# Physico – Chemical & Microbial Studies on Different Varieties of Asafoetida & Defining Process Flow for Commercial Production.

A Dissertation Thesis submitted

to

Institute of Science, Nirma University

In partial fulfillment of degree of

MASTER OF SCIENCE

IN

MICROBIOLOGY

SUBMITTED BY

Himisha Patel (21MMB018)

Under the Guidance of

Mr. Sonu Sharma – Manager (Q, FSR & NPD)

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Dr. Heena V. Dave

INSTITUTE OF SCIENCE, NIRMA UNIVERSITY

Ahmedabad

NIRMA UNIVERSITY
INSTITUTE OF SCIENCE
NAAC ACCREDITED 'A' GRADE

# CERTIFICATE

This is to certify that the thesis entitled **"Physico – Chemical and Microbial Studies on Different Varieties of Asafoetida & Defining Process Flow for Commercial Production"** submitted to the Institute of Science, Nirma University in partial fulfilment of the requirement for the award of the degree of M.Sc. in Microbiology, is a record research work carried out by Himisha Patel (21MMB018) under the guidance of Dr. Heena V. Dave. No part of the thesis has been submitted for any other degree or diploma.

**Prof. Sarat K. Dalai**

Director

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**Dr. Heena V. Dave**

Dissertation Guide

28/4/2023

# DECLARATION

The above Dissertation project was carried out by **Himisha Patel (21MMB018)** under my guidance.

28/4/2023

**Dr. Heena V. Dave**

(Assistant Research Scientist, ISNU)

Place: Ahmedabad

Date: 28/4/2023

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# CERTIFICATE OF COMPLETION

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## CERTIFICATE OF COMPLETION

This certificate is gladly presented to **Ms. Himisha Patel** for completion of her academic internship cum Research and Development project on **Physico – Chemical & Microbial Studies on Different Varieties of Asafoetida, Defining Process Flow for Commercial Production of Compounded Asafoetida Powder.**

During her tenure with us, we found her Punctual, Hardworking and Result Oriented.

We wish her all the best for future endeavours.

**With Regards**
**For,**

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Thanking you,

**Himisha Patel (21MMB018)**

## DECLARATION

I, Himisha Patel, Roll. No. 21MMB018, student of M.Sc. Microbiology batch 2021 – 2023 Institute of Science, Nirma University, Ahmedabad hereby declare that dissertation work entitled as: "Physico – Chemical & Microbial Studies on Different Varieties of Asafoetida & Defining Process Flow for Commercial Production" is a result of my own research work. I shall be solely responsible for my dissertation work. I assert that the statement made, and conclusion drawn is outcome of my research work only. I declare that my dissertation does not contain any part or any work that has been submitted for an award concerning toward any other degree certificate in this institute or any other institute in India or Abroad.

**Date:**

**Place:** Ahmedabad

**Himisha Patel (21MMB018)**

## Table of Contents

# ABBREVETIONS

g : Gram

ml : Milliliter

CSIR : Council of Scientific and Industrial Research

IHBT : Institute of Himalayan Bioresource Technology

% : Percentage

°C : Degree Celsius

pH : Negative logarithms of hydrogen ion concentration

N : Nitrogen

P : Phosphorus

K : Potassium

FSSaI : Food Safety and Standard Authority of India

EMB : Eosin Methylene Blue Agar

Mac : MacConkey Agar

SS : Salmonella – Shigella Agar

DCA : Deoxycholate citrate agar

GPB : Glucose Phosphate Broth

SC : Simmon Citrate

$Fecl_3$ : Ferric Chloride

$H_2O_2$ : Hydrogen Peroxide

Wt. : Weight

& : And

**INDEX OF FIGURES: -**

## INDEX OF TABLES: -

## ABSTRACT: -

*Ferula Asafoetida* is AKA Hing, Devil's drug, stinking gum, food of good is vital spices belong from umbelliferon family. From the ferula plant, oleo – resin or gum of asafetida has been ejected through the root. Asafoetida brings the Sharpe, powerful, nauseating, and sulfurous aroma. Asafetida is vernacular to Afghanistan and Iran. Asafetida is non vernacular of India. Hence India imports the asafetida from the Afghanistan and Iran. It is widely cultivated in the unproductive cold and dry desert. Asafetida is widely used in flavoring dal, curries, pickles, sauces, meat, etc. Generally, asafetida is available in the powder form, compound form, granule form, oil form, oleo-resin form, and sphere (goli) form. The major chemical constituent of asafetida is resin (40-60%), endogenous gum (25%), and essential oils (10-17%). Asafetida consists of phytochemicals including carbohydrates, fiber, iron, potassium, calcium, volatile oil. Gum and phytochemical of asafetida is widely used in ayurvedic as well as in unani therapeutic practices. Asafetida is beneficial to cure respiratory disorder, digestion problem, cardio - vascular health, maintain blood pressure, women aliments, etc. Asafetida shows the antimicrobial, antibacterial, antifungal, antiviral, anticarcinogenic, antitumor, antioxidant, antidiabetic, hepatoprotective, neuroprotective, antispasmodic properties. Asafetida is mostly propagated by seed sown either in spring or winter. Asafetida is mature after the five years of planting, and it became ready to harvest and yield of individual plant is 900g.

## Keywords:

Asafoetida, Antimicrobial Activity, Physico – Chemical, Organoleptic and Microbiological Properties, Alcohol Soluble Extract, Commercial Production.

# LITERATURE REVIEW:

**1: Asafetida:**

Asafetida is also known as Hing, a dried latex which was excreted through root or creeping rootstalk of the asafetida plant. Ferula asafetida is belonging from the Umbelliferae family. Asafetida is vernacular from Iran, Afghanistan, Turkey, Uzbekistan, Kazakhstan, especially on the cold dry desert and mountain area. In India city of asafetida are known as Hathras in Uttar Pradesh. There are variety of asafetida based on their climate, soil, moisture etc. Asafetida consists various phytochemical chemical constituents and due to these it shows various medicinal properties like antimicrobial, antibacterial, antifungal, antiviral, neuroprotective, anticarcinogenic, anti – obesity, etc. not only in medicinal it is also used for culinary usage.

**2: First time cultivation of asafetida in India:**

India imports 1500 ton of asafetida per year. Hence the cost of asafetida is almost 948cr INR. To become self-sufficient, India has tried to cultivate the asafetida at the Kawring village of Lahula and Spiti valley, Himachal Pradesh. Dr. Sanjay Kumar, he was director of CSIR – IHBT, planted the first shrub of asafetida in India.

**3: Harvesting:**

There are three different methods for the harvesting of asafetida, based on that production of asafetida is depending. A): Concave cutting method B): Conventional cutting method C): Surface cutting method. For the harvesting of asafetida best method is concave cutting method for the mass production.

**4: Varieties of asafetida:**

There are several varieties of asafetida based on different country, climate, moisture, soil, etc. Different asafetida has their different properties.

# CHAPTER – 1: INTRODUCTION

## 1.1 Spices of India:

India ranked first for the highest production of spices among all the world, hence India has its own identity as the home of spices. Diversity in spices is grown all over India. Cultivation of spices occurred in varies weather in different parts of the country, many of which are native to the subcontinent. Spices are used in the form of seed, bark, flower, leaves, bulbs, and roots and produce variety of flavors like hot, sour, sweet, aromatic, mild, and tart. The best example of native of Indian spices is Pepper, turmeric, cardamom, cumin etc. While some other spices imported from different countries. The best example of the imported spices is Asafoetida.

**Figure 1**: Spices of India

Spices has been used in form of complete form, cut up form, powder form, baked form, frizzled, and in a matter of topping. For extraction of the nutrients and make up delicious form of dish they blend food. Individual spice has its own distinctive appearance, peculiar odor, & amplifying characteristic which make food delicious. Indian spices possess a prolong deep - history of business with the prehistoric civilization of Italy along with China. India is the largest domestic market on the globe considering spices.

### 1.1.1: History of Indian Spices: -

Spices of India shows the antiquity from last thousands of years. Indian tribes use herbs and spices from the period of human civilization of spices. The whole narration of Indian spices has been mentioned in the Rigveda, Samaveda, Yujurveda, Atharveda. At the time of vedic period, the knowledge of spices has been transformed from generation to generation via hyms. The antiquity of Indian spices is embedded in Mother Nature's lavishness and goodwill.

