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## ECOTYPIC VARIATIONS IN INDIAN POPULATIONS OF *ERYNGIUM FOETIDUM* L.

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### ABSTRACT

**Aim:** The ecotypic variations with respect to total phenolics, flavonoid, tannin and Vitamin-C content in three different populations of *E. foetidum* collected from Andaman, Darjeeling (West Bengal) and Hassan (Karnataka) have been studied.

**Methodology:** The total phenolic and total tannin content was estimated by FC method against standard pyrogallol and gallic acid respectively. Vitamin C was extracted with 4% oxalic acid solution and estimated using 2, 4- dinitrophenyl hydrazine reagent alongside standard ascorbic acid. The total flavonoid content was determined by Aluminum chloride colorimetric method with standard Quercetin (1mg/ml). The values were expressed as mg/g equivalents of respective standards.

**Results:** A significant difference ( $p=0.05\%$ ) in the concentration of secondary metabolites among all the studied populations was observed. The total phenolic content was highest in the Darjeeling population, flavonoid and tannin content was maximum in Andaman population and Vitamin C content was predominantly high in the Karnataka population.

**Conclusion:** The variations in phytochemical constituents could be attributed to the possible interaction of the plant populations with their geographical location and their subsequent adaptations to the same.

**Keywords:** *E. foetidum*, ecotypes, total phenols, flavonoid, tannin, vitamin C.

### INTRODUCTION

*Eryngium* is a large and taxonomically complex genus of the family Apiaceae comprising nearly 250 species distributed in many parts of Asia, America, Africa and Australia (Worz *et al*, Calvino *et al*). *Eryngium foetidum* L. commonly named as “wild coriander” (Seaforth *et al*) is the most popular herb under this genus extensively used for seasoning of a variety of continental dishes like salads, soups, sauces, noodles, and ceviche owing to its unique, pungent coriander-like aroma in most of the tropical regions. The herb has a wide range of usage in traditional medicine for treating cold, cough, fever, seizures, ear ache, hypertension, worms, infertility complications, snake bites, arthritis and malaria

(Mitchelle *et al*, Shavandi M A *et al*). The scientific validation for the medicinal properties of the plant has been established for analgesic and anti-inflammatory properties (Garcia *et al*, Saenz *et al*, Mekhora C *et al*), anti-helminthic (Forbes WM, 2009) and anti-clastogenic activity (Promkum C *et al*). The essential oil distilled from this plant has also been valued in perfumery industry for its aromatic properties in international and domestic markets (Wong *et al*, Pino *et al*, Ignacimuthu S *et al.*, 2004).

In recent years, much attention has been given to the intake of phenolic acids, tannins, flavonoids and vitamin C in the human diet and analyzing the possible health benefits due to their antioxidant nature. These secondary metabolites are believed

to be responsible for the wide spectrum of pharmacological activities seen in many plants. Nowadays, these are receiving considerable attention because of their reported protective role against cancer, heart disease, health promoting effects like anti-inflammatory, anti-microbial, antioxidant, prevention of osteoporosis, as well as protection of plants from pests and diseases. Vitamin C (a water soluble vitamin) acts as a coenzyme for large number of metabolic activities in the living organisms and also possess antioxidant property (Amina Abd El – Hamid ALY 2010, Daniel Modniki, Maciej Balcerek, 2009; Daniel Modniki, Maciej Balcerek 2009, Min B.R *et al.*, 2008).

In India, occurrence of *E. foetidum* is rare, endemic and is localized to small pockets of Tamil Nadu, Kerala, Karnataka, North East India and Andaman Island (Chandrika R *et al.*, 2011, Kar & Borthakur 2007). The restricted distribution of the plant is attributed to the long dormant period before seed germination and the dependence of vital phenological events on first showers of monsoon (Chandrika *et al.*, 2013). Hence, the present study was conducted to observe the effect of ecological variations and its impact on the production of secondary metabolites (total phenolics, flavonoids, tannin and vitamin C) in various Indian populations.

