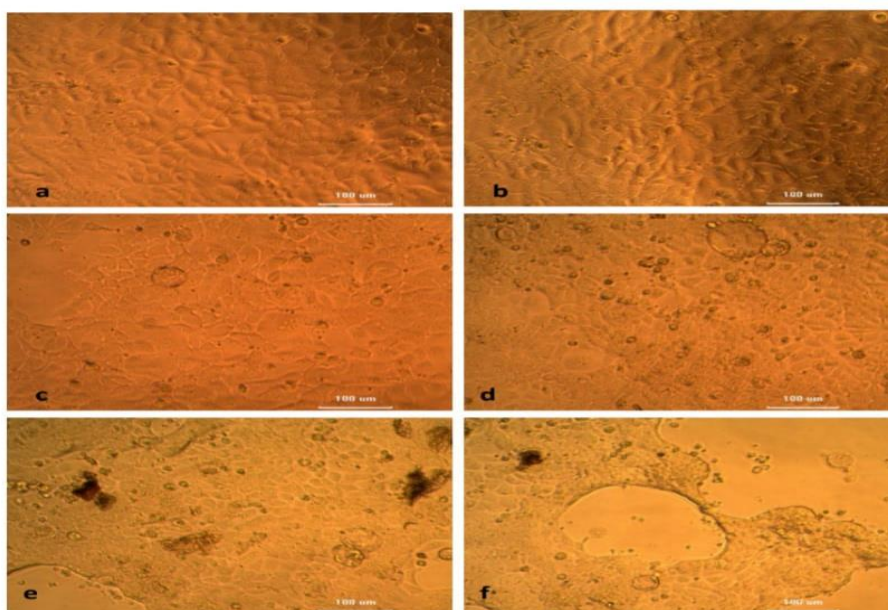
The experimental results demonstrate that various concentration of extract has the ability to inhibited cell proliferation in a dose dependent manner. The IC_{50} values of extract against human breast cancer cells were calculated as 142.352 μ g/ml. The control cells did not show any remarkable changes on their morphology. However, in the presence of extract the cells shows the improved cell shrinkage, membrane blebbing and forms floating cells in a dose-dependent manner. It is well accepted that cytological investigations elucidate the antiproliferative effect routed through membrane blebbing, membrane instability and distressing the cytoskeleton of the cells by the extract. The results showed that the methanolic extract of *Eryngium foetidum* has significant anticancer activity (Figure-3).

Figure 3: Shows the cytotoxicity of methanolic extract of *Eryngium foetidum* determined through MTT assay



Evaluation of cytotoxic effects of four turkish species of *Eryngium* was studied by Berna *et al* in 2019. Cytotoxic activities on prostate carcinoma and endometrial cancer cells were analyzed by cytotoxic activity assay. The plants exhibited cytotoxic effects on endometrial cancer cells. Kevin *et al*, 2017 studied the cytotoxic effects from the essential oils of *Eryngium campestre* and *Eryngium amethystinum*. They evaluated their cytotoxic effects on human cancer cells namely A375, MD A-AB 231 cells and HCT 116 cells by MTT assay. The results turned out to be highly cytotoxic on tumor cells with IC_{50} values comparable or close to those of the anticancer drug cisplatin.

***In vitro* cytotoxicity effect of various concentration of methanolic extract of *Eryngium foetidum* against human breast cancer cell line (MCF-7)**



- a) Control
- b) Treated with methanolic extract (6.25mg)
- c) Treated with methanolic extract (12.5mg)
- d) Treated with methanolic extract (25mg)
- e) Treated with methanolic extract (50mg)
- f) Treated with methanolic extract (100mg)

CONCLUSION

The medicinal properties of the plant mainly depend on phytochemical constituents that have great pharmacological significance. It has great potential to be developed as a drug by pharmaceutical industries. The present study was undertaken to find out the phytochemical screening, antioxidant activity and cytotoxic activities of the plant *Eryngium foetidum* in methanolic extract.

