

# PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND HYPOLIPIDEMIC POTENTIAL OF AQUEOUS EXTRACT OF *FERULA ASAFOETIDA*-AN *IN VITRO* STUDY

Type of study: Original research

Running title: Phytochemical and antioxidant activity of aqueous extract of *Ferula asafoetida*

## ABSTRACT

**Aim:** To analyse the preliminary phytochemical, antioxidant, anti-cholesterol potential of aqueous extract of *Ferula asafoetida*.

**Background:** Hyperlipidemia is considered as one of the leading causes behind the occurrence of deadly disorders like diabetes, cardiovascular diseases, atherosclerosis etc. It is characterised by elevated levels of plasma lipids, mainly total cholesterol. Antioxidants are compounds which can inhibit oxidative damage. is the herbaceous plant belonging to the family Umbelliferae. It is used as spice in food and also used as digestive aid. It is used in the treatment of asthma and whooping cough and it also helps to reduce blood pressure.

**Methods:** Aqueous extract of *Ferula asafoetida* was prepared by hot percolation method. The screening of phytochemical constituents, assessment of in vitro antioxidant activity and anticholesterol activity were done using standard procedures and the data were analysed statistically using one-way analysis of variance (ONE-WAY ANOVA) and the significance was considered at the levels of  $p < 0.05$ .

**Results:** *Ferula asafoetida* extract was rich in phytochemicals and possessed potent in vitro antioxidant activity. Anti-cholesterol activity of *Ferula asafoetida* extract was examined and it was observed that the plant extract exhibited significant anti cholesterol potential in a dose dependent manner with an IC 50 value of 400  $\mu\text{g/ml}$ .

**Conclusion:** The study established the *in vitro* antioxidant and hypolipidemic potential of aqueous extract of *Ferula asafoetida* . It is concluded that the extract of *Ferula asafoetida* possesses potent antioxidant and anticholesterol activity.

**Keywords:** *Ferula asafoetida*, anti- cholesterol, antioxidant, phytochemical screening, Innovative technology, Novel method

## **INTRODUCTION**

*Ferula asafoetida* is a herbaceous plant belonging to the Umbelliferae family. It has been used as a spice in food for more than thousands of years. It is also used as a digestive aid. It can change the dull food into an appetizing meal by their colour, flavor and pungency (1). The resin gum obtained from *Ferula asafoetida* is used to treat whooping cough and ulcer. It is used in the treatment of cancer ,women's allergies, blood pressure, and chemoprotive therapy (2). It is used in modern treatments in whooping cough , asthma, and also it reduces cholesterol . The oil of *Ferula asafoetida* is reported to have antifungal activity. It has cardioprotective effects in low doses and cardiotoxic effects in higher doses. It is also used in organic farming to kill several insect pests. It helps to treat gastrointestinal, neurological and respiratory disorders (3).

Phytochemicals are the compounds produced in the plants. Secondary metabolites are present only in plants possessing various biological activities(4). They help in the normal processing and are capable of prevention or treatment of various disorders(5). Phytochemicals generally are regarded as an important compound of research interest because of their possible health effects(6). Antioxidant is the material which terminates the chain reaction by removing the free radical(7). Antioxidants reduce the risk of cancer, cardiovascular diseases, diabetes and other diseases which are caused by oxidative stress(8).

Hyperlipidemia is a secondary metabolic disorder. It is characterised by elevated levels of serum triglycerides, cholesterol and low density lipoproteins and decreased serum levels of high density lipoproteins(9). Hyperlipidemia is regarded as a major risk factor for the premature development of cardiovascular disease like atherosclerosis, hypertension, coronary heart disease etc. (10). It is also associated with other metabolic disorders like obesity, diabetes mellitus etc

(11). Statin drugs are widely used for the treatment of hypercholesterolemia as it is an inhibitor of the key enzyme HMG CoA reductase, which is involved in the cholesterol biosynthesis (12). Our team has extensive knowledge and research experience that has translate into high quality publications(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28), (29),(30), (31),(32)Hence the study aimed to assess the anticholesterol and antioxidant activity of *Ferula asafoetida* extract by *in vitro* methods.

## **MATERIALS AND METHODS**

### **1. Preparation of Aqueous extract of *Ferula asafoetida*:**

*Ferula asafoetida* was purchased from a herbal health care centre. Crushed and made into powder. 80% aqueous extract was obtained. The extract was prepared by a hot percolation method. Phytochemical analysis, assessment of in vitro antioxidant and anti cholesterol potential was evaluated (33).