## MATERIALS AND METHODS

### Plant collection

The populations of *E. foetidum* were collected from Hassan, Karnataka ( 12°. 967N and 75°.783 E), Andaman Islands (16° to 14° N and 92° to 94° E) and West Bengal, Darjeeling (27° 01' 59'' N and 88° 16' E) during August-September 2012. The climatic conditions of all the locations were recorded for purpose of interpretation of the present experimental findings (Table 1). The plants were identified and a voucher specimen of each population was deposited at the Regional Research Institute, Bangalore.

### Preparation of plant extract:

About 20g of dried plant material was extracted with 200 ml of methanol with occasional agitation at RT for 24 h. The extract was filtered, centrifuged at 8000g for 10 min, supernatant collected, rotary evaporated at 25<sup>0</sup> C and stored in air tight bottles at 4<sup>0</sup> C till further use.

### Determination of total phenolic, total tannin and total flavonoid content:

The total phenolic and total tannin content was estimated by F-C method as according to Singleton VL and Rossi JA (1965) N and Tamilselvi *et al.*, (2012) with 1mg/ml of pyrogallol and gallic acid as a standard. The unknown extract values are expressed as mg/g of pyrogallol and gallic acid equivalents. The total flavonoid content was determined by Aluminum chloride colorimetric method (Chang C. *et al.*). Quercitin (1mg/ml) was used as a standard and the values are expressed as mg/g of Quercitin equivalents.

### Extraction and estimation of total Vitamin C content:

Vitamin C was extracted with 4% oxalic acid solution and estimated by using 2, 4- dinitrophenyl hydrazine reagent with A540 nm (S Sadashivam and A Manickam, 1997). Standard ascorbic acid at 5mg/ml was used to derive a calibration curve and the results are expressed as mg/g of ascorbic acid equivalents.

### Statistical analysis

The variation in secondary metabolite content was statistically analyzed by applying ANOVA followed by Tukey HSD test and results are expressed as mean  $\pm$  S.E (n=10) with p=0.05 as a limit of significance.

## RESULTS

The mean variations in secondary metabolites from different populations are represented in Table 2 and graphically in Fig. 1 with Tukey HSD analysis at 0.05% level of significance. The total phenolic content in the dried plant extract was significantly high in Darjeeling population

followed by Andaman and least in Karnataka population. Likewise the total flavonoid and tannin content was elevated in Andaman, followed by Darjeeling and lowered in Karnataka. Finally, the total vitamin C content was notably high in Karnataka population followed by Andaman and least in Darjeeling. In comparison with other secondary metabolites, Vitamin-C content was found to be quantitatively high in all the populations studied.

## DISCUSSION

The variations among the ecotypes for Vitamin-C and secondary metabolite content are due to the biological diversity of *E. foetidum* populations in India. In the present study, all the ecotypes of *E. foetidum* showed very high concentration of Vitamin-C when compared to other secondary metabolites (total phenolics, flavonoid and tannins). Vitamin C is found to be the major constituent distributed in the plant leaves of *E. foetidum* (Campos *et al.* 2007; Promkum *et al.* 2012). Vitamin C was higher in the Karnataka population with significant difference ( $P < 0.05$ ) compared with other two ecotypes. The Darjeeling population showed significantly lower Vitamin-C content than the other ecotypes. Variations in the content of Vitamin-C have been reported by Koziol (1992) while studying *Chenopodium quinoa* Willd ecotypes. According to Jimenez *et al.* (2009) the variations can be attributed to genetic or environmental growth conditions. Lee and Kader (2000) reported more Vitamin- C production when grown under cool temperatures rather than extreme hot and cold temperatures. The variation in Vitamin C content has also been observed with extended storage, higher temperature, low relative humidity, physical damage, chilling injury, large genotypic variations and climatic conditions (Lee and Kader, 2000; Dumas *et al.*, 2003; Xu *et al.*, 2008). According to Wall (2006), the levels of Vitamin-C in plants is directly proportional to the availability of light to the plant. In contrast, an excess of soil Nitrogen or

Phosphorus tend to decrease ascorbic acid content. Hence it is possible to infer that the Karnataka population growing in a favorable moderate climatic condition contained high Vitamin-C content. Whereas, Darjeeling region with extreme cold conditions and limited supply of sun light yielded considerably low yield of Vitamin- C. The climate of Andaman Islands being hotter with high precipitation and humidity levels would have favored relatively higher production of Vitamin- C content in the populations of that region. The high Vitamin- C content reported among the populations in this investigation was similar to the data reported by Campos *et al.* (2007) in *E. foetidum*, Ruales & Nair (1993) and Dini *et al.* (2010) in *Chenopodium quinoa* Willd.