Before the Greek and roman civilization, the antiquity of Indian spices makes it way to Egypt, Mesopotamia, and Arabia centuries. At the later time, Greek dealer buy the opulent condiments from the south India. During the time of 1300, in Europe 1 pound of nutmeg has been more valuable compared to gold. In 1498, Vasco da Gama journey to India via southernmost point of Africa. He landed at the India's Malabar cost, which is the center of spices trading and start export directly to Europe and southeast Asia. In short period, Portuguese had gained authority of the spice producing region in 15$^{th}$ centennial and set up an ownership on Indian spice trade, while it has been continuing for over a century and remarkable it has been profitable for the Portuguese empire. The important product import to Lisbon was black pepper and at that time pepper was valuable as gold.

At the time of 16$^{th}$ centennial, over half of the Portugal state income arrived from Indian pepper and West African gold. At that time value of spice is more compared to the gold. Hence spices from East are more valuable, at the time of middle ages, the price of one pound ginger worth an one sheep, while the price of one pound mace worth three sheep. While the pepper is most valuable spice. Asafetida is another example of the most valuable spices, but it is not vernacular of India. Hence India imports asafetida from other countries.

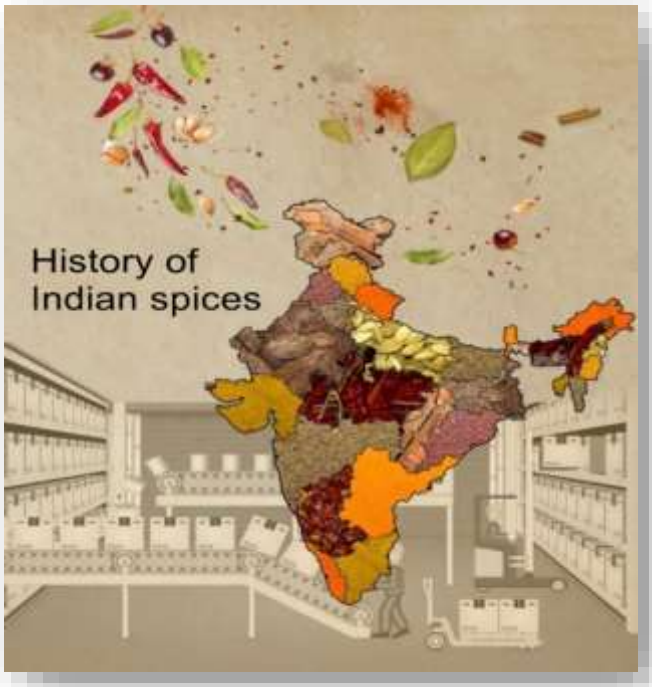



**Figure 2**: India is Home of Spices

## 1.2: Asafoetida: -

*Ferula Asafoetida,* AKA either asafetida or Devil's Drug. Locally in India asafetida are known even as Hing or Hingu. Asafetida are dried latex or oleo gum resin. It is obtained by scraping the stem of ferula plant. Commonly asafetida is dried and yellow powder form, and it is also used for the purpose of pharmacological, culinary, and for various traditional aspects. Asafetida is available majorly in two forms: mass & tear. Mass form of asafetida are easily available in the market.

As their name suggest, Asafetida has fetid smell and nauseous taste, due to this reason they are known as Devil's Dung and stinking gum. Astonishingly, in Persia asafetida are known as "food of the gods". Asafetida herb is significant constitute in Hingashtak: which is a polyherbal ayurvedic medicine used as a digestive / gastro - intestinal aid. Spices is utilized for millennium like food appendages that escalate diligence and standard value of food. By conveying the colour, pungency, and flavor, they can alter flavorless food into delicious, aromatic and appetizing meal.

In Latin Ferula means "vehicle" or "carrier" while asa = resin and foetidus = fetid smell.

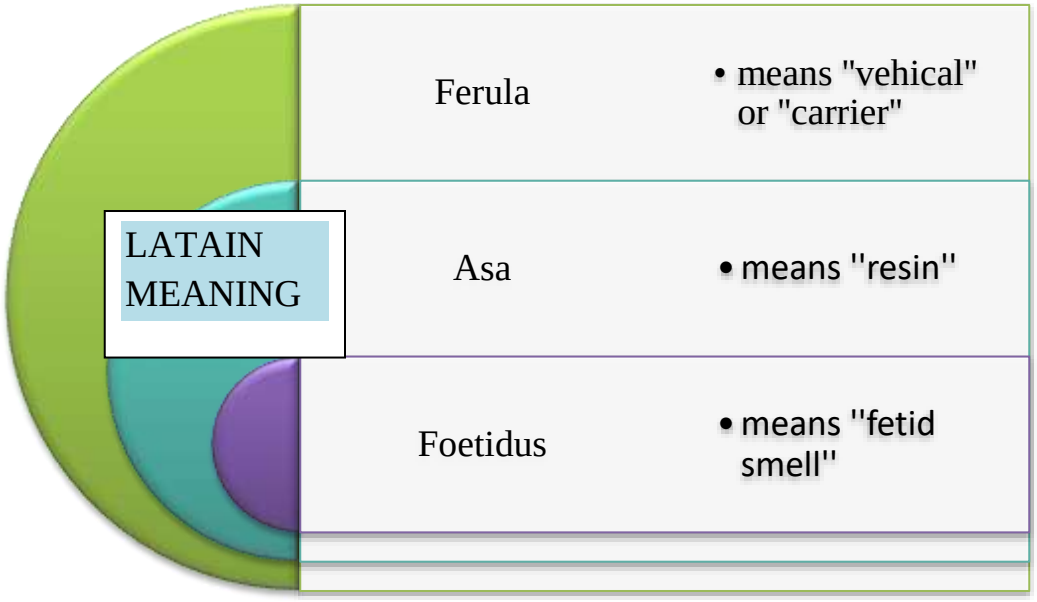



**Figure 3**: Latin meaning of Asafetida

There are two main types of Ferula asafetida:

- Hing Kabuli sufaid – which is milky white in colour.
- Hing Lal – which is red in colour.

**Figure 5**: Hing Kabuli sufaid

**Figure 4**: Hing laal

Asafetida is dignified into the hing and hingra. Hing is water soluble and white-pale in colour while Hingra is oil soluble and darker in colour compared to hing. The ferula plant brings the strong, powerful, pungent, and sulfurous aroma due to inherent higher concentration of sulfur.

Ferula asafetida is widely used as the flavoring agent in various dishes including dal, curries, gravies etc. In India, asafetida is used widely to make veg and non – veg cuisine more prominent due to its aroma and natural flavor and taste enhancing properties. In Ayurveda, Ferula asafetida is used as antidote for heroin, while it is also used as the appetizer and reliever to common gastro intestinal issues. Ferula asafetida is used as perfumes and flavoring agent by United States and European countries.

During antiquarian Rome, asafetida is store in container along pine nuts, that are utilized to flavoring the delicate dishes, which increase taste in mushroom and vegetable dishes.

Asafetida is a dense-sticky gum, when it is in a fresh condition then it appears grayish-white, while it growing older it becomes yellow, red, and finally it becomes dark cream.

Gum like asafetida trades in form of chunks and slices and more constantly sold in a fine yellow powder.

While asafetida is non – vernacular from India. Hence India imports asafetida mainly from Afghanistan. Other than Afghanistan, India also imports from Iran, Uzbekistan, Kazakhstan, Turkey.

- There are different names of asafetida based on their country and different languages.

