



RESEARCH ARTICLE

EVALUATION OF ANTI-OXIDANT ACTIVITY OF *ELETTARIA CARDAMOMUM* AND *FERULA ASSA-FOETIDA* LEAVES WITH SPECIAL EMPHASIS ON PHYTOCHEMICALS AND HPLC FINGERPRINTING

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ABSTRACT

In present study the methanolic extracts were evaluated for phytochemical composition, total Phenolic content, flavonoid content, antioxidant potential by Total antioxidant assay, and Reducing activity assay. Methanolic extracts of two traditionally used Indian medicinal plants namely *Elettaria cardamomum* and *Ferula assa-foetida* were selected for the study. Phytochemical analysis of plant extracts indicated the presence of major phyto constituents, including phenolics, alkaloids, flavonoids, and saponin. Total phenolic content (1.71 mg/ml and 1.40 mg/ml expressed as gallic acid equivalents) was observed in *Elettaria cardamomum* and *Ferula assa-foetida* respectively. Antioxidant activity was measured by Phosphomolybdenum method. The methanolic extract of *E. Cardamomum* and *F. Assa-foetida* showed total antioxidant capacity and it was 25.60 mg/ml and 18.43 mg/ml respectively calculated as Ascorbic acid equivalents. Both the extracts exhibited the higher Reducing activity. The higher scavenging activity was observed in *Ferula assa-foetida* (82.55%) as compared with *Elettaria cardamomum* (54.0%). The results of one-dimensional TLC analyses show that different phenolic compounds, flavonoids and phenolic acids, are present in the investigated extracts. A largest number of flavonoids (rutin, quercetin and some unidentified flavonoid-glycosides) and phenolic acids (chlorogenic, caffeic, coumaric and vanillic acid) was found in methanol extract. The extracts contain Rutin, some unidentified flavonoid-glycosides, coumaric, caffeic and chlorogenic acid. HPLC analysis showed that the extracts of *E. Cardamomum* and *F. Assa-foetida* leaves contain various secondary metabolites. The standards used for analysis were tannic acid, vanillin and catechol. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

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INTRODUCTION

Oxidation is one of the most important processes, which produce free radicals in food, chemicals and even in living systems. Free radicals have an important role in the processes of food spoilage, chemical materials degradation and also contribute to more human disorders in human beings. Active oxygen and in particular, free radicals are considered to induce oxidative damage in bio molecules and to play an important role in aging, cardiovascular diseases, cancer and inflammatory diseases (Gupta *et al.*, 2010). They are believed to cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Antioxidants, on the other hand, significantly prevent tissue damage that stimulates wound healing process (Fitzmaurice *et al.*, 2011).

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There are available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxytoluenes (BHT), tertiary butylated hydroquinone and gallic acid esters, but have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. There has been an increasing interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury since it is the belief that the antioxidant property is the main contributory factor to the therapeutic benefit of many medicinal plants (Nayak *et al.*, 2006). As a result, many vegetables, fruits and many other plant species have already been exploited commercially either as antioxidant additives or a nutritional supplements (Schuler *et al.*, 1990) or have been investigated in the search for novel antioxidants (Chu, 2000; Koleva *et al.*, 2002). It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds (Pokornyns *et al.*, 1997; Subhangkar *et al.*, 2012) such as

flavonoids which are a group of polyphenolic compounds with known properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Shetty *et al.*, 2008). Some evidences suggest that the biological actions of these compounds are related to their antioxidant activity (Halliwell, 1981). Medicinal Plants with antioxidant or free radical-scavenging activity thus can play a significant role in cancer (Kamath *et al.*, 2003) and in the correction of many human disorders. The selected plants were *Elettaria cardamomum* and *Ferula assa-foetida* (concentration-1mg/ml). Literature has revealed that several anti-cancerous plants also possess considerable antioxidant properties as evident from the results of various *in vitro* and *in vivo* assays. The current study was therefore designed to explore the potential sources of natural antioxidants from the leaves of *Elettaria Cardamom* and *Ferula assa-foetida* plants in order to examine the significant role it plays not only in cancer but its contribution to health in general. In view of this screening project, studies on the *in vitro* activity on methanolic leaf extracts of two medicinal plants of India were investigated to assess their antioxidant properties in different antioxidant property determination assays include Reducing activity assay, Total antioxidant activity, Total Phenol test and Total Flavonoids test were studied in this report. Ascorbic acid, Quercetin and Gallic acid were used as antioxidant standard compounds respectively.

