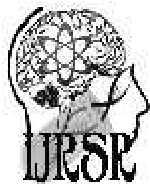

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Research Article

EVALUATION OF LIPID PEROXIDATION ACTIVITY OF ELETTARIA CARDAMOMUM AND FERULA ASSAFOETIDA LEAVES

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ABSTRACT

The medicinal properties of plants are due to the presence of active principles. These bioactive secondary metabolites are synthesized by two principal pathways: shikimic acid or aromatic amino acid, and mevalonic acid. Alkaloids, phenolics and terpenoids constitute many pharmacologically active compounds. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Epidemiological studies specify that intake of fruits and vegetables have the ability to inhibit the damaging behaviour of free radicals in the human body.

Free radicals which induce oxidative damage of cellular lipids, nucleic acids and proteins are thought to be one of the major risks for diseases such as cancer, atherosclerosis, diabetes mellitus and various other degenerative diseases. Numerous natural free radical scavengers and antioxidants can protect biomolecules against the attack of free radicals or may suppress injury caused by them. Two leaf extract of *Elettaria cardamomumand Ferula assa-foetida* leaves, were prepared in methanol and subjected to test the extent of inhibition of in vitro lipid peroxidation by using lipid membrane preparations of RBC ghosts. Both the extracts caused a substantial decline in the extent of LPO in RBS ghosts. The decrease in LPO was more pronounced in *Ferula assa-foetida* leaves extract.

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INTRODUCTION

Free radicals are highly reactive atomic or molecular species that can damage vital cellular molecules like nucleic acids, lipids and proteins resulting in subsequent cell death (Pauwels *et al*., 2007). In the biological system, lipids are the immediate targets of oxidative moieties and DNA molecules are the ultimate targets (Balakrishna *et al*., 2009). Membrane lipids present in subcellular organelles are highly susceptible to free radical damage and cause peroxidation of polyunsaturated fatty acids in the membranes (Kim *et al*., 2005). When reactive oxygen species attack polyunsaturated fatty acids on the cell membrane of living organisms in the presence of molecular oxygen, a chemical cascade is triggered. This eventually leads to the disintegration of fatty acids and the formation of malondialdehyde (MDA) which is called as lipid peroxidation (LPO) (Cemek *et al*., 2006). MDA, the end product of lipid peroxidation, has also been demonstrated to be a mutagenic and genotoxic agent that can contribute to the development of human cancers (Ajith, T.A.2010). The damage caused by LPO is highly detrimental to the functioning of the cell (Skrzydlewska *et al*., 2005). Antioxidants are compounds which have the ability to transform reactive oxygen species into stable and harmless compounds or to scavenge both reactive oxygen and nitrogen species with a redox-based mechanism (Niki, E. 2009). Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases, and their consumption lowers the lipid peroxidation (Muanda, F *et al*., 2009). The present study was undertaken to test the extent of inhibition of in vitro lipid peroxidation by the methanoic extracts of *Elettaria cardamomum and Ferula assa-foetida* leaves using RBC ghosts. These plants, *Elettaria cardamomum and Ferula assa-foetida* plants are known to have many therapeutic remedies; it cures digestive disorders and has antimicrobial activities as well.

MATERIALS AND METHODS

Chemicals and Materials Used

Reagents and solvents used were of analytical grade of the highest commercial quality and without further purification. Particularly, methanol and distilled water were used as solvents. Folin-Ciocalteu reagent. gallic acid powder, methanol and sodium carbonate powder were purchased from Merck.,

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gallic acid powder were purchased from Sigma-Aldrich Delhi (INDIA)

Plant material collection

The leaves of *Elettariacardamomum* 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 *Elettariacardamomum* 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 Whattmanfilter no1 and the extract was collected and concentrated by evaporation to dryness in evaporating dish (Trease and Evans, 1997). Keep it foe 50-72 hours in desiccator. The dried methanol extract was stored in refrigerator for studying the effects of E. Cardamomum and F. Assa-foetida Leaves extracts onoxidative induced damage to lipids.

Percent of yield calculated for *Elettariacardamomum* was 3.2%w/w.
Percent of yield calculated for *Ferula assa-foetida* was 8.28%w/w.

Evaluation of the effects of E. Cardamomum and F. Assa-foetida Leaves extracts on oxidative induced damage to lipids.

Lipid peroxidation (LPO), a well-established mechanism of cellular injury, is used as an indicator of oxidative stress (Sangeetha, R at al., 2010). In the present study, the extent of lipid peroxidation was assessed in the membrane model, namely goat RBC ghosts (plasma membrane lipids).