    - Different names of asafetida based on different country: -

| **Country name** | **Different name of Asafetida** |
|---|---|
| Afghanistan | Kama, Anguza |
| Bangladesh | Hing |
| England | Asafetida |
| United States | Asafetida |
| Myanmar | Sheingo |
| Denmark | Dyvelsdrak |
| Russia, Italy, Poland, France, Germany, Finland, Spain | Asafetida |

**Table: 1**- Country wise different names of Asafetida

    - Different names of asafetida on basis of Indian languages: -

| **Different Indian languages** | **Different name of asafetida** |
|---|---|
| Gujarat, Hindi, Bengali, Assamese | Hing |
| Tamil | Perungayam |
| Kannada | Ingu |
| Kashmiri | Yang |
| Malayalam | Kayam, perungayam |
| Sanskrit | Hengu, Raamnath |
| Punjabi | Hing, Hingra |

**Table: 2**- Indian state wise different names of Asafetida

### 1.2.1: Botanical characteristic of Ferula asafetida: -

*Ferula asafetida* is monoicous, perpetual, and herbaceous plant belongs from the apiaceous family. Due to the higher concentration of sulfur, it possesses bitter taste and fetid smell. Asafetida are oleo - gum resin, and it is extracted through thick taproot of ferula plant. It is growing about 2m in height and 12.5 to 15cm diameter on the crown. It will take 4-5 years for production of oleoresin on ferula herbaceous plant. Roots is enormous, broad, & juicy. On superficial surface of bark of ferula plant is brownish to blackish and crumpled; while inner surface is fleshy and white consist of the milky white juice.

The leaves of ferula plant are glossy, ovoid, tripinnate, and grow prior to 45cm in length, while the stem is solid, smooth and 10cm thick. Hence cortex region consists of several duct, and it possess the gum of asafetida. The flower of asafetida is slime, even, and yellow in colour. Fruits of Ferula asafetida are ovoid, narrow, uneven, slightly hairy, and russet in colour. The white discharge of fruit is pure, crystal clear, and aromatic.

| **Taxonomic Rank** | **Taxon** |
|---|---|
| Kingdom | Plantae |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Family | Umbelliferae |
| Genus | Ferula |
| Species | Asafoetida |
| Common name | Hing, Hingu |

**Table: 3**-Taxonomy of Asafetida

**Figure 6**: Plant of Asafetida

## 1.2.2: Origin and distribution of *F. Asafetida*: -

*Ferula asafetida* is the vernacular spices of region of Mediterranean & central Asia. Asafetida cultivates at height of 600-120m beyond SL. Specifically in central Asian countries including Iran, Afghanistan, Uzbekistan, Kazakhstan, Tajikistan, Turkey, western Pakistan, Zagros and Elburz Mountain of Persia gulf, North Africa, and Europe. Iran and Afghanistan are predominant countries in production of asafetida; hence both these countries have ability to export asafetida all over the world.

Major Asafetida producing countries are highlighted in world map.

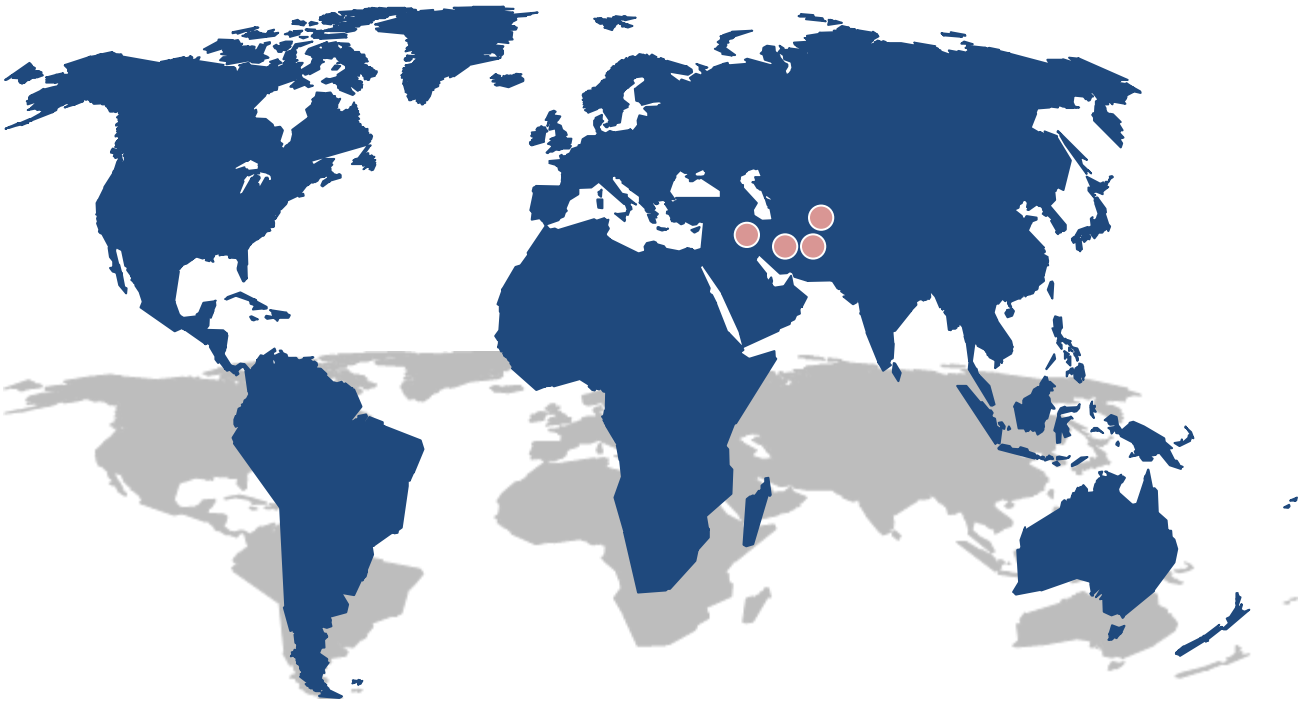


**Figure 7**: Shows major countries producing asafetida.

Asafetida majorly found in the Western mountains of Afghanistan and eastern desert of Iran. Asafetida is not vernacular spices of India, but still it is used by age old time for culinary and therapeutic properties. Asafetida is widely grown at Mount Telesm of Kermanshah province, and Mazar area in Bedzhestan district of South Khorasan province of Iran. Kunduz and Samangan province of Afghanistan is the major producer of asafetida in the world.

## 1.2.3: Phytochemical constituents of Asafetida:

There is numerous chemical constituents exist in the *F. asafetida*, which are shown in following pie chart. Asafetida having bitter taste, sulfurous order in their pure form. Asafetida consists of four most important fragments:

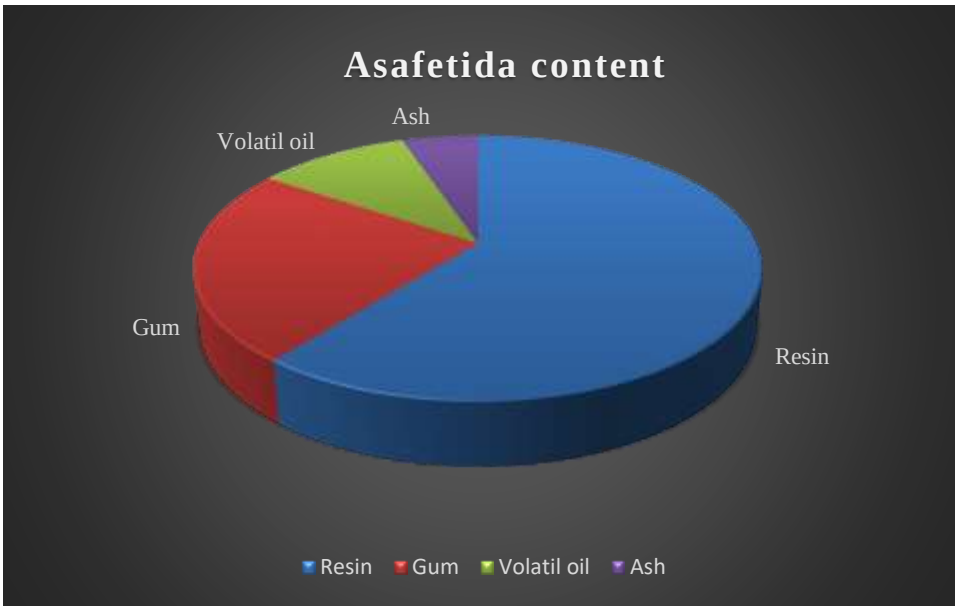
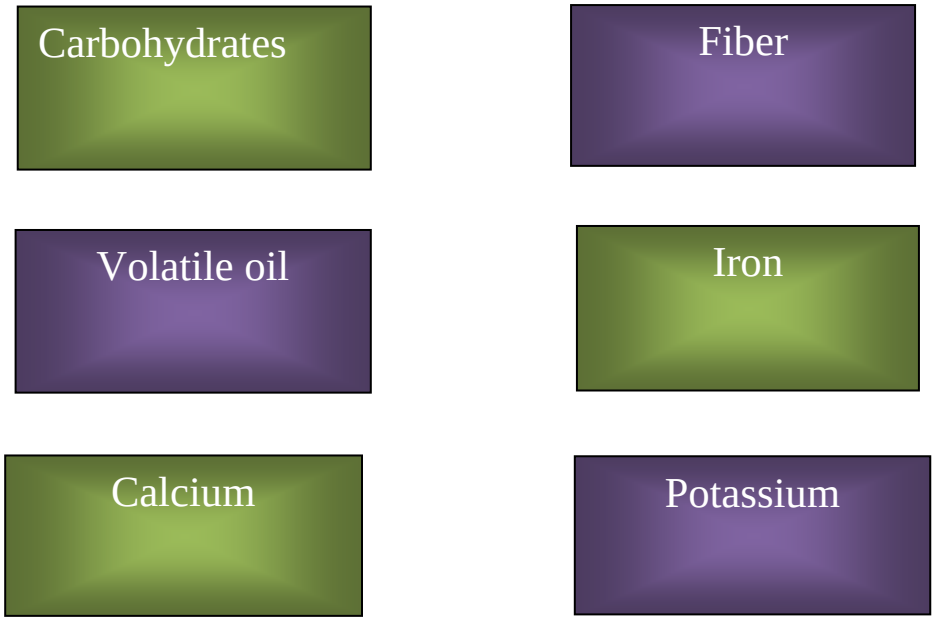