Objective of Research

Antioxidants are vital substances which own the ability to protect the body from damage caused by free radical induced oxidative stress. Epidemiological studies postulate that intake of fruits and vegetables have the ability to inhibit the damaging behavior of free radicals in the human body. No study has been conducted on the antioxidant activity of *Elettaria Cardamom* and *Ferula assa-foetida* leaves. Hence, the present study was carried out to explore these properties in leaf extracts of both plants.

MATERIALS AND METHODS

Chemicals and Materials Used

Reagents and solvents used were of analyticals grade of the highest commercial quality and without further purification. Particularly, methanol and distilled water were used as solvents. Folin-Ciocalteureagent. Gallic acid powder, methanol and sodium carbonate powder were purchased from Merck., gallic acid powder were purchased from Sigma-Aldrich Delhi (INDIA)

Plant material collection

The leaves of *Elettaria cardamomum* and *Ferula assa-foetida* were collected from Hapurchungi Road nursery, Ghaziabad. The material was identified by the Plant taxonomist NaliniVemurai in the Department of botany, Venketeshwar college, Delhi University.

Preparation of methanolic extract of leaves

The collected leaves *Elettaria cardamomum* and *Ferula assa-foetida* were cut into small pieces when got properly dried with the help of mixer.

Extraction of the powdered leaf of the plant

Maceration

The powdered leaves (5 g) was weighed and soaked in 100 ml of methanol in a conical flask. The flask containing the leaves was shaken, corked and left to stand on shaker for 48 h at room temperature. After 48 h, the mixture was filtered by Whatman filter no1 and the extract was collected and concentrated by evaporation to dryness in evaporating dish (Trease and Evans, 1997). Keep it for 50-72 hours in desiccator. The dried methanol extract was stored in refrigerator for phytochemical screening and antioxidant study.

Percent of yield was calculated as follows:

$$\text{Extract yield \%} = (W1/W2) \times 100$$

Where, W1 is net weight of powder in grams after extraction and

W2 is total weight of wood powder in grams taken for extraction.

II) Qualitative photochemical screening

The first phase of the study was to carried out to identify the methanolic extract of the leaves of *Elettaria cardamomum* and *Ferula assa-foetida*. screened for the presence of phytochemicals according to the method of Khandelwal (2002) Table 1.

III) Quantitative study

1) Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method (Singelton *et al.*, 1999). In brief, 0.1 ml of each extract was mixed with 4.9 ml distilled water, 0.5 ml of FolinCiocalteu reagent was added to the mixture. After 5min of incubation, 5 ml of 7% of aqueous Na₂CO₃ solution was added. The mixture was allowed to stand for 30 minutes and the absorbance was measured at 760 nm using a UV-Vis Double Beam spectrophotometer. The standard curve was prepared by gallic acid (1mg/ml) in methanol: water (50:50, v/v). Total phenolic content was expressed as of gallic acid equivalent mg GAE/mg of extract.

2) Determination of flavonoid content

Aluminum chloride method was used for flavonoid determination (Quettier *et al.*, 2000). Briefly, 0.1ml of each extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415nm. Quercetin (1mg/ml) was used as a standard. Total flavonoid content was expressed as mg QE/mg of extract.