In this phase, an attempt was made to study the extent of oxidative damage to cellular biomolecules like membrane lipids and DNA and its protection by *E. Cardamomum and F. Assa-foetida* leaf extract in cell-free systems and intact cells. The effect of *E. Cardamomum* and *F. Assa-foetida* leaves extract on different types of cells subjected to oxidative stress was also studied. The methodology adopted is given below.

Principle

The extent of formation of thiobarbituric acid reactive substances (TBARS) from the damaged lipids by oxidizing agents can be used as a measure of damage to membrane lipids.

***Evaluation of LPO in RBC ghosts* reagents**

1. Isotonic KCl (1.15%)
2. Hypotonic KCl (0.5%)
3. Tris buffered saline (TBS) (10mM Tris, 0.15M NaCl, pH 7.4)
4. Ferrous sulphate (10µM)
5. Thiobarbituric acid (TBA) (1%)
6. Ascorbic acid (0.06mM)
7. Ethanol (70%)
8. Acetone

Preparation of goat RBC ghost

Goat blood (50ml) was collected fresh in a sterile container. The blood was immediately defibrinated using acid-washed stones. The defibrinated blood was transferred to another sterile container and diluted 1:1 with sterile isotonic KCl and transferred to the laboratory on ice. The RBCs were pelleted by centrifuging at 3000 x g for 10 minutes at 4°C. The supernatant was discarded and the pellet was washed thrice with isotonic KCl. The cells were then lysed at 37°C for one hour in hypotonic (0.5%) KCl. After lysis, the lysate was centrifuged at 5000 x g for 10 minutes at 4°C. The pellet obtained was washed repeatedly with hypotonic KCl until most of the haemoglobin was washed off and a pale pink pellet was obtained. The pellet was suspended in 1.5ml of TBS and 50µl aliquots were used for the assay, as described by (JT Dodge *et al*., 1963).

Procedure

The reaction mixture contained 50µl of RBC ghosts, 50µl of *E. Cardamomum and F. Assa-foetida*l Leaves extracts, 50µl FeSO4 and 100µl of ascorbate in a total volume of 500µl, which was made up with TBS. A blank was prepared without the plant extract and lipid source, but containing only FeSO4, ascorbate and TBS in a final volume of 0.5ml. An assay medium corresponding to 100% oxidation was prepared, which contained all the other constituents except the plant extract. The experimental medium corresponding to auto-oxidation contained only RBC ghosts. All the tubes were incubated at 37°C for one hour. After incubation, the reaction was arrested by adding 0.5ml of 70% alcohol to all the tubes.

Then 1.0ml of TBA was added to all the tubes and heated in a boiling water bath for 20 minutes. After cooling to room temperature, the tubes were centrifuged and 0.5ml of acetone was added to the supernatant. The pink colour developed was measured at 535nm in a UV-VIS Double Beam Spectrophotometer (Iitachi U-2000).

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

Determination of in vitro Lipid peroxidation inhibition

The extracts tested were effective in reducing the production of TBARS in a dose –dependent manner. The in vitro LPO was inhibited to a good extent by the *Elettaria cardamomum* and *Ferula assa-foetida* extracts and the extent of inhibition being higher in the RBC membrane model in *Elettaria cardamomum.* (See table 1)

Table No 1 % LPO Inhibition *E.Cardamom* and *F. Asafoetida*

% LPO Inhibition			
S.No.	Concentration of plant extract (µg)	*E.Cardamom*	*F. Asafoetida*
1	50	27.61 ± 0.11	38.34 ± 0.21
2	100	42.91 ± 0.01	53.98 ± 0.11
3	150	47.54 ± 0.21	55.27 ± 0.03
4	200	51.23 ± 0.10	59.52 ± 0.01
5	250	76.99 ± 0 .04	64.42 ± 0.79

*Values are represented as mean ± SD of triplicates.

The Lipid peroxidation (LPO) has been broadly defined as the oxidative deterioration of polysaturated lipids. Peroxyl and hydroxyl radicals are important agents that mediate lipidperoxidation, thereby damaging cell membranes (Halliwell B *et al*., 1981). A number of toxic compounds are generated during this process of LPO. TBARS are produced as by-products of LPO that occurs in the hydrophobic core of biomembranes (Fraga C *et al*., 1987).

A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom (Khanam S *et al*., 2004). The model of RBC ghost (plasma membrane) was used as it contain different lipid composition. Mammalian cells have evolved migrate interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism (Khajuria A 1997).