### **2. Phytochemical Screening test:**

#### **Test for phlobatannin:**

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

#### **Test for Carbohydrates**

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

#### **Test for Flavonoids**

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

#### **Test for Alkaloids**

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

#### **Test for Terpenoids**

2 ml of sample along with 2ml of chloroform and 3 ml of con.H<sub>2</sub>SO<sub>4</sub> was added. Red color ppt obtained indicates the presence of terpenoids.

#### **Test for proteins**

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

#### **Detection of saponins**

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam (34).

#### **Test for steroids**

One ml of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

#### **3. *In vitro* antioxidant activity (DPPH free radical scavenging activity)**

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{control OD}} \times 100$$

#### 4. *In vitro anti-cholesterol activity:*

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000  $\mu\text{L}$  of R1 reagent and 10  $\mu\text{L}$  of cholesterol as sample. Twenty microliter of distilled water and 2000  $\mu\text{L}$  of R1 reagent were used as blank. Negative control consisted of 20  $\mu\text{L}$  cholesterol and 2ml R1; standard consisted of 20  $\mu\text{L}$  simvastatin and 2mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control} - \text{Sample}}{\text{Negative OD}} \times 100$$

#### 5. Statistical analysis:

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of  $p < 0.05$ .

## RESULTS

### Phytochemical screening of *Ferula asafoetida*

The results of phytochemical analysis showed the presence of proteins, flavonoids, alkaloids, terpenoids, saponins and steroids in *Ferula asafoetida*. The carbohydrates and the amino acids are absent in *ferula asafoetida* (Table 1). The presence of phytochemicals in the extract might be the underlying reason for the therapeutic properties of *Ferula asafoetida*.

Table 1: Phytochemical screening of *Ferula asafoetida* aqueous extract.

Phytochemicals	Presences
Proteins	+

Flavonoids	+
Alkaloids	+
Terpenoids	+
Saponins	+
Steroids	+
Carbohydrates	-
Amino Acids	-

+ = It indicates the presence of the phytochemical in the *ferula asafoetida*.

- = It indicates the absence of the phytochemical in the *ferula asafoetida*.

#### **Antioxidant activity of aqueous extract of ferula asafoetida:**

Antioxidant activity of aqueous seed extracts of *Ferula asafoetida* was determined by performing DPPH free radical scavenging assay (Figure 1). The extract showed a dose-dependent increase in the antioxidant activity although its activity is less compared to the standard vitamin C. The P value is  $p < 0.05$ .