**Figure 8**:Chemical constituent of asafetida

1) Resin in asafetida (40-64%):
    - Resin contains ferulic acid and its esters, coumarins, sesquiterpene and thier terpenoids.
2) Gum in asafetida (25%):
    - Gum consists of glucose, galactose, 1-arabinose, rhamnose, glucuronic acid, polysaccharides, and glycoproteins.
3) Volatile oil in asafetida (10-17%):
    - It consists of sulfur-containing compounds, monoterpenes and other volatile terpenoids. Asafetida contains around 68% of carbohydrates, 16% of moisture, 7% of minerals, 4% of protein, 4% of fiber, 1% of fat.




Ferula asafetida shows the numerous biological activities, and it is also considered as the medicine due to its phytochemical constituents. The mineral and vitamin contents of asafetida consist specific amount of calcium, phosphorus, iron, carotene, riboflavin, and niacin. Because the presence of phytochemical constituents, Ferula asafetida brings diversified pharmacological and therapeutic properties including, antibacterial, antimicrobial, antitumor, anticarcinogenic, antispasmodic, antiviral, antifungal, and antidiabetic. Traditionally, asafetida is used to treat various disease including women aliments, respiratory disorder, cough, digestion problem, Neuroprotective effect due to its phytochemical constituents.

### 1.2.4.: Value Addition:

By making cut on the thick taproot of the Ferula asafetida, milk juice has been obtained and further it becomes brown in colour by drying. After the processing of the asafetida, it has been marketed either in powdered or clump form. The cluster or clump of asafetida is most known as pure asafetida. The common form commercially available in markets is combination of pure form of asafetida with edible gum and grains (Wheat / Rice) is known as compounded Asafetida. Hence compounded asafetida consisting of the 50% or more of the rice flour or wheat flour and the Arabic gum. Arabic gum plays a vital role in the powdered asafetida, which prevents lump formation in powder. By the steam distillation of the raw asafetida, oil of asafetida can be obtained.

### 1.2.5: IMPORTANCE

Asafetida is used as the culinary as well as in the form of medicinal herb. Gum, resin and volatile oil of gum also used as the medicinal herb to treat various diseases.

### 1.2.5.1: Medicinal Importance: -

Asafetida is filled with phytochemical including calcium, potassium, fiber, iron, carbohydrates, and volatile oil. Such kind of phytochemical play a vital role in therapeutics properties.

- Carbohydrates: -
  - It stimulates metabolism. It is useful to maintain the good ingestion, cardiac health and in improving brain activity.
- Fiber: -
  - Asafetida maintains the digestion, regular bowel movements, decrease hyperglycemia and control the blood sugar levels in the body.
- Iron: -
  - Iron is powerful blood purifier. It cures the anemia by increasing the concentration of hemoglobin and red blood cells. And stimulate the immunity.
- Potassium: -
  - It acts as the electrolyte. Maintain the balance of sodium for maintaining the blood pressure. Potassium blocks the shrinking of arteries and decreases the risk of blockage and maintains the good cardio vascular health.
- Calcium: -
  - Calcium boosts the good bone and dental health. Calcium fortifies the teeth and bone; and decreases the risk of colon cancer and kidney stone.
- Volatile oil: -
  - Due to the anti-inflammatory, anti-microbial, anti-fungal properties of asafetida, asafetida treats the cough, asthma, and block dental problems.

Medicinal properties of Asafetida: -


**Figure 9**: Medicinal properties of asafetida

**1.2.5.1(A): Digestive enzyme activity:**

Predominantly spices are used to enhance the salivary flow and gastric juice secretion, which helps indigestion. Asafetida is the ancient medicine to treat the dyspepsia including irritable bowel syndrome (IBS), Helminths, flatulence, gas, and bloating.

In instance of flatulence and distension of the stomach, by adding asafetida within lukewarm water, soak the cloth, and this remedy is used for provoking the abdomen.

**Figure 10:**Asafetida use in digestion problem

Asafetida is also known as the digestive aid. Asafetida essentially provokes pancreatic amylase and intensifies the pancreatic lipase activity. Asafetida shows anti-spasmodic and anti-inflammatory in response to such kind of digestive issues. Asafetida shows the anti- microbial activity; hence it regulates the growth of micro flora in intestine and prevent from infection.

**1.2.5.1(B): Effect of asafetida on respiratory disorder:**

Asafetida can as well aid in alleviate respiratory disorders including asthma, bronchitis, and pneumonia, whooping cough. Asafetida is an aid in alleviate chest obstruction and liberating cough. By applying the past of asafetida and water on the chest, it has ability to reduce the obstruction and treat the cough. With that past also add the dry ginger and honey, by consuming this mixture get the comfort from respiratory disorder.

**Figure 11:** Asafetida use as aid in Respiratory dis

### 1.2.5.1(C): Effect of asafetida on blood pressure levels:

Asafetida is also known as the natural blood thinner, and it is also used as aid to reduce the blood pressure. It is given with coumarin, which regulate the blood flow and block the formation of clots.

Asafetida regulates the production of insulin from pancreatic cells with the help of essential nutrient present in asafetida and maintains the diabetes under control.

**Figure 12**: Asafetida control Blood pressure levels

### 1.2.5.1(D): Effect of asafetida on women's ailments:

Ferula asafetida is contemplate convenient as the therapeutic of various complications with respect to women including sterility, unwanted abortion, pre-mature labor, and excessive menstruation and leucorrhea. Asafetida is used to relieving the menstrual aching and contractions at below the stomach and back.

**Figure 13**: Asafetida used to cure Women's ailments.

As Asafetida is an anticoagulant agent, it assist blood flow evenly without obstruct any part of body. Asafetida enhance easy blood flow; by stimulate progesterone hormone secretion as a result provides relaxation from pain. Prepare the mixture of the pinch of asafetida, fenugreek powder, and salt; add this mixture into the buttermilk. During the periods taking this mixture with buttermilk as a result it is providing the relief from cramps and pain. Asafetida is useful for women during the post delivery period. While in the south India, mixture of the asafetida and rice flour are given to women after delivery.

**1.2.5.1(E): Effect of asafetida on headache:**

The powder form of asafetida consists of the antioxidant & anti-inflammatory activity, that assist in decompressing the throbbing blood vessel. Asafetida consist the antidepressant properties which helps to improve the mood and help to become a stress free.

**Figure 14**: Asafetida gave relief from Headache.

**1.2.5.1(F): Asafetida is best in consideration of cardiac health:**

As asafetida consist coumarins which show the anti - coagulant activity, hence it does not allow for clot and decrease the high blood pressure. As the asafetida is rich in potassium content there was less chance of blockage or strokes in the body.

