

# Invitro analysis of phytochemicals and investigation of antimicrobial activity using crude extracts of *Ferula assa-foetida* stems.

Sagar Bashyal<sup>1</sup>, Shubham Rai<sup>2</sup>, Osama Abdul<sup>2</sup>

<sup>1&2</sup> Student, Department of Biotechnology,

<sup>1&2</sup>IILM College of Engineering and Technology/Dr. A.P.J Abdul Kalam Technical University, U.P

\*\*\*

**Abstract** - Many living organisms show sensitivity to the various crude extracts of the plants found biologically that consist various types of pharmaceutically important phytoconstituents. These phytoconstituents have become the valuable step in the detection of the bioactive compounds which may lead to the development of new drugs resulting from the mitigation of numeral diseases. In this research, the phytoconstituents present in the stems of *Ferula assa-foetida* were identified and the antimicrobial activity was tested against *E. coli* invitro. The four different plant extracts were screened for the test of terpenoids, flavonoids, alkaloids, saponins, glycosides, steroids, proteins, tannins, carbohydrates. Glycosides presence was seen in all extracts. Terpenoids and carbohydrates presence was found in chloroform extract. Flavonoids were present in methanol and acetone extracts while Saponins was found in chloroform and aqueous extracts. Proteins were found in methanol and aqueous extracts. Alkaloids and tannins were found in aqueous extract. All four extracts showed an absence of steroids. The prepared extracts were used for antimicrobial analysis against *E. coli* using agar well diffusion technique where Streptomycin was taken as a control. All the extracts showed the antibacterial activity against *E. coli* giving variable zones of inhibition (in mm).

**Keywords:** *Ferula assa-foetida*, plant crude extracts, phytochemical analysis, antimicrobial assay.

## 1.INTRODUCTION:

Since ancient ages, herbal plants, also called medicinal herbs have been continuously used to treat multiple diseases. There is number of reports available as a literature which describes the sensitivity of various fungi and bacterial species towards the various plant leaves, stems, oils, and roots. *Ferula asafetida* Linn: *Asafetida*, is the herbaceous plant that belongs to the Umbelliferae family. This plant is famous for the resin known as oleo gum resin obtained from the root of the plant. It is a perennial plant which is in the range of 3.3 to 4.9 ft. tall. The species is native to the deserts of Iran and mountains of Afghanistan and is mainly cultivated in nearby India [1]. The Latin name *ferula* means "carrier" or "vehicle". *Asa* is a Latinized form of Farsi *asa* "resin", and Latin *foetidus*

means "smelling, fetid". The aroma of the plants is used in the food as a flavoring agent. It is also known as devil's dung, *asant*, food of the gods, *jowani badian*, stinking gum, *hing*, *hengu*, *ingu*, *kayam*, and *ting*. [2] In Persia this herb is so highly esteemed as a condiment, it is mixed with almost all their dishes. Many commercial preparations of *asafoetida* use the resin ground up and mixed with a larger volume of other neutral ingredients, such as gum arabic, wheat flour, rice flour, and turmeric. [3]

Afghanistan people at earlier used the gum orally for a hysteria and whooping cough and to treat ulcers.[4 Hot water extract of the dried root is taken orally as an antispasmodic, a diuretic, a vermifuge and an analgesic in Egypt.[5] The decoction of the plant is taken orally as a vermifuge in China.[6] Gum is chewed for amenorrhea in Malaysia[7] and as antiepileptic in Morocco.[8] In Saudi Arabia, dried gum is used medicinally for whooping cough, asthma, and bronchitis.[9] and water extract of the resin in Nepal is taken orally as an anthelmintic[10] and In Brazil hot water extract of the dried leaf and stem is taken orally by males as an aphrodisiac[11] and oleoresin powder, crushed with the fingertips, is used as a condiment.[12] Fluid extract of the resin is taken orally as an emmenagogue, a stimulating expectorant, an anthelmintic, an aphrodisiac, and a stimulant to the brain and nerves and claimed to be a powerful antispasmodic in United State.[13] Gum resin with salt and the bark juice of *Moringa pterygosperma* is used externally for stomachaches.[14]

## Taxonomical classification [15]

<b>Kingdom:</b>	Plantae
<b>Class:</b>	Angiosperms
<b>(unranked):</b>	Eudicots
<b>(unranked):</b>	Asterids
<b>Order:</b>	Apiales
<b>Family:</b>	Apiaceae
<b>Genus:</b>	<i>Ferula</i>
<b>Species:</b>	<i>F. assa-foetida</i>

