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Phytochemical investigation and heavy metal analysis of a miracle plant *Eryngium foetidum Linn.*

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Abstract

Herbs are rich source of secondary metabolites that have been found to have medicinal properties. The present study was conducted to evaluate the phytochemical and heavy metal analysis whole plant of *Erygium foetidium Linn*. The obtained results revealed that Petroleum ether and Chloroform extract contains less phytochemical constituents when compared with, Ethyl acetate and Ethanolic extracts in maximum quantity. The Heavy metals anlaysis for powder were determined by atomic absorption spectroscopy method (AAS). The results showed doesn't contains high toxic levels of heavy metals; (Arsenic-0.022 mg/L; below the detectable level, Chromium 0.005 mg/L; and Lead 0.006 mg/L below the detectable level).

Keywords: Phytochemical Constituents, Atomic Absorption Spectroscopy Method, *Eryngium foetidum Linn.*

Introduction

Medicinal herbs have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. They often contain highly active pharmacological principles including minerals and trace metals(1). According to World Health Organization (WHO) estimates, nearly 70–80% of the world population still primarily relies on nonconventional medications, mostly derived from herbs(2,3). India is known for its rich tradition of herbal medicine, and Indians are quite familiar with the medicinal and flavoring properties of several herbs. Herbs are traditionally used for the treatment and prevention of chronic ailments. The toxicity of herbal plants may be related to contaminants such as pesticides, microbes, heavy metals, chemical toxins, and adulterants(4,5). In general, the geography, the geochemical soil characteristics, contaminants in the soil, water, and air, and other growth, transport, and storage conditions can significantly affect the properties and the quality of the herbal plants and their constituents(6,7).

Materials and Methods

***Plant Materials*:** The whole plant of *Erygium foetidium Linn*. was collected from the Marthandam, Kanya kumari Dist., Tamilnadu in the month of December. Healthy and mature plants were selected for the study. The taxonomic identification of plant was authenticated by Dr. C. Madhava Chetty, Professor, Dept. of Botany, SV University, Tirupati, Chittoor Dist., A.P, India and deposited to the department as a sample herbarium. The plants were thoroughly washed with tap water followed by second distilled water to remove the dirt and specks of dust.

***Drying*:** The cleaned plants were cut into small pieces and were left for shade drying for 15 days. Afterwards, they were dried in hot air oven at 40°C for 2 h to remove the equilibrium moisture before the extraction process.

***Extraction*:** Extraction was performed by Soxhelt extraction technique(11). Whole plants of *Erygium foetidium Linn*. were pulverized by using a mechanical grinder, which was later sieved through mesh size 80 to get the powder of uniform size. Around 10 g of the powder was packed in a thimble of whatmann's filter paper. The apparatus was then assembled and the extractions were carried out using 250 ml each of as Petroleum ether, Chloroform, Ethyl acetate

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and Ethanol as solvent. The temperature was maintained at 35-40°C. The extraction was continued for 10 h for Ethanol, 5 h for Petroleum ether, Chloroform, Ethyl acetate following the color of the solvent collected in the thimble chamber. Ten milliliters each of concentrated extract was kept for phytochemical screening and remaining extracts were dried. The dried extract was used for isolation of phytoconstituents and Pharmacological activities. Preliminary Characters and percentage yield of Petroleum ether, Chloroform, Ethyl acetate and Ethanol extracts of *Erygium foetidium L*. is presented in Table 1 & Fig. 1

Phytochemical Screening: Phytochemical screenings were carried out for Petroleum ether, Chloroform, Ethyl acetate and Ethanol extracts as per the standard methods(6,7,8,9) and following tests were performed:

Test for Alkaloids

a. Mayer's Test: To 2 ml of each extract, few ml of 2N HCl along with few drops of Mayer's reagent was added. Gelatinous white precipitation confirms the presence of alkaloids.

b. Wagner Test: To 2 ml of extract few drops of Wagner reagent was added, a reddish brown precipitation observed confirms the presence of alkaloids.

Test for Saponin

Froth Flotation Test: 2 ml of sample was added in a test tube with few ml of water, a froth observed and persisted on constant shaking confirms the presence of saponin.