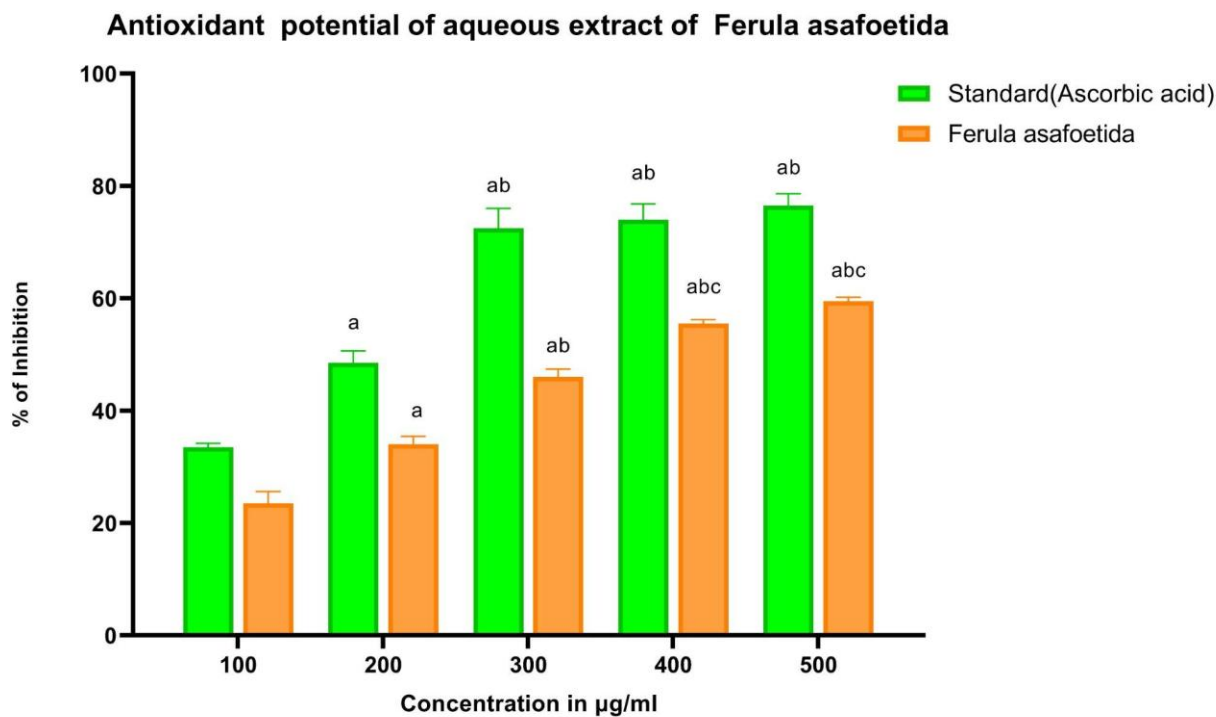


Figure 1: The figure represents the antioxidant potential of aqueous extract of *Ferula asafoetida* by DPPH assay. The X axis represents the concentration in ug/ml and the Y axis represents the % of inhibition. Green bar represents ascorbic acid, orange bar represents aqueous extract of *Ferula asafoetida* . Each bar represents the Mean±SEM of 3 independent observations. Significance at  $p < 0.05$ .

#### **AntiCholesterol activity of aqueous extract of ferula asafoetida:**

anticholesterol activity of *Ferula asafoetida* aqueous extract revealed a dose dependent increase in the percentage of inhibitory activity. The extract showed a potent anti cholesterol activity with a  $IC_{50}$  value of 450 µg/ml (Figure 2). The activity of the extract is less compared to the standard drug simvastatin in all the tested concentrations. Simvastatin is a standard anti-cholesterol drug which acts as HMG CoA reductase inhibitor. It showed that the extract is showing less activity compared to simvastatin. The p value is  $p < 0.05$ .