## 2.METHODS:

### 2.1 Plant Material Collection and Authentication:

Plants of *Ferula assa-foetida* was collected from the local region of Greater Noida for the test purpose. The plant was authenticated by Associate Professor Dr. Avijit Guha of Department of Biotechnology at IILM College of Engineering and Technology in the month of August 2017. The stems of the plants were oven dried at the controlled temperature of 170°C. Morphological studies were based on the shape, size, color, odor. The dried part of the stem was weighed and grinded using electrical grinder until the coarse powder was formed.

### 2.2 Preparation of crude extracts:

5 gm of coarse powder sample in each 4-conical flask(200ml) was Soxhlet with distilled water (50 ml), ethanol and water (7:3, v/v), chloroform and acetone (70%) in successive mode for 48 hours in successive mode using Soxhlet apparatus (Fig. 1) after loading on an orbital shaker at a speed 120 rpm for 24hrs.



**Fig. 1 Soxhlet Apparatus**

The extracts obtained was further concentrated using rotary evaporator (Rotavap, Heidolph Laborotechnik VV 2000) with the water bath set at 55°C. The dried extracts obtained was weighed and percentage extracted was calculated which was then transferred to airtight jars and stored at 4°C in the refrigerator for future use. The obtained crude extracts were further used for the

evaluation of the antimicrobial activity and phytochemical analysis.

### 2.3 Maintenance of Test Organisms & Sterilization of Materials:

Agar plates were prepared and bacteria were cultured continuously to produce active *E. coli* cells and the plates were left to incubate for 24 hours. Before each experiment, the organism was activated by successive sub-culturing and incubation. The media in conical flask and Petri dishes, and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170°C for 1 h at each occasion. Laminar air flow was cleaned with 70% ethanol before starting the culturing of microbes. The obtained crude extracts were further used for the phytochemical and antimicrobial test.

## 3.PHYTOCHEMICAL TEST:

Standards methods were employed to test the presence of the phytochemicals present in the *Ferula assa-foetida* extracts based upon the methods given. [16,17,18,19].

### 3.1 Tests for steroids and terpenoids

#### 3.1.1 Salkowski Test

0.5 ml of each extract was treated in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, red color at the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicates the presence of terpenoids.

### 3.2 Test of flavonoids

#### 3.2.1 Alkaline reagent test

To 0.5 ml of plant extracts few drops of sodium hydroxide solution was added. A yellow coloration which turns to colorless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

### 3.3 Test for alkaloids

#### 3.3.1 Dragendorff's test

To 0.5 ml of plant extracts the Dragendorff's reagent was added. (Potassium bismuth iodide solution). A reddish-brown precipitate confirms that test as positive.

### 3.4 Test of saponins

#### 3.4.1 Froth test

A pinch of the dried powdered plant was added to 3 ml of distilled water. The mixture was shaken vigorously. Formation of a foam indicated the presence of saponin.

### 3.5 Tests for glycosides

#### 3.5.1 Borntrager's test

1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the plant extracts. A reddish pink color indicated the presence of glycosides.

### 3.6 Test of proteins

#### 3.6.1 Biuret Test

To 0.5 ml of plant extracts, 4% NaOH solution and few drops of 1% CuSO<sub>4</sub> solution were added. Violet color appears, indicating the presence of protein.

### 3.7 Test of tannins

#### 3.7.1 Ferric Chloride Test

To 0.5 ml of plant extracts, few drops of 0.1% ferric chloride solution was added. Formation of brownish green or a blue-black coloration indicated the presence of tannins.

### 3.8 Test of carbohydrates

#### 3.8.1 Benedict's test

0.5 mg of plant extracts was shaken with 2.5 ml of water, filtered and the filtrate was concentrated. To this 1.25 ml of Benedict's solution was added and boiled for 5 minutes. Brick red precipitate indicated the presence of carbohydrates.

## 4. EVALUATION OF ANTIMICROBIAL ACTIVITY:

### 4.1 Test microorganisms and control:

The extracts of the seeds of *Ferula assa-foetida* was tested against *E. coli*. The sample of *E. coli* was obtained from the sample taken from clinical sites. The isolated culture in nutrient agar medium was sub-cultured in a nutrient broth

which was kept at 37°C for 24 hours. Ciprofloxacin was used as the control for *E. coli* cells.