Test for Tannins and Phenolic Compound: To a 2 ml of extract few drop of 5% $FeCl_3$ solution was added, blue black precipitation confirms the presence of tannins and phenolic compound.

Table 1: Preliminary Characters and % Yield of Various Extracts of *Erygium Foetidium Linn.*

S No	Solvent	Color of Extract	Percentage Yield
1.	Petroleum ether	Dark Green	33.3%
2.	Chloroform	Light Green	8.41%
3.	Ethyl acetate	Light Green	8.31%
4.	Ethanol	Brown	20.0%

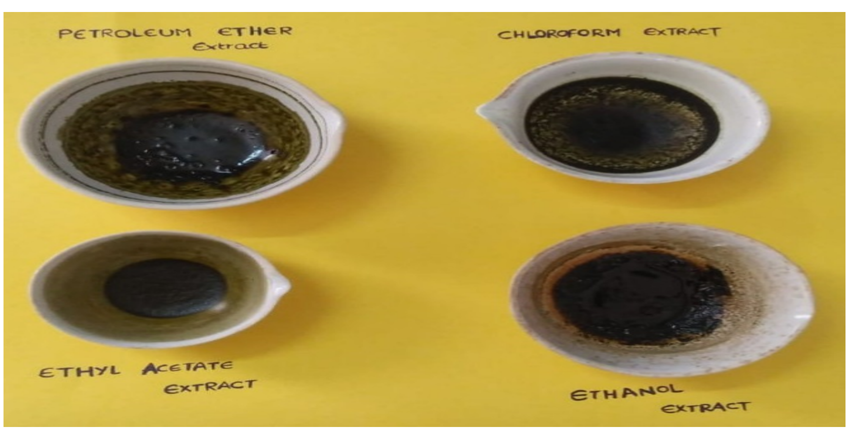

Fig. 1. *Preliminary Characters of Various Extracts of Erygium Foetidium Linn.*

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***Test for Flavonoids*:** To 2 ml of extract, 1 ml of lead acetate solution was added. An intense yellow color was appeared which confirms the presence of flavonoids.

***Test of Cardiac Glycoside*:** To 2 ml of plant extract, 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml of concentrated H_2SO_4 was added. Appearance of a brown ring indicates the presence of cardiac glycoside.

***Test for Reducing Sugars*:** Fehling test: 1ml each of Fehling's A and Fehling's B solutions was mixed and boiled for 1 min and equal volume of test solution was added. The whole solution is heated in a boiling water bath for 5–10 min. Formation of brick red ppt. confirmed the presence of carbohydrate.

***Test for Glycoside (Killer Killiani Test)*:** To 2 ml of extract, 1 ml of glacial acetic acid, few drop of $FeCl_3$, and few drops of concentrated H2SO4 were added. Green/blue precipitation indicates the presence of glycoside.

***Test for Emodins*:** To 2 ml of extract, 2 ml of NH_4OH and 3 ml of benzene were added. Red coloration indicates the presence of emodins.

***Test for Phlobatannins*:** To 2 ml of extract, 2 ml of 1% HCl was added and heated. Red precipitate indicates the presence of phlobatannins.

***Test for Terpenoids*:** To 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added. A reddish brown coloration indicates the presence of terpenoids.

***Test for Protein*:** To 2 ml of extract, few drops of concentrated H_2SO_4 was added on it. White precipitate indicates the presence of protein.

***Test for Steroid (Salkowski Test)*:** To 2 ml of extract, 2 ml of $CHCl_3$ and 2 ml of conc. H_2SO_4 were added on it. A reddish brown ring at the junction indicates the presence of steroid.

Heavy Metal Analysis

The quantification of heavy metals in the plant powder was done by flame AAS technique equipped in ICE 3000 series atomic absorption spectrometer. For this, 1.0 g of dried plant powder was taken in a 250ml conical flask, and 5 ml of conc. HNO_3 (GFS Chemicals Inc., Columbus, 69%) was added slowly. The mixture was heated on the hot plate till the brown fumes disappeared yielding the white fumes. Water was added to make the solution, and it was then filtered in a 50 ml volumetric flask. Finally, the volume was adjusted to 50 ml by adding triple distilled water up to the mark(16,17). This filtrate was then introduced in flame AAS for the detection of metals.