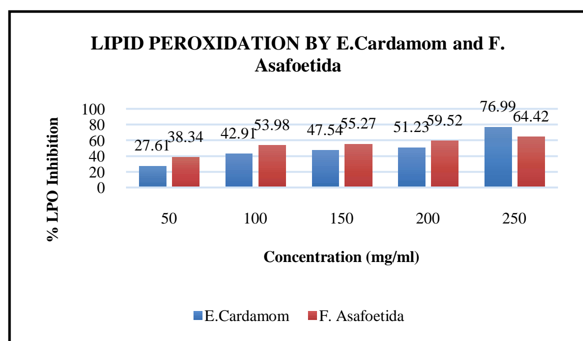

Fig 1 Lipid peroxidation by *E.Cardamom* and *F. Asafoetida*

The effect of mushroom extracts on LP show significant inhibition of TBARS formation. The present finding strongly suggests that the use of *E.Cardamom and F. Asafoetida* extracts prevent LP leading to membrane damage consequent to radiation and to certain chemicals which generate potent ROS. (See fig 1 and fig 2)

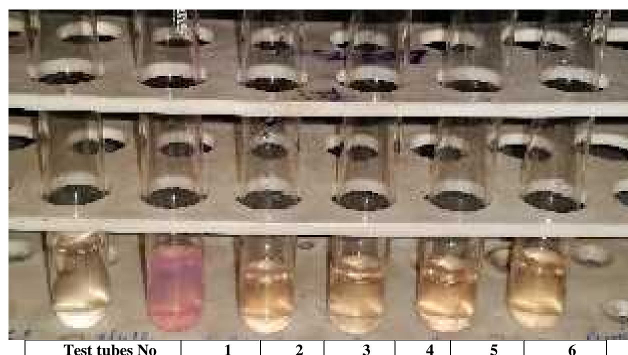

Fig 2 Lipid peroxidation with Blank (Test tube 2), *E.Cardamom* (Test tube 3-4) and *F. Asafoetida* (Test tube 5-6)

DISCUSSION

The medicinal properties of plants are due to the presence of active principles. These bioactive secondary metabolites are synthesized by two principal pathways: shikimic acid or aromatic amino acid, and mevalonic acid. Alkaloids, phenolics and terpenoids constitute many pharmacologicallyactive compounds. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Epidemiological studies specify that intake of fruits and vegetables have the ability to inhibit the damaging behaviour of free radicals in the human body.

Additionally in our present study the methanolic extracts were evaluated for phytochemical composition, total Phenolic content, flavonoid content, antioxidant potential by Total antioxidant assay, Reducing activity assays, Super oxide and hydroxyl scavenging activity, H2O2 decomposition assay. Efforts were also made to study Antimicrobial, Antifungal Activity and Anti-tumor activity. 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. Antioxidantactivity was measured by Phospho molybdenum method. Free radicalscavenging activity was evaluated using Superoxide anion scavenging activity, Hydroxyl scavenging activity and Hydrogen peroxide decomposition. The extract of *Elettaria cardamomum and Ferula assa-foetida* showed total antioxidant capacity and it was *25.60 mg/ml* and *18.43 mg/ml* calculated as Ascorbic acid equivalents respectively. 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%). Mild hydrogen peroxide decomposition has been observed in both the plants having 9.83 % and 9.60 % in *Ferula assa-foetida* and *Elettaria cardamomum* respectively. The *Elettaria cardamomum* and Ferula assa-foetida extracts showed concentration dependent hydroxyl radical scavenging activity and both plants Elettaria cardamomum and Ferula assa-foetida showing 33.94 % and 44.95 % respectively. The present finding strongly suggests that the use of *E.Cardamom and F. Asafoetida* extracts prevent LP leading to membrane damage consequent to radiation and to certain chemicals which generate potent ROS.

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 unidetified 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 ware determined based on the standard HPLC graphs. The standards used for analysis were tannic acid, vanillin and cathechol.

Plasmid Relaxation experiment was the first report concerning the protective effect of E. Cardamomum and F. Assa-foetida on DNA damage-induced by hydroxyl radical. Furthermore, our study showed that the extract exhibited good antioxidant activity with a high content of polyphenol and flavonoid compounds. These findings indicate that the potent antioxidant activity *of E. Cardamomum* and *F. Assa-foetida* extract partly contributes to the amount of polyphenol and flavonoid compounds.

In addition Methanoic extract of *Elettaria cardamomum* and *Ferula assa-foetida* revealed anti microbial and effective anti-tumor activity. We have also carried out Molecular DOCKING with the help of DOCK BLASTER- An Online tool for docking and it provided us with molecules having least energy level ranging -24 k/cal/mol to -31 k/cal/mol (phytochemicals as proteins and ligands associated with different Cancers.) that could have anti cancerous activity.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Epidemiological studies specify that intake of fruits and vegetables have the ability to inhibit the damaging behaviour of free radicals in the human body. The present finding strongly suggests that the use of *E.Cardamom* and *F. Asafoetida* leaves extracts prevent LP leading to membrane damage consequent to radiation and to certain chemicals which generate potent ROS.

The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

Conflicts of interest

The authors report no conflicts of interest.

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