3) Total antioxidant activity

The total antioxidant activity was eluted by using the method described by Prieto *et al.* (1999). Plant extracts were dissolved in methanol to obtain a concentration of 1 mg/ml. 3 ml of

extract was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 95°C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of the each solution was measured by using UV-Visible spectrophotometer at 695 nm against blank. The experiment was performed in triplicate (Joseph Francis Morrison *et al.*, 2010). A calibration curve was constructed, using ascorbic acid (0.1-0.7 mg/ml) as standard and total antioxidant activity of extract (mg/ml) expressed as ascorbic acid equivalents.

4. Reducing Power Activity

2.5 ml of different concentrations of extract/standard was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and potassium ferricyanide (2.5 ml, 1%). This was incubated at 50°C for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid was added. 2.5 ml of the reaction mixture was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). The solution absorbance was measured at 700 nm. The experiment was performed in triplicate. Vitamin C was used as positive control. Increase in absorbance of the reaction mixture indicated the increased reducing power of the samples (Pandey Manisha *et al.*, 2009).

5. Thin Layer Chromatography

The TLC was performed on precoated 20×20 cm and 0.25 mm thick plates. The plates were prepared by using silica gel G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100°C for 1hr. Cold alcoholic extract was plotted on TLC plates. The plates were dried and developed in suitable solvents for rapid screening chloroform / methanol in the ratio 5:1. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by UV light at 254nm. Different bands were observed and corresponding Rf values are determined. Rf value of each spot was calculated as:-

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

HPLC fingerprinting

1gm of sample kept overnight for extraction in HPLC grade methanol. This extracts were sonicated for 20 min in sonicator 20 ul from sonicated extracts was passed through 0.45 mm filter. Filtrate was used for HPLC analysis. Qualitative and quantitative HPLC analysis of the sample was performed according to the method of Sarma *et al.* (2002). The HPLC system (Agilent 1100 series HPLC) was used. The software package used for analyzing results was Agilent 1100 series HPLC control and sampling.

Chromatographic analysis was carried out using a Reverse Phase C18 (150mm x 6mm) ID – 5um at temperature: 25 degree celsius ambient. Running conditions included: injection volume 20ul; mobile phase: Acetonitrile + Water + Glacial Acetic Acid (5:1:5), flow rate 1 ml/min; Run Time was 50 minutes and detection at UV Wavelength 280 nm by Chemstation detector. Samples were filtered through an ultra membrane filter (pore size 0.45um) prior to injection in the sample loop.

Statistical analysis

Results are presented as mean ± SD of three independent experiments. Statistical analyses were performed by Student's t-test (Microsoft Excel 2013). The values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Yield percentage of plants

a) Percent of yield of *Elettaria cardamomum* was calculated as follows:

$$\text{Extract yield \%} = (W1/W2) \times 100 = (0.16/5) \times 100 = 3.2\% \text{w/w}$$

b) Percent of yield of *Ferula assa-foetida* was calculated as follows:

$$\text{Extract yield \%} = (W1/W2) \times 100 = (0.29/3.5) \times 100 = 8.28\% \text{w/w}$$

Where, W1 is net weight of powder in grams after extraction and W2 is total weight of plant powder in grams taken for extraction.

Table 1. Yield percent (g)

<i>Elettaria cardamomum</i>	<i>Ferula assa-foetida</i>
3.2 %	8.3 %

Percent of yield of *Elettaria cardamomum* and *Ferula assa-foetida* was estimated as 3.2 % w/w and 8.28 %w/w respectively.

Qualitative study

Phytochemical constituents of *Elettaria cardamomum* and *Ferula assa-foetida* were investigated qualitatively and results are summarized in Table 2.

Table 2. Phytochemical Screening of Methanoic Plant Extracts

Test Name	Alkaloids	Flavonoids	Phenolic compounds	Saponins
<i>E. cardamomum</i>	+	+	+	+
<i>F. assa-foetida</i>	+	+	+	+

Qualitative estimation of *Elettaria cardamomum* and *Ferula assa-foetida* shows alkaloids, flavonoids, phenolic and Saponin compounds were positive.