**Figure 15**: Asafetida use in Heart issues

**1.2.51(G): Effect of asafetida on beauty benefits:**

Asafetida support to decrease acne due to its anti-inflammatory properties, while its antibacterial properties show the inhibition of the growth of the pimple and rash. Add pinch of asafetida in Multani Miti with rose water and apply on affected area as a result it reduces the problem of acne and pimple. Asafetida support raises the oxygen and providing into the facial tissues and as a result it gives the shinning glow.

## 1.2.5.2: Asafetida in Ayurveda:

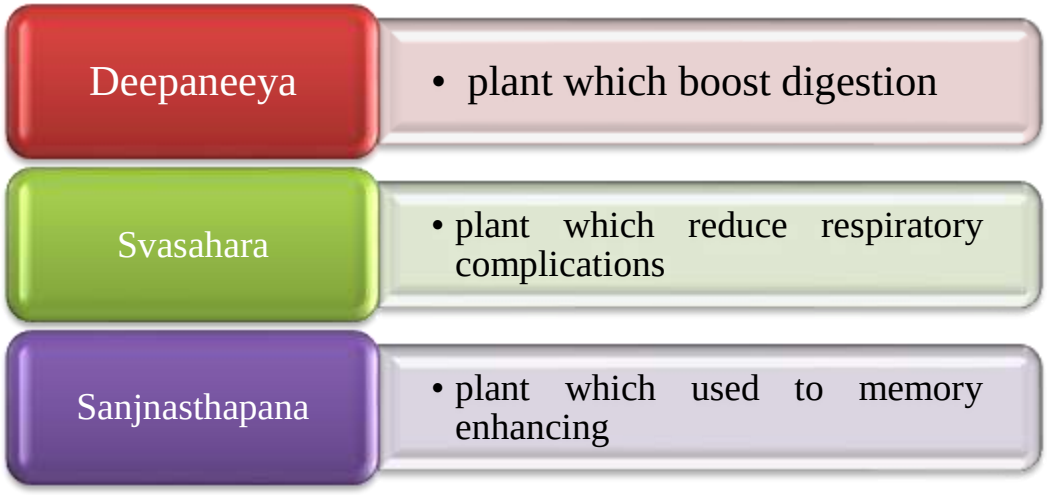
In Sanskrit language asafetida is known as Ramaha, Badhika, Ugragandha and Sahasravedhi.

Ugragandha - designated due to its powerful sulfurous aroma

Sahasravedhi - designated due to, herb to treat of 100+ diseases

According to Ashtanga Hridaya, a prehistoric Ayurvedic document stated that, asafetida is a strong, natural remedy in contrast to digestive issues, flatulence, tooth problem, impotency etc. Widely asafetida has been utilized in the baked form due to untreated form of asafetida create irritation and inflammation.

Charaka Samhita, a prehistoric Sanskrit collection on Ayurvedic medicines classify asafetida like.




Asafetida is utilized to cure nervous disorder & considerably it is functional treatment for hysteria. Asafetida is powerful in smell, hence in ayurveda this is known as Khatu rasa. Asafetida is adorned with all three qualities.

: easy to digest

: consist of oil

: penetrate deep into tissues

After digestion, asafetida shows warming and powerful properties. Asafetida also maintain to balance Kapha,Vata, and Pitta. Asafetida is widely accessible in several forms and used in the form of Powder, oil, and tablets. Ayurveda also acknowledgement of asafetida for several remarkable properties are listed below in table.

| **Ayurvedic medicine names** | **Treatment** |
|---|---|
| Anulomana | Reduce bloating |
| Hridaya | Potent in cardiac issues |
| Chakshushya | Help to cure in eye problem |
| Krimighna | Cure anthelmintic agent |
| Vaajikaran | Used as aphrodisiac |
| Aartjanan | Help to better in female reproductive health. |
| Balya | Assist in brain build - up |
| Jwarghna | Antipyretic agent |
| Shoolprashan | Reduced edema |
| Vedanasthapan | Relieving pain |
| Udarshool | Prevent from gastrointestinal diseases |
| Kaas-shvash | Cure respiratory disorder |

**Table: 4**- Remarkable properties of Asafetida

### 1.2.5.3: Culinary usage of asafetida: -

Asafetida is widely used for the vegetarian dishes, curries, gravies and sauces. Asafetida is used or added in any dishes in proportion of the pinch. While the undiluted form of asafetida is used in much smaller quantity. Asafetida is broadly used for Indian cuisine, which intensify the aroma and taste of any dish.

As Asafetida are used by the Brahmins and Jain because the use of onion and garlic is forbidden hence, to enhance the flavor of food they use asafetida. By adding the asafetida gum special flavor of Worcestershire sauce is acquired. Asafetida is also used in non – veg food where onion and garlic are in bulky or in whole form.

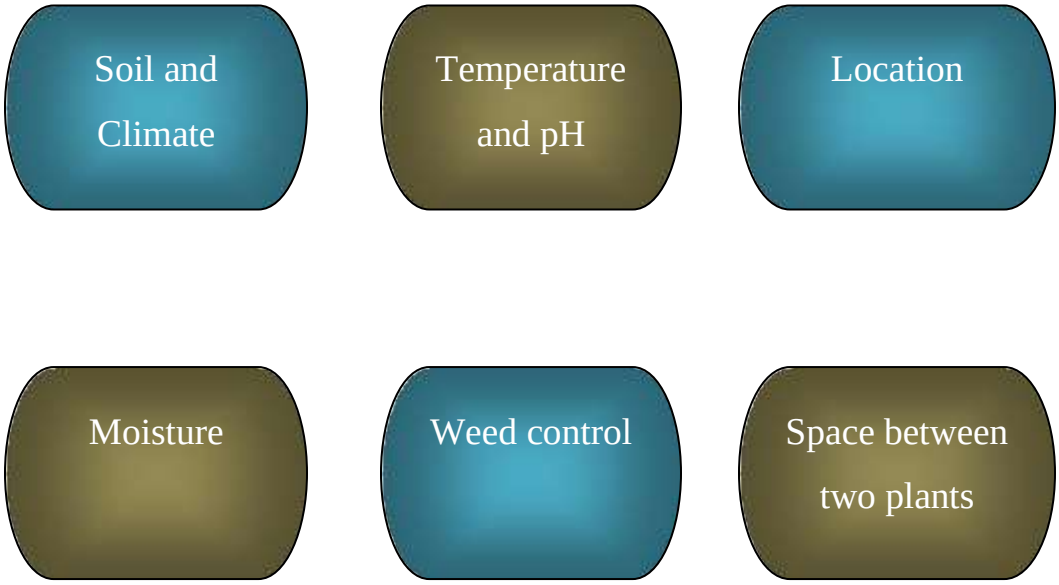
During the time of Mughal period, singers at Agra & Delhi ate a spoonful of Hing along butter to enhance their voice and exercise on the coast of Yamuna. Add pinch of asafetida powder in the hot oil and enhance the aroma of dishes like dal, rasam, curries, pickles, snacks, Kachori, etc. In non – veg dishes in which egg and meat are predominant then to intensify the flavor asafetida or Hing are commonly used.

**Figure 16**: Culinary uses of Asafetida

# CHAPTER – 2: CULTIVATION & HARVESTING OF ASAFETIDA

## 2.1: Cultivation of Asafetida: -

Asafetida has been widely obtained in the gum form. For the cultivation of asafetida few factors are required for their farming,




i. Soil and climate: -

For the cultivation of the asafetida plantation, soil should be in mixes form including, sandy, loamy or clay soil. The soil must be well evacuated. While for the asafetida desert region are more suitable especially cold and dry desert for the best growth of asafetida.

ii. Temperature and pH: -

Asafetida has ability to bear acidic, alkaline, and neutral ph. The cultivation of asafetida is best at the time of august, which having 20-30 °C.

iii. Location for asafetida farming: -

Select the area for asafetida farming where the sunshine interacts with the plant. Hence ferula plant require ample amount of sunshine. The asafetida does not grow in the dark or shaded region.

iv. Spacing between two plants:

There must be 5 feet distance between two plants of Ferula asafetida.

v. Moisture: -

For the growth and the germination of the Ferula asafetida optimum moisture must be necessary. The growth of plant and germination is reduced due to the less moisture content. 40-70% is the optimum moisture for the growth and germination.

vi. Weed control: -

Weed control must be necessary for the plant growth, during the early stage.