## Anticholesterol potential of aqueous extract of *Ferula asafoetida*

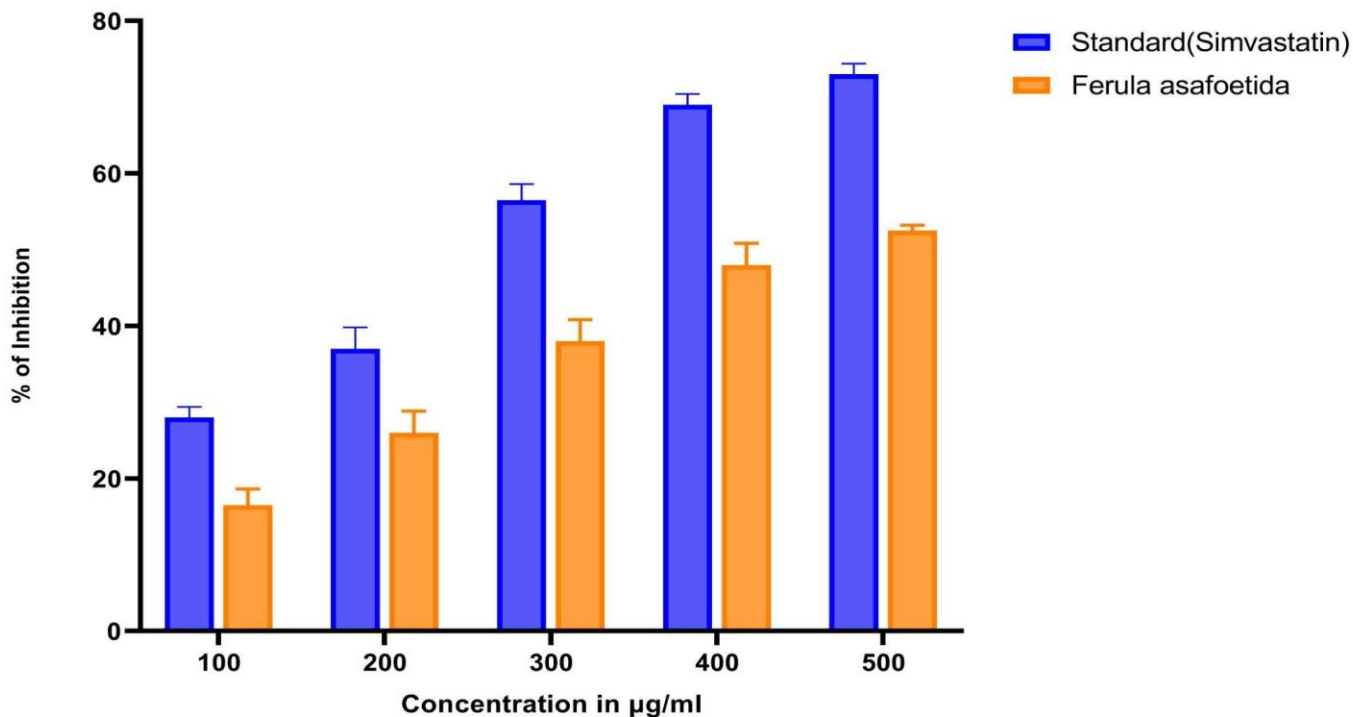


Figure 2: The figure represents the hypolipidemic potential of aqueous extract of *Ferula asafoetida*. The X axis represents the concentration in µg/ml and the Y axis represents the % of inhibition. Blue bar represents the standard Simvastatin, orange bar represents aqueous extract of *Ferula asafoetida*. Each bar represents Mean  $\pm$  SEM of 3 independent observations. Significance at  $p < 0.05$ .

## DISCUSSION

Plants are an excellent source of biologically active compounds known as phytochemicals. It is well established that free radicals are the major cause of various chronic and degenerative diseases, like coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (35). The medicinal value of plants is related to the content of phytochemicals including phenolic compounds, flavonoids, alkaloids, tannins etc. (36). The results of phytochemical analysis showed the presence of proteins, flavonoids, alkaloids, terpenoids,

saponins and steroids in *Ferula asafoetida* (Table 1). The presence of phytochemicals in the extract might be the underlying reason for the therapeutic properties of *Ferula asafoetida*.

Antioxidant activity of aqueous seed extracts of *Ferula asafoetida* was determined by performing DPPH free radical scavenging assay (Figure 1). The extract showed a dose-dependent increase in the antioxidant activity although its activity is less compared to the standard vitamin C. Free radicals are molecules possessing unpaired electrons. Antioxidants are the most effective ingredients to eliminate free radicals which can create oxidative stress and are possible protective tools that protect the cells from reactive oxygen species (37). They retard the progress of many diseases as well as lipid peroxidation. Additionally, they also possess anti-inflammatory, anti-viral and anti-cancer properties ((37,38). *Ferula asafoetida* extracts which are rich in phytochemicals which have great importance in free radical scavenging. The effect of antioxidants on the DPPH free radical scavenging was considered due to their hydrogen donating ability. The extract of *Ferula asafoetida* exhibited a significant antioxidant potential with standard IC<sub>50</sub> value of 380 µg/ml Further studies may be needed to find out the potential health benefits of the extracts in prevention and scavenging of free radicals.

Investigation of anticholesterol activity of *Ferula asafoetida* aqueous extract revealed a dose dependent increase in the percentage of inhibitory activity. The extract showed a potent anti cholesterol activity with a IC<sub>50</sub> value of 450 µg/ml (Figure 2). The activity of the extract is less compared to the standard drug simvastatin in all the tested concentrations. Simvastatin is a standard anti-cholesterol drug which acts as HMG CoA reductase inhibitor (39). Although statin groups of drugs do well in many people, the use of it is associated with potential adverse effects which include cognitive loss, neuropathy, pancreatic and hepatic dysfunction, and sexual dysfunction (40). Hence our study showed that even though the extract is showing less activity compared to simvastatin, since the extract is natural in origin it can avoid the adverse side effects created by the synthetic drugs.

## CONCLUSION

The present study can be concluded that the extract of *Ferula asafoetida* possesses potent antioxidant and anticholesterol activity. Further detailed studies on in vitro cell lines and *in vivo*

experimental animal models need to be done on the plant extract for the formulation of the anti-cholesterol drug towards the clinical utility.

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**Statement of conflict of interest:**

The authors reported no conflict of interest while performing this study .

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