Quantitative study

Phytochemical constituents of *Elettaria cardamomum* and *Ferula assa-foetida* were investigated quantitatively and results are summarized in Tables below:

1. Total Phenolic Content

Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free

radicals, quenching singlet and triplet oxygen or decomposing peroxides (Schuler *et al.*, 1990). Figure: 1 shows total phenolic content of standard Gallic acid.

The methanolic extract of *Elettaria cardamomum* showed higher total phenolic content and it was 1.71 mg/ml calculated as Gallic acid equivalent of phenols was detected. The total phenolic content of methanolic extracts exhibited the following order:

Table 3. Total Phenolic Content of methanolic plant extracts: (100µl of 1mg/ml)

<i>Elettaria cardamomum</i>	<i>Ferula assa-foetida</i>
1.71 ± 0.173*	1.40 ± 0.181*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$

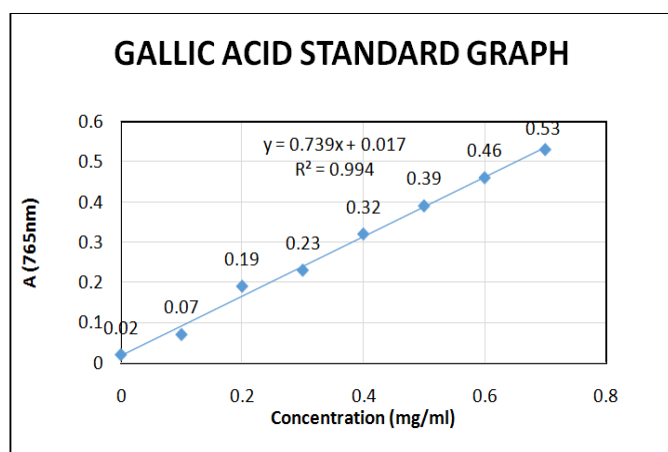


Figure 1. Gallic Acid Standard

Total phenolic content (1.71 mg/ml and 1.40 mg/ml expressed as gallic acid equivalents) was seen in *Elettaria cardamomum* and *Ferula assa-foetida* respectively.

2. Total flavonoid content

Flavonoids comprise the most widespread and diverse group of polyphenolic plant secondary metabolites. These compounds play an important role in biological activities includes antibacterial, antiviral, and anti-inflammatory, anti allergic, antithrombotic, vasodilatory actions and also exhibit free radical scavenging properties by either through scavenging or chelating process (Quettier *et al.*, 2000, Joseph Francis Morrison *et al.*, 2010 and Pandey Manisha *et al.*, 2009).

Figure: 02 shows total flavonoids content of standard Quercetin. The extract of *Ferula assa-foetida* showed higher total flavonoids content and it was 5.55 mg/ml calculated as Quercetin equivalent flavonoids was detected.

Table 4. Total flavonoid content of methanolic plant extracts: (100µl of 1mg/ml)

<i>Elettaria cardamomum</i>	<i>Ferula assa-foetida</i>
1.11 ± 0.278*	1.36 ± 0.24*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$

Total flavonoids content (1.11 mg/ml and 1.30 mg/ml expressed as gallic acid equivalents) was seen in *Elettaria cardamomum* and *Ferula assa-foetida* respectively.

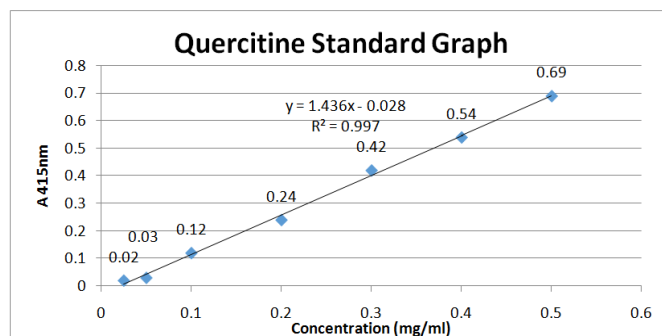


Figure 2. Quercitine standard graph

Table 5. Total phenolic and flavonoid content of Methanolic extracts

S.No.	Extracts (100µl of 1mg/ml)	Total phenolic content (mg GAE/mg of extract)	Total flavonoid content (mg GAE/mg of extract)
1	<i>Elettaria cardamomum</i>	1.71 ± 0.173*	1.11 ± 0.278*
2	<i>Ferula assa-foetida</i>	1.40 ± 0.181*	1.36 ± 0.24*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$