### 4.2 Antimicrobial assay:

Agar well diffusion method was used to determine the antimicrobial activity. *E. coli* suspension was seeded on two MHA (Muller Hinton Agar) plates maintained in the sterilized condition. In each of these plates, two wells were punched using the sterilized corn borer. Using a micropipette 100 µl of ethanol extract and Streptomycin was loaded in the first plate (well 1 and 2) and again, the same concentration of acetone, chloroform, and aqueous extract was loaded in the second plate in respective numbered wells. Plates were incubated for 24 hours at 37°C.

Diameter measurement method of inhibition zone formed around well was used to analyze antimicrobial activity. The effects were compared with that of the standard antibiotic Streptomycin.

## 5. RESULT & DISCUSSION:

### 5.1 Phytoconstituents screening:

Phytochemical test of four different extracts is shown in Table 1. Glycosides presence was seen in all extracts. Terpenoids and carbohydrates presence was found in chloroform extract. Flavonoids was present in methanol and acetone extracts while Saponins was found in chloroform and aqueous extracts. Proteins were found in methanol and aqueous (aqueous) extracts. Alkaloids and tannins were found in aqueous extract. All four extracts showed an absence of steroids.

**Table 1: Phytochemical test results of four different extracts of *Ferula assa-foetida***

2	Phytochemical.	Methanol	Acetone	Chloroform	Water
1.	Terpenoids	-	-	+	-
2.	Flavonoids	+	+	-	-
3.	Alkaloids	-	-	-	+
4.	Saponins	-	-	+	+
5.	Glycosides	+	+	+	+
6.	Steroids	-	-	-	-
7.	Proteins	+	-	-	+
8.	Tannins	-	-	-	+
9.	Carbohydrates	-	-	+	-

'+' sign indicates the presence and '-' sign indicates absence.

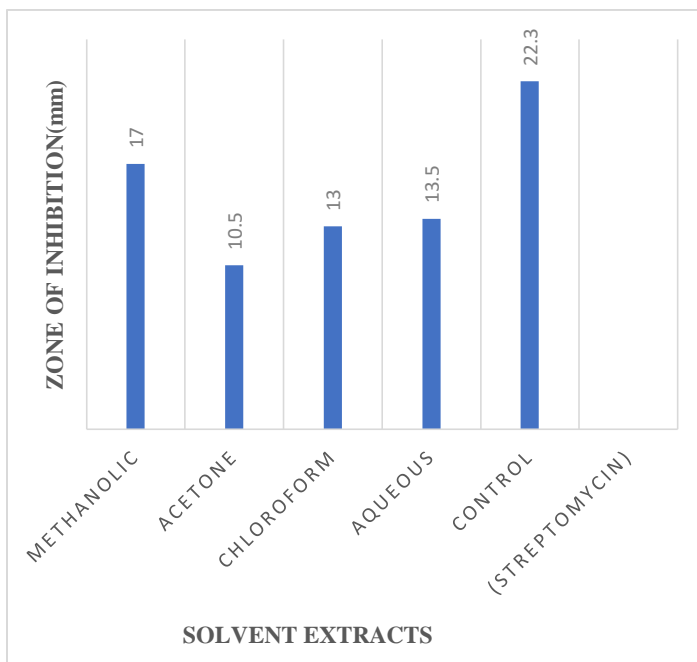
## 5.2 Antimicrobial activity:

After incubation for 24 hours from the time of loading of extract, inhibition zones were measured. The different zones of inhibition signified that different extracts of the plant so different antimicrobial capability. The well loaded with the streptomycin showed inhibition zone of 22.3mm, the methanolic, acetic, chloroform, and aqueous extracts showed inhibition zone of 17mm, 10.5mm, 13mm and 13.5mm (Table 2, Fig1). Maximum zone of inhibition was found in the methanolic extract.

**Table 2. Inhibition zone of four different extracts of *Ferula assa-foetida* on *E. Coli***

Solvent Extract	Zone of Inhibition (mm)
Methanolic	17
Acetone	10.5
Chloroform	13
Aqueous	13.5
Control (Streptomycin)	22.3

**Fig. 2: Chart showing the zone of inhibition and different solvent extracts.**



## 6. CONCLUSION:

The study revealed that *Ferula assa-foetida* stem has potential antimicrobial activity and can be used for

pharmacological evaluation and treatment of various infectious diseases. These seeds contain alkaloids, carbohydrates, glycosides, flavonoids, proteins, terpenoids, tannins, phenols which have the high medicinal purpose. The high zone of inhibition was seen in the methanolic extract which signifies the high antimicrobial action than other three extracts. Therefore, the plant can be used in the treatment of ulcers, used as an analgesic, and treatment of a whooping cough, asthma, and bronchitis.