For the asafetida cultivation, the excellent technique for plant propagation is seed. In case of seed, germination begins within 20days of sowing. The removal of seed coat from 1 year old seed and then sow as a result it shows the 84% germination has been appears in 4 days in spite at 20°C. Hence the freshly collected seed are sow, then it expresses the long-term dormancy. Hence their germination is not occurring under the optimum conditions. And their germination as completed within a month.

**Figure 17**: Seeds of asafetida

The seed of asafetida has poor germination and long-term dormant phase. Cold stratification is the best process to boost the germination of the seed. The time and temperature of the stratification control the germination of seed. Hence at 4°C for the 60 days are the enough to released embryo from the dormant phase.

In green house, seeds of asafetida are sow, and seeds are ripe at the time of autumn. Seed sowing of asafetida is done in winter or spring. As the seed sow in the winter season then as a result higher amount of the gum yielding is occurred compared to spring season.

Winter – December to January

Spring – April

When the seed are sown 4cm deep in soil then the rate of development is higher compared to the seed sowing in the 2cm of depth. In first year of planting cow manure and chemical fertilizer including N, P, K, should be given for the seedling and plant growth development. Hence dose is raised gradually up to the ten years. Due to the water deficient condition, the rate of plant growth and their productivity has been reduced. For their seed germination, the availability of moisture is necessary. During the summer, water irrigation must be required, to prevent the drying of plant. Hence it is sufficient to giving water once in a week.

**Figure 18**: Cultivation of Asafetida

During the early time, weed which grows strenuously should be detached. Commonly in a year, two-time weeding must be necessary in a month of June-July and October-November. And once in a year one time digging is required in a month of August-September. After 4-5 years of plant then weeding and digging are not required.

## 2.2: Harvesting: -

Within 5 years of time, a plant grows 2m of height and the gum of asafetida has been obtained from the thick rhizome of the ferula plant. During the March – April, it is the time that plant bears the flower before that time make the root of plant uncovered and applies the vertical cut on stem of living rhizome. As a result, milky white juicy resin has been acquired from that cut on stem. Initially the resin is in the juicy form, after the drying it encounters air it becomes hard jelly like consistency. Hence perform this process continuously after every 4-5 years.

**Figure 19**: Harvesting of Asafetida

Generally during the earlier time, the gum from the plant has been obtained by using scalpel without considering plant regeneration & cut the branch and bud of the plant, as a valuable plant is not survived anymore. Earlier the method for collecting gum from plant is direct incision which is lethal for the plant.

The revenue of gum is ducted by number of cuts and method of cutting.

There are three different cutting methods for the asafetida.

1) Concave cutting method.
2) Conventional cutting method.
3) Surface cutting method.

1) Concave cutting method: - By using concave method, highest yielding of gum has been obtained within 10 cuts and without destroying the valuable plant. And the best regeneration of the plant is being occurred.

**Figure 20**: Concave cutting method

**Figure 21**: Regeneration from concave method

2) Conventional cutting method: - This method is the highly applied method. This method demolishes the bud at crown; as a result, consequence of death of plant is occurred.

**Figure 22**: Conventional cutting method

**Figure 23**: Regeneration from conventional method

3) Surface cutting method: - By using the surface cutting method, lower yield of gum has been obtained as compared to, other cutting methods. While in this cutting method regeneration of plant has been achieved.

**Figure 24**: Surface cutting method

**Figure 25**: Regeneration from surface cutting method

Hence concave cutting method is the widely used while the surface cutting method is the rarely used method for the harvesting of asafetida. After completing the whole three months process, an individual asafetida plant has able to produce 900g of asafetida gum. When the gum is fresh it appears grayish white in colour and as the gum is interacting with the air it becomes dried and hard then it appears amber in colour.

## 2.3: Yielding:

Approximately, one individual asafetida plant can produce 40-900g of fresh gum. The rate of dried asafetida gum in global market is 140-160 $USD. The essential oil obtained is 2.43-20.85% from ferula plant. Several factors are affecting the yield of asafetida including, location of cultivation, climate etc. Based on the different variety of asafetida, their rate also has been different.

# CHAPTER– 3: CULTIVATION OF ASAFETIDA IN INDIA:

## 3.1: First time cultivation of asafetida in India:

Asafetida is one of the most important condiments in Indian cuisine. One of the best ingredients in kitchen is Asafetida. India does not produce Asafetida. In India there was great demand of Asafetida, hence every year India import the 1500 tons of Asafetida from Iran and Afghanistan worth Rs 942 crore. India has first time farming the Asafetida, in strive to become self-sufficient in the production of the spice and suppress its imports.

CSIR said In India due to scarcity of planting equipment of Ferula asafetida plants was a crucial constriction in farming of this crop. IHBT imports the asafetida from the different countries and develop the agro-technology.

With the help of National Bureau of Plant Genetic Resources, New Delhi, CSIR-IHBT has import the six acquisitions of asafetida from Iran and develops the systematic protocol according to the Indian conditions. This project has been initiated by the scientists of CSIR-IHBT and designates the Lahula and Spiti valley as cold and dry desert. Primarily the IHBT pinpoint the 300 hectors land for the plantation.

**Figure 26**: Dr. Sanjay Kumar, Director of CSIR-IHBT planting the first asafetida plant at Lahula valley.

On 15 October 2020, first time plantation of asafetida has been done by Dr. Sanjay Kumar, Director of CSIR-IHBT at kawring village of Lahula valley. In course of trial, for the cultivation of asafetida only 7 farmers of valley have been provided by the asafetida plant. Optimal condition for the plant growth is powdery soil, very less moisture content and less than 200mm rainfall per annum. Cold desert area is the absolute land including several region of Himachal Pradesh, Uttarakhand, & Arunachal Pradesh for plantation of asafetida. During inadequate weather condition plant undergo for the dormant state**.** India imports 90% of asafetida from Afghanistan.

In last three decades, this was the first attempt to introduce the asafetida seed in country. Although the difficulty for scientist is the seed of asafetida having only 1% of rate of germination and seed undergo for longer time of dormant state. When farmer would be successful in finish the five year of cycle and after studying its result then further expansion of asafetida cultivation will be been recommended.

**Figure 27**: Raw and dried asafetida

Sanjay Kumar who is director of CSIR-IHBT inform that: "It'll cost farmers nearly Rs 3 lakh per hectares over next five years and give them a net return of minimum Rs 10 lakh from fifth year onwards. We will in collaboration with state governments provide support to farmers with finance and technical know-how. It'll be a gamechanger for farmers in cold desert region of the country." (ETNOWNEWS.COM, 2020)

## 3.2: Hathras Hing: -

In India large scale production of Hing is accomplished by Hathras in Uttar- Pradesh for the last 10 decades. Raw form of asafetida is imported from Afghanistan, Iran, Uzbekistan, and Turkey. India is the largest consumer of the asafetida from Afghanistan. Hathras is one of the best examples of one district one product in Uttar Pradesh.

The main aim of the of one district one product by UP's government to inspire domestic and specialist product and handicrafts which are not available in any other regions.