Anti- Oxidant Potential

Total antioxidant activity

The Phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in plant extracts (Nayak *et al.*, 2006) ascorbic acid. The extract of *Elettaria cardamomum* showed higher total antioxidant capacity and it was 25.60 mg/ml calculated as Ascorbic acid equivalents was detected and *Ferula assa-foetida* showed total antioxidant capacity and it was 18.43 mg/ml calculated as Ascorbic acid equivalents was detected Figure: 3 shows total antioxidant activity of standard

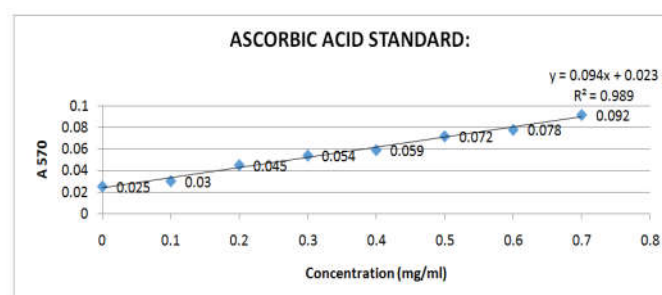


Figure 3. Ascorbic Acid Standard (1 mg/ml)

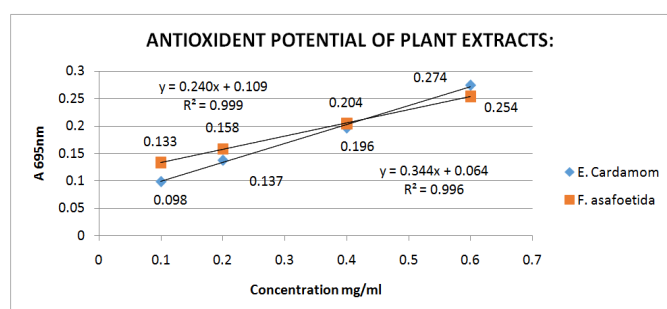


Figure 4. Anti-oxidant activity of *Elettaria cardamomum* and *Ferula assa-foetida*

Total antioxidant activity

Total antioxidant activity of both plants *Elettaria cardamomum* and *Ferula assa-foetida* was estimated as 25.60 mg/ml and 18.61 mg/ml at 500 μ l of 1 mg/ml of plant extracts.

Reducing Power Activity

For the measurements of the reductive ability it has been investigated from the Fe³⁺ to Fe²⁺ transformation in the presence of extract samples using the method described by Oyaizu. Figure 4 shows reducing power of methanolic extracts and standard ascorbic acid. The reducing power of methanolic extracts and standard compound ascorbic acid exhibited the following order: **Ascorbic acid > Ferula assa-foetida > Elettaria cardamomum**

The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain or by donating a hydrogen atom.