The preliminary phytochemical screening reveals the presence of alkaloid, flavanoid, glycoside, polyphenol and tannin. The quantitative analysis revealed that the *Eryngium foetidum* extract had the highest concentration of tannins with a value at 29.5mg/L and polyphenols content with a value at 19.16mg/L. In DPPH assay, the IC-50 value of the methanolic extract was 62.27mg/L and in ABTS assay IC-50 value was 53.33mg/L. The results obtained for the antioxidant screening of plant showed that they had appreciable amount of bioactive components. The methanolic extract of *Eryngium foetidum* has selectively cytotoxic to human breast cancer cell line (MCF-7). The significant cytotoxicity of the study plant is described as IC₅₀ value of 142.352 µg/L.

In conclusion, it is apparent that the pharmacological activities of *E. foetidum* reflect its uses in traditional medicine. The plant exhibited good relationship between phenolic groups and flavonoids content and its antioxidant activity. The plant showed significant antioxidant and cytotoxicity activities with very low toxic effects. Consequently, the isolation of bioactive compounds from this plant might be our future research.

REFERENCE

- Aswathy P M and Saj O P (2014). Carminative, phytochemical and antioxidant potentialities of the leaf extracts of *Eryngium foetidum* L.(Apiaceae). *World journal of pharmacy and pharmaceutical sciences*, 3(6): 2269-2280.
- Celik B O, Kara E M, Guzel C B, Genc G E, Genc I, Anil S K and Melikoglu G (2019). Evaluation of antimicrobial and cytotoxic effects of four Turkish species of *Eryngium* L. *Bangladesh Journal of Botany*, 48(2): 271-278.
- Cianfaglione K, Blomme E E, Quassinti L, Bramucci M, Lupidi G, Dall'Acqua S and Maggi F (2017). Cytotoxic essential oils from *Eryngium campestre* and *Eryngium amethystinum* (Apiaceae) growing in central Italy. *Chemistry & biodiversity*, 14(7):186-195.
- Dai X, Cheng H, Bai Z, and Li J (2017). Breast cancer cell line classification and its relevance with breast tumor subtyping. *Journal of Cancer*, 8(16): 3131.
- Fraternale D, Ricci D, Calcabrini C, Guescini M, Martinelli C and Sestili P (2013). Cytotoxic activity of essential oils of aerial parts and ripe fruits of *Echinophora spinosa* (Apiaceae). *Natural product communications*, 8(11): 114-126.
- Harborne J B (1973). Phytochemical methods. *London: Chapman and Hall, Ltd*, pp. 49-188.
- Karakaya S, Ozdemir O, Koca M, Demirci B, Aksakal O, Turkez H and Baser K H C (2020). Cytotoxic effect and molecular docking studies of essential oils of *Cymbocarpum erythraeum* (DC.) Boiss.(Apiaceae) as potential inhibitors of cholinesterase. *Journal of Essential Oil Research*, 32(5): 436-448.
- Lingaraju D P, Sudarshana M S, Mahendra C and Rao K P (2016). Phytochemical screening and antimicrobial activity of leaf extracts of *Eryngium foetidum* L(Apiaceae). *Indo American Journal of Pharmaceutical Research*, 6(2): 4339-4344.
- Malik T, Pandey D K, Roy P and Okram A (2016). Evaluation of Phytochemicals, Antioxidant, Antibacterial and Antidiabetic Potential of *Alpinia galanga* and *Eryngium foetidum* Plants of Manipur (India). *Pharmacognosy Journal*, 8(5): 459-464.
- Tharasaraswathi K J, Chandrika R, Jayalakshmi N R and Mythri B (2014). Ecotypic variations in Indian populations of *Eryngium foetidum* L. *International Journal of Current Research and Review*, 6(4): 1-6.
- Thi N Q N, An T N T, Nguyen O B, Dung L T, Minh L V and Nhan L T H (2020). Phytochemical Content and Antioxidant activity in aqueous and ethanolic extracts of *Eryngium foetidum* L. *Materials Science and Engineering* 991(1): 26.
- Thomas P S, Essien E E, Ntuk S J and Choudhary M I (2017). *Eryngium foetidum* L. essential oils: chemical composition and antioxidant capacity. *Medicines*, 4(2): 24.
- Wang P, Su Z, Yuan W, Deng G and Li S (2012). Phytochemical constituents and pharmacological activities of *Eryngium* L.(Apiaceae). *Pharmaceutical Crops*, 3: 99-120.