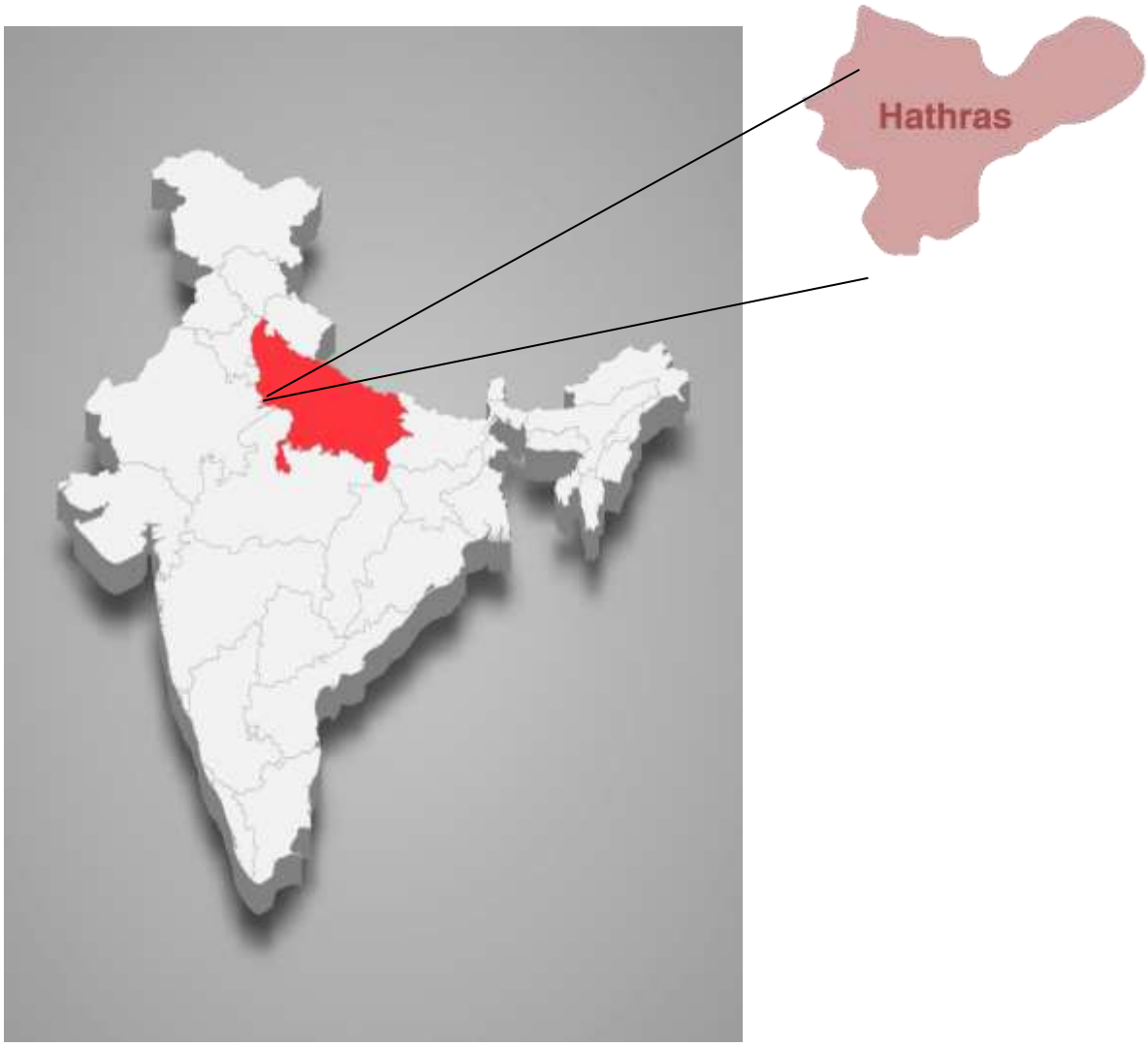


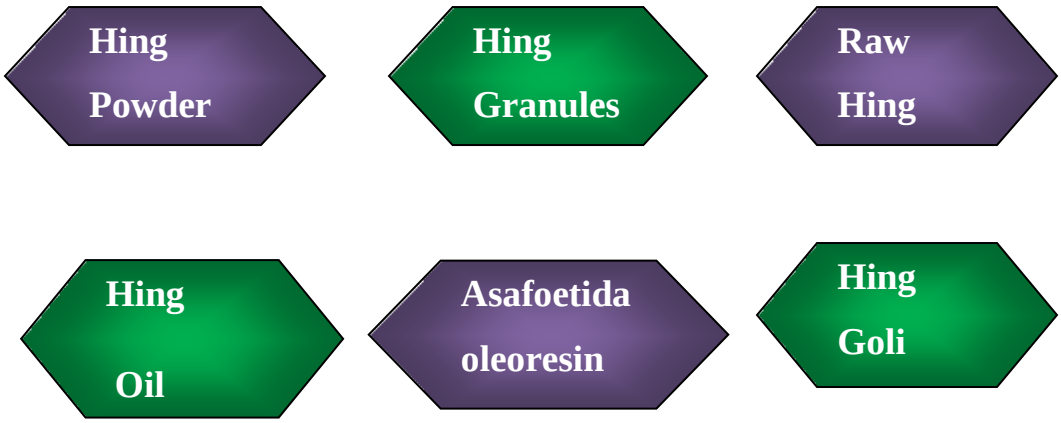

**Figure 28**: Shows the Hathras, which are major asafetida producing district in India.

Hathras in UP is predominate producing asafetida in India. Hathras has its unique identity due to large production of Hing. Historically and in accord with Purans, Hathras can be of the era of Mahabharata. During the British era Hathras are known to be an industrial hub. Hathras region, possessing of about 135 manufacturing unit of Asafoetida and about 60-70 crore valuation of business. From Hathras Asafoetida is being exported to Kuwait, Saudi Arabia, and Bahrain, United Kingdom, Eudaimon Arabia, East Africa, Malaysia, Oman, Switzerland, United Arab Emirates, countries of Europe like Belgium and various additional countries.

During time of 1920s, there was clamor for refined hing over maximal southern merchandise, chiefly in region of Tanjore, Tamil Nadu. Then various units have been set up for the processing of the asafetida major at Chennai, Kumbakonam and Nasik. Hence the main producing unit of asafetida from last 100 years is the Hathras district, in Uttar Pradesh.

### 3.3: Different forms of Asafetida: -

There are different forms of the Hing, accomplished by the asafetida industry at Hathras. Raw hing, granule, powder, oil, goli, and oleoresin form of asafetida are available.



1) Hing Powder:

Initially excreted juicy like gum resin from the thick root of ferula plant then after drying it becomes in hard form then after crushing it, its yellow powder form has been obtained.

**Figure 29**:Powder of asafetid

2) Hing Granule:

Hing granule is effectual, and it is believed as the digestive spices and used to observe as the medicinal treatment. It is effective to treat the various
Stomach disorders like acidity, gas, and constipation.

**Figure 30**:Granules of asafetida

3) Raw Hing:

It is excreted from the dried resin from Ferula plant. Due to its pleasant smell and flavor raw Hing is important cuisine in the Indian kitchen. Generally, is added into the legume and gas producing vegetable.

**Figure 31**:Raw Hing

4) Hing Oil:

Normally Hing oil is extricate by the process of steam distillation from the root of the small asafetida plant. Hing oil appears like pale yellow, dark amber or golden yellow in colour.

**Figure 32**:Oil of Hing

5) Asafoetida Oleoresin:

It is dried latex obtain from the thick taproot of the ferula plant. Oleoresin have feature of savory smell and acidic taste. The pure form of oleoresin is strong and having unpleasant aroma. Hence oleoresin is used in their diluted form.

**Figure 33**:oleoresin of asafetida

6) Hing Goli:

Hing goli is manufactured by mixing the raw asafoetida with rich natural and organic herbs in an appropriate amount.

**Figure 34**:Hing Goli

# CHAPTER- 4: TYPES OF ASAFETIDA

1. Uzbeki Naukra: -

Uzbeki naukra asafetida is in dried and crystal form. This type of asafetida has been excreted from the asafetida crystal plant and having the yellow to natural brown in colour. Basically, they are known as naukra hing. Uzbeki naukra hing are native of Uzbekistan. Hence it is widely used for enhancing the taste in meals.

**Figure 35**:Uzbeki Naukra

2. Tazaki Dana Hing: -

Tazaki dana hing is the strongest asafetida and it is non-dried and semisolid in form. Commonly it is known as Tazaki dana. When it is excreted then it appears white to yellow to brown in colour, while the flavor is chilly like. Hence it is native from the Tazakistan. This type of hing is widely used for the culinary and medicinal purposes. Tazaki Hing is known as the king of hing.

**Figure 36**:Tazaki Dana Hing

3. Excel Hing: -

Usually, it is in a dried and granules form and appears wooden like brown in colour.

**Figure 37**: Excel Hing

4. Sahabandi Hing: -

It is liquid in form. It appears like white in colour and sweet and spicy in flavor. Widely used to enhance the taste in food. Known for its long lasting sweet and spicy taste.

**Figure 38**:Sahabandi Hing

5. Tazaki Sarkas Hing: -

Tazaki sarkas hing is non-dried and liquid from. Commonly it is known as Tazaki sarkas. When it is excreted from the root of the ferula plant then it appears like pinkish in colour. Hence is native from the Tazakistan. This type of hing is widely use for the cooking and enhance the taste in food. And known for good self-life.