All the ecotypes of *E. foetidum* presently studied showed that the variation in secondary metabolite production. Previous studies by Shui *et al.* showed that variation in secondary metabolite was related to the balance between carbohydrate source and sink. The greater the source-sink ratio, higher the production of secondary metabolites, which is directly linked to the plant growth in a particular ecotype. The presence of higher primary nutrients in the soil like nitrogen, phosphorous and potassium increases the secondary metabolite production (Tucker, 1999) and an even minor variation significantly varies the concentration of these metabolites (Jaleel *et al.* 2008). This implies that the increased nutrient content in the soil could have enhanced the production of total non structural carbohydrates which in turn would have contributed towards higher production of secondary metabolites (Ibrahim MH, 2010) in *E. foetidum* populations from Darjeeling and Andaman populations.

## CONCLUSION

In the present study the variation in the polyphenol and vitamin C contents in *E. foetidum* populations from India were determined. This finding helps us to know the importance of secondary compounds showing significant variation among different

ecotypes. The significance involved in relating external and edaphic factors to trace out the performance of secondary metabolite production in the ecotypes needs further emphasis.

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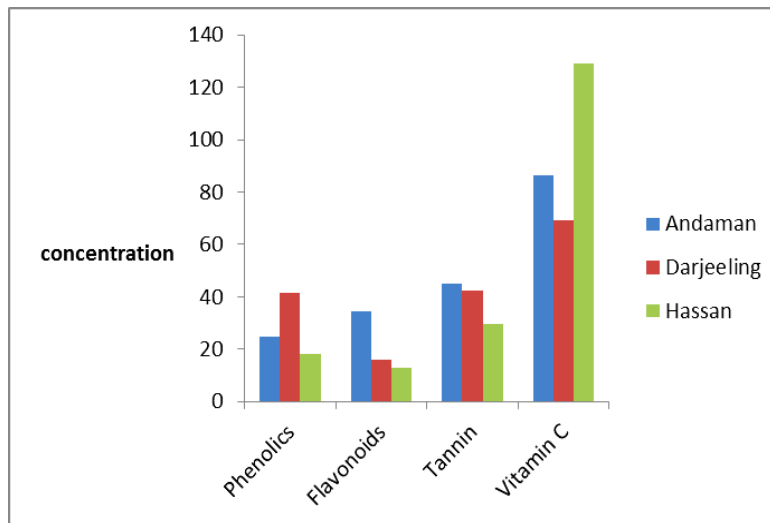
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**Table-1: Climatic conditions of the various geographical locations from where *E.foetidum* populations were collected**

Climatic factors	Hassan (Karnataka)	Andaman Islands	Darjeeling (West Bengal)
Temperature ( <sup>0</sup> C)	24-27(max); 12-14(min)	29-31(max);19-21(min)	11-19 (max); 6-7(min)
Precipitation (mm/yr)	2900-3100	3176-4200	2000-3200
Humidity (%)	80-90	79.4-85	80-95

**Table-2: Variation in Mean±S.E values of total phenolics, flavonoid, tannins and vit-C content in populations of Andaman, Darjeeling and Karnataka (P=0.05% level of significance, Tukey HSD)**

Ecotypes	Total Phenolics mg Pyrogallol/g	Flavonoid mg QE/g	Tannins mg GAE/g	Vitamin C mg /g
Andaman	24.953±0.33466	34.358±0.19004	45.026±0.2295	86.314±0.2160
Darjeeling	41.61±0.29059	15.765±0.18223	42.333±0.2182	69.066±0.2700
Karnataka	18.099±0.2319	12.957±0.22044	29.55±0.2121	129.09±0.2460



**Fig-1: Concentration of phenols, flavonoid, tannin and vitamin C in populations of Andaman, Darjeeling and Karnataka**