## 7. ACKNOWLEDGEMENT

The authors are thankful for the Head, Department of Biotechnology, IILM College of Engineering and Technology, Greater Noida to provide necessary laboratory facilities to conduct this research work.

## 8. REFERENCES:

- [1] "Archived copy". Archived from the original on January 4, 2012. Retrieved January 7, 2012.
- [2] Literature Search Unit (January 2013). "Ferula Asafoetida: Stinking Gum. Scientific literature search through SciFinder on Ferula asafetida". Indian Institute of Integrative Medicine.
- [3] Vandevi Hing (Asafoetida): list of ingredients <https://www.amazon.com/dp/B000JMDJ5C>
- [4] Mahran GH, El Alfy TS, Ansari SM. A phytochemical study of volatile oil of Afghanistan asafetida. Bull Fac Pharm Cairo Univ. 1973;12:101-7.
- [5] Buddrus J, Bauer H, Abu-Mustafa E, Khattab A, Mishaal S, El- Khrisy EA, et al. Foetidin, a sesquiterpenoid coumarin from *Ferula assa-foetida*. Phytochemistry. 1985;24:869-70.
- [6] Duke JA, Ayensu ES. Medicinal plants of China. Vol. 1. Algonac, Michigan: Reference Publications Inc; 1985. pp. 52-361.
- [7] Gimlette JD. A dictionary of Malayan medicine. New York, USA: Oxford University Press; 1939.
- [8] Bellakhdar J, Claisse R, Fleuret J, Younos C. Repertory of standard herbal drugs in the Moroccan Pharmacopoeia. J Ethnopharmacol. 1991;35:123-43. [PubMed]
- [9] Bhattarai NK. Folk Anthelmintic drugs of central Nepal. Int J Pharmacol. 1992;30:145-50.

[10] Seabrook WB. Adventures in Arabia among the Bedouins, Druses, whirling dervishes and Yezidee devil worshipers. New York: Blue Ribbon Book; 1927. pp. 99–105.

[11] Elisabetsky E, Figueiredo W, Oliveria G. Traditional Amazonian nerve tonics as antidepressant agents: *Chaunochiton kappleri*: A case study. *J Herbs Spices Med Plants*. 1992;1:125–62.

[12] Seetharam KA, Pasricha JS. Condiments and contact dermatitis of the finger-tips. *Indian J Dermatol Venerol Leprol*. 1987;53:325–8.

[13] Anon . Lilly's handbook of pharmacy and therapeutics. 5th rev. Indianapolis: Eli Lilly and Co; 1898.

[14] Subrahmanyam V, Sastry VL, Srinivasan M. *Asafoetida*. *J Sci Ind Res B*. 1954;13:382–6.

[15] 1897 illustration from Franz Eugen Köhler, Köhler's *Medizinal-Pflanzen*

[16] Mameta S., Jyoti S. Phytochemical screening of *Acorus Calamus* and *Lantana Camara*. *Int Res J Pharm*, 2012;3(5): 324-326.

[17] Sanjay Parihar, Kartik D. Virani, E. A. Pithawala, M. D. Shukla, S. K. Lahiri, N. K. Jain and H. A. Modi. Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of wild edible mushroom *Pleurotus ostreatus*. *Int. Res. J. Pharm*. 2015; 6(1):65-69 <http://dx.doi.org/10.7897/2230-8407.06115>

[18] Mumtaz, F., Shahid Massod R., Zubair A., Iftikhar A. and Musaddique H. Qualitative phytochemical analysis of some selected medicinal plants in local area of Faisalabad Pakistan. *Journal of Pharmacy and Alternative Medicine*, 2014;3(3):17- 23.

[19] Reddy S., Ammani,Ch., RoseMary K., Nikhil Rajesh T., Aravind G. and Bala Sekaran Ch. Phytochemical and GC-MS analysis of *Commiphora caudata* (Wt&Arn.) Eng. Bark, *Indian Journal of Advances in Plant Research*, 2014;1(5):24-29.