Table 7. Reducing power assay of plants:(A700)

<i>E. Cardamomum</i>	<i>F. Assa-foetida</i>
48.67% \pm 0.194*	70.23% \pm 0.285*

*Each value is expressed as mean \pm standard deviation (n= 3); P < 0.05)

Ascorbic acid standard (1 mg/ml)- same as Figure 3

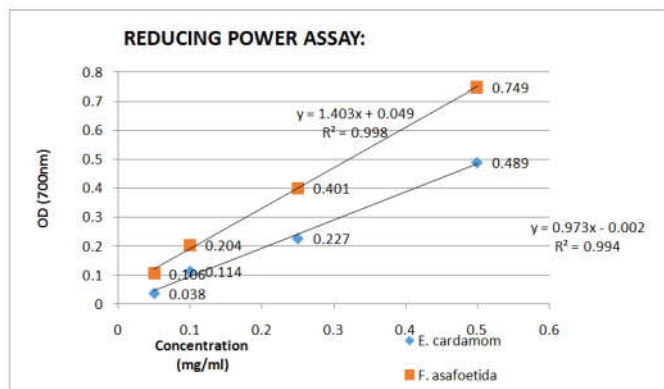


Figure 5. Reducing Power Activity of *Elettaria cardamomum* and *Ferula assa-foetida*

Reducing Power Activity of *Elettaria cardamomum* and *Ferula assa-foetida* was estimated as 48.67% and 70.23% at 50 μ l of 1mg/ml of plants extract respectively.

Correlation between Antioxidant Capacity and Total Phenolic Content

Despite the presence of a wide range of the total antioxidant capacities and total phenolic contents among the selected plants, linear positive relationships could be found between the FRAP value and the total phenolic content, as shown in Figure 6.

Thin Layer Chromatography

The results of one-dimensional TLC analyses show that different phenolic compounds, flavonoids and phenolic acids, are present in the investigated extracts. A largest number of flavonoids (rutin, quercetin and some unidentified flavonoid-

glycosides) and phenolic acids (chlorogenic, caffeic, coumaric and vanillic acid) was found in methanol extract. Rutin and some unidentified flavonoid-glycosides are present in the Solvent used: Chloroform: Ethyl acetate: Formic acid Ratio - (10:9:2). The extracts also contain coumaric, caffeic and chlorogenic acid when second solvent Methanol : Chloroform Ratio-(5:1) was used. Iodine balls vapour served as spraying method. Three spots have been observed. 1st- Brown, 2nd- Yellow and 3rd- Green.

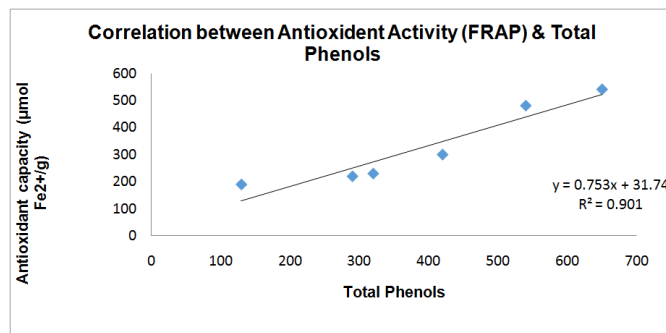


Figure 6. Correlation between the antioxidant capacity and total phenolic content of the selected plants. Antioxidant capacity was measured by the FRAP assay. (r2 = 0.9013)

1) Solvent used: Chloroform: Ethyl acetate: Formic acid Ratio - (10:9:2)

Table 14. *Elettaria cardamomum*

No of spots.	Rf	Colour	Compound
1	0.66	B	chlorogenic acid/ rutin
2	0.89	Y	caffeic acid
3	0.98	G	coumaric acid

Table 15. *Ferula assa-foetida*

No of spots.	Rf	Colour	Compound
1	0.081	B	flavonoid-glycoside
2	0.21	Y	flavonoid-glycoside
3	0.84	Y	coumaric acid/caffeic acid
4	0.97	G	Quercetin*

Solvent used : Methanol : Chloroform Ratio - (5:1)
Spray used : Iodine balls vapour

Table 16. *Elettaria cardamomum*

No of spots.	Rf	Colour	Compound
1	0.71	B	chlorogenic acid
2	0.82	Y	caffeic acid / quercetin
3	0.97	G	Quercetin* / coumaric acid / vanillic acid