Results and Discussion

The phytochemical screening revealed the presence of phenols were present in all extracts. Flavanoids, were present in all three petroleum ether, Ethyl acetate and ethanol extracts(10). Terpenoids were present in Chloroform, Ethyl acetate and ethanol extracts and but were absent in petroleum ether extract. Anthraquinones, emodins and glycoside were present only in Ethyl acetate extract and tannins were present only in ethanol extract and absent in all three extracts. The concentration of alkaloid by Mayer's test was medium in the ethanol extract. Similarly, in Wagner's test, it was high in ethanol and low in petroleum ether extract. The reducing sugar was at high concentration in petroleum ether and low in ethanol. Proteins were absent only in petroleum ether extract and remaining have extracts with low concentration. The report of phytochemical screening is presented in (Table 2).

Environment, pollution, atmosphere, soil, harvesting and handling are some of

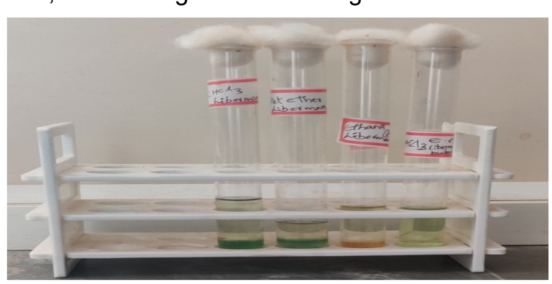

Figure. 2 Test for Steroids Various Extracts of *Erygium Foetidium Linn.*

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Table 2: Phytochemical Compositions of Various Extracts of *Erygium Foetidium Linn.*

Chemical Test	*Eryngium foetidium* Extract			
	Pet. Ether	Chloroform	Ethyl Acetate	Ethanol
Test for alkaloid				
1. Mayer's test	_	_	+	++
2. Wagner test	_	_	+	++
Test for saponin	_	_	_	+++
Test for phenol	+	+	++	++
Test for flavonoids	+	_	++	+++
Test for glycoside	_	_	++	+++
Test for reducing sugar	+	+	+	_
Test for steroid	+	_	+	++
Test for tannins	_	_	_	+
Test for protein	_	_	_	_
Test for terpenoids	_	+	+	+++
Test for emodins	_	_	_	_
Test for anthraquinone	_	_	+	_

Table 3: Heavy Metal Analysis of Dried Powder of Plant Erygium *Foetidium Linn.*

S. No.	Heavy Metal	Wavelength	Concentration	Normal Level
1.	Arsenic [As]	188.979	0.022 mg/L [BDL]	0.053mg/L
2.	Chromium [Cr]	267.716	0.005 mg/L	0.007 mg/L
3.	Lead [Pb]	220.353	0.006 mg/L	0.042 mg/L

the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Therefore it is necessary to measure and establish the levels of metallic elements in the herbal plants as these elements when consumed at higher levels become toxic(10-12). The dried powder of plant *Erygium foetidium L*. analyzed for the presence of important heavy metals such as Arsenic, Chromium and Lead in this study. The results showed doesn't contains high toxic levels of heavy metals; (Arsenic-0.022 mg/L; below the detectable level, Chromium 0.005 mg/L; and Lead 0.006 mg/L below the detectable level) (Table 3). The heavy metal content did not exceed the limit given according to the WHO guidelines 2005(12-15).

Conclusion

The outcome of the present studies revealed that various phytochemicals including alkaloids, steroids, terpenoids, glycosides, anthraquinones, etc., are present while tannins

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and emodins were found absent in this plant. Heavy metals like As, Cr, Pb are below the detectable level according to the WHO guidelines, which provide biological significance of the studied plant. The studies therefore suggest that the folklore use though is very resourceful; the authenticity yet needs serious research. The closely resembling species may sometimes create ambiguity that even leads to fatal disorders. With more resources and time, further investigation and isolation of phytochemical constituents of plant *Erygium foetidium L*. can be revealed.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement

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