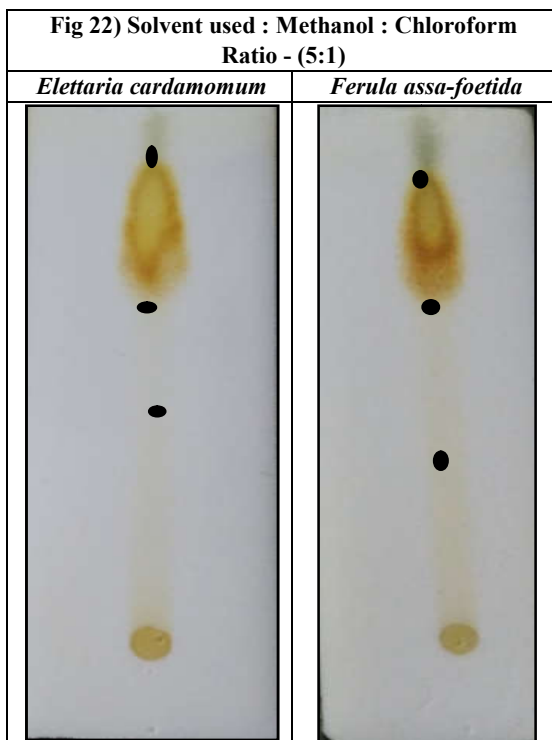
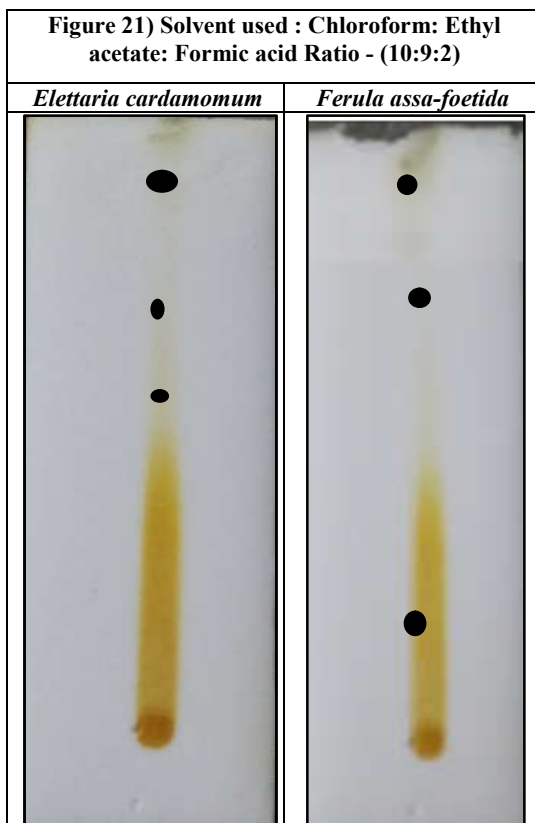
Table 17. *Ferula assa-foetida*

No of spots.	Rf	Colour	Compound
1	0.69	B	rutin /chlorogenic acid
2	0.77	Y	caffeic acid
3	0.97	G	coumaric acid / quercetin* /vanillic acid/

HPLC Fingerprinting

HPLC has been carried out on 24th September'2014atAllele lifesciences by the Director Mr. K.B.Singh. According to published research papers phenols and flavonoids have anti cancerous activity. So HPLC has been carried out to know the

amount of phenolic and flavonoids present in both plant extracts. We will use these results in Molecular Docking by DOCK BLASTER.



(Total length of glass slide used was 6.2 cm)

Table 18. HPLC results

Components present	<i>Elettaria cardamomum</i>	<i>Ferula assa-foetida</i>
Tannic acid	2.270	2.421
Vanillin	2.08	2.025
Cathechol	4.824	2.667

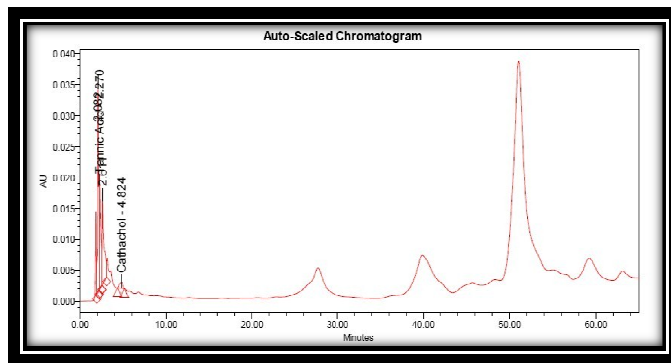


Figure 23. Chromatogram-1 of *E. Cardamomum*

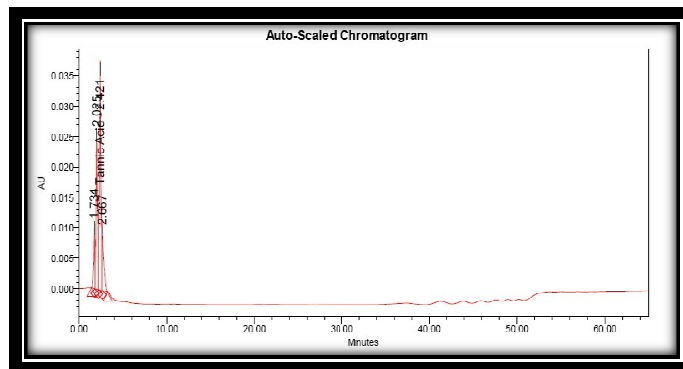


Figure 24. Chromatogram-2 of *F. Assa-foetida*

The HPLC analysis showed that the extracts of *Elettaria cardamomum* (chromatogram-1) and *Ferula assa-foetida* plant (chromatogram-2) contain various secondary metabolites. The concentration of the various secondary metabolites present in the extracts were determined based on the standard HPLC graphs. The standards used for analysis were tannic acid, vanillin and catechol. The HPLC graphs for each sample are shown in Figure 12. Thus the analysis showed that both plants are rich source of secondary metabolites which are used as medicines and also commercially in various processes. Thus this method can also be used for production of other secondary metabolites.

Conclusion

Research has demonstrated that nutrition plays a vital role in the inhibition of chronic diseases, as most of them can be related to diet. Functional food enters the concept of considering food not only necessary for living but also as a source of mental and physical wellbeing, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions (Gupta *et al.*, 2010). Thus present study was carried out with two medicinal plants in search for effective, nontoxic natural compounds with antioxidative activity. The antioxidant potential, total phenolic and flavonoid contents of *Elettaria cardamomum* and *Ferula assa-foetida* commonly consumed in human diet were studied. The results of one-dimensional TLC analyses show that different phenolic compounds, flavonoids and phenolic acids, are present in the investigated extracts. A largest number of flavonoids (rutin, quercetin and some unidentified flavonoid-glycosides) and phenolic acids (chlorogenic, caffeic, coumaric and vanillic acid) was found in methanol extract. Rutin and some unidentified flavonoid-glycosides are present in the Solvent used : Chloroform: Ethyl acetate: Formic acid Ratio - (10:9:2). The extracts also contain coumaric, caffeic and

chlorogenic acid when second solvent Methanol : Chloroform Ratio-(5:1) was used. Iodine balls vapour served as spraying method. Three spots have been observed. 1st- Brown, 2nd- Yellow and 3rd- Green. The HPLC analysis showed that the extracts of *Elettaria cardamomum* and *Ferula assa-foetida* plant contain various secondary metabolites. The concentration of the various secondary metabolites present in the extracts were determined based on the standard HPLC graphs. The standards used for analysis were tannic acid, vanillin and catechol. The findings showed that both plants under study are a good source of dietary phytochemicals as shown by their total phenolic and flavonoid contents. These plants can be seen as a potential source of nutraceuticals. They may help in combating oxidative stress.

Conflicts of interest

The authors report no conflicts of interest.

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