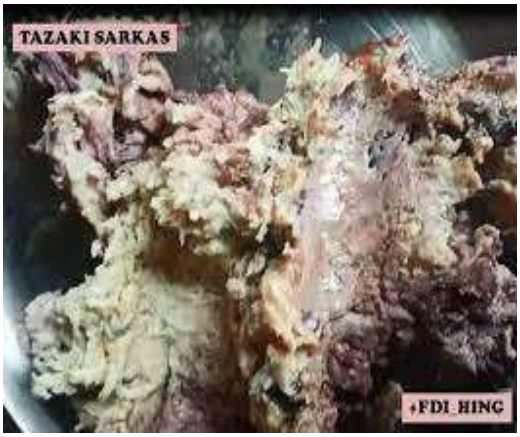



**Figure 39**:Tazaki Sarkas Hing

6. Pinexir white Hing: -

Pinexir hing is non-dried and liquid form. When it is excreted from the plant then it appears like white in colour, while the fragrance of hing is like spicy. Commonly it is known as Talab hing. Hence it is native from Kazakhstan and Uzbekistan. Widely used for household purpose especially in papad and pickles making.

**Figure 40**: Pinekxir white Hing

7. Hadda Hing: -

Commonly it is known as Irani hing. It is dried and solid crystal in form. When it is excreted from the plant then it is appearing as like golden to natural brown in colour and flavor of hing is like spicy. Hence it is native from Iran. Generally, it is used for making papad, pickles, and widely used as spices.

**Figure 41**: Hadda Hing

8. Hingra Hing: -

It is liquid in form and native from Iran. When the asafetida is excreted then it appears light brown in colour. Commonly they are known as Hingra. Widely used for the medicinal and cooking purpose.

**Figure 42**:Hingra Hing

9. Kabuli Hing: -

It is non-dried and liquid in form. When it is excreted then it is white to golden in colour. Hence it is native from the Tazakistan. And this of asafetida is used for the making snacks, spices, and pickles. Having strong aroma and prolong shelf-life.

**Figure 43**: Kabuli Hing

10. Mazari Hing: -

Mazari hing is native from the mazari sharif and it is liquid in form. When it is excreted then it appears like white in colour and chilly like flavor. Widely used for the namkeen, papad and pickles making. Mostly used in south India.

**Figure 44**:Mazari Hing

11. Pinexeer Nukra Hing: -

It is dried and crystal in form, while it is having white and golden in colour. Pinexeer nukra hing is native from the Kazakhstan and Uzbekistan. Commonly it is known as the pinexeer and Talab Hing. And it is widely used for the cooking and agricultural purposes. Pinexeer has main advantage of long-lasting smell.

**Figure 45**: Pinexeer Naukra Hing

12. Hadda Paste Hing: -

Commonly it is known as the Irani hing and hadda hing. It is paste in form and golden in colour having sweet, spicy in flavor. Hadda hing is native from Iran. Widely used for the cooking and the agricultural use.


**Figure 46**: Hadda Paste Hing

13. Russian: -

It is non-dried and paste form. Russian Hing are cream to pinkish in colour. Russian Hing having the chilly like flavor. And this type of Hing is widely used for the cooking including many dishes like papad, pickles, etc.

**Figure 47**:Russian Hing

14. Peenakshir Brown: -

It is brown in colour, and it is widely in the paste form. widely it is used in the papad making industry. While peenakshir brown type of Hing are having the prolong self-life. It consists of the yellow colour of oil. The percentage of oil in peenakshir brown is high compared to other.

**Figure 48**: Peenakxir Brown

15. Afghani Sheera: -

This type of Hing consist higher amount of volatile oil. It is non-dried and paste form, while the colour of Hing is dark creamish. This type of Hing having the spicy flavor.

**Figure 49**: Afghani Sheera

## Table of types of asafetida

| **Product Details** | **Uzbeki Nukra** | **Tazaki Dana Hing** | **Tazaki Sarkas Hing** | **Pinexir white Hing** | **Hadda Hing** |
|---|---|---|---|---|---|
| **Botanical name** | Asafetida crystal | Asafoetida | Asafoetida | Asafoetida | Asafetida |
| **Common Name** | Nukra Hing | Tazaki Dana | Tazaki Sarkas | Talab Hing | Irani Hing |
| **Form of asafetida** | Crystal & Dried | Semi-solid & Non Dried | Liquid & Non Dried | Liquid & Non Dried | Solid Crystal & Dried |
| **Place of origin** | Uzbekistan | Tazakistan | Tazakistan | Iran | Iran |
| **Colour** | Yellow & natural brown | White, brown, yellow | Pinkish | white | Golden To natural Brown |
| **Flavour** | - | Pungent | Pleasant | Highly pungent | Pungent |
| **Uses** | Brings taste in food | House-hold, Medici-nal | cooking | House-hold for cooking | Pappd, spices, pickels |

| **Product Details** | **XL Hing** | **Pinexeer Nukra Hing** | **Hadda Paste Hing** | **Russian** | **Peenaksir Brown** |
|---|---|---|---|---|---|
| **Botanical name** | - | Asafoetida | Asafoetida | Asafoetida | Asafoetida |
| **Common Name** | - | Peernexir, Talab Hing | Irani Hing, Hadda hing | - | Peenaksir Hing |
| **Form of asafetida** | Dried & Granules | Dried & Crystal | Liquid & Non-Dried | Paste & Non-Dried | Paste & Non-Dried |
| **Place of origin** | Iran | Kazakhstan Uzbekistan | Iran | Uzbekistan | Iran |
| **Colour** | Brownish | White, golden | Golden | Pinkish | Brown |
| **Flavor** | - | - | Less Pungent | Pleasant | Mordantly Pungent |
| **Uses** | Papad Making | Cooking | Spices, pickles, agricultural | Cooking, medicinal. | Papad, Namkeen making. |

| Product Details | Afghani Sheero | Sahab-andi hing | Mazari Hing | Hingra Hing | Kabuli Hing |
|---|---|---|---|---|---|
| **Botanical name** | Asafoetida | Asafoetida | Asafoetida | Asafoetida | Asafoetida |
| **Common Name** | Afghani Hing | Sahabandi Hing | Afghani Hing | Hingra | Kabuli Hing |
| **Form of asafetida** | Paste & Non-Dried | Liquid & Non-Dried | Non-Dried | Liquid | Liquid & Non-Dried |
| **Place of origin** | Afghanistan | - | Uzbekistan, Kazakhstan | Iran | Tazakistan |
| **Colour** | Dark creamish | white | White | Light brown | White to golden |
| **Flavor** | Mild Pungent | Mild Pungent | Mordantly Pungent | Highly Pungent | Mild Pungent |
| **Uses** | Cooking. | Enhance taste in food. | Namkeen, papad, pickels | Medicine, cooking | Snacks, spicey, pickels |

**Table: 5**-Types of asafetida

# CHAPTER- 5: MATERIALS & METHODS

## 5.1: Aim & Objective: -

## 5.1.1: Aim:

To check antimicrobial activity using alcoholic extraction of asafetida on bacterial growth. Hence asafetida gave highest antimicrobial activity, then this may be more used for the routinely usage in food, in form of Asafoetida powder. And uncovering of procedure to make compounded production of asafetida.

### 5.1.2: Objectives:

- The chief objective of this study is to build up the several potential significant of Asafoetida. In this study, antimicrobial activities of Alcohol Soluble Extracts of Asafoetida have been checked against divergent microbes.
- It is noticed that Alcoholic Extraction of Asafoetida exhibit remarkable result against flora of fertile farm soil, garden soil, equipment, and machine swab by agar disc diffusion method. The alcoholic extract showed a broad spectrum of antimicrobial activities by inhibiting the specific growth of microbes.
- Agar disc diffusion technique shows antimicrobial activity capitulate the zone of inhibition up to 5 to 15 mm diameter for Alcoholic Extracts of Asafoetida. Hence current research study has massive role in medicinal properties.
- Asafoetida as it's not directly consumable due to its acrid taste and other organoleptic properties. Varieties and combination of varieties shows vast difference as far as organoleptic properties are concerned therefore it's an exigent task to define best possible combination of different varieties of Asafoetida for commercial production of Compounded Asafoetida. Results of Physico – Chemical and Organoleptic studies help well to distinguish best among other varieties.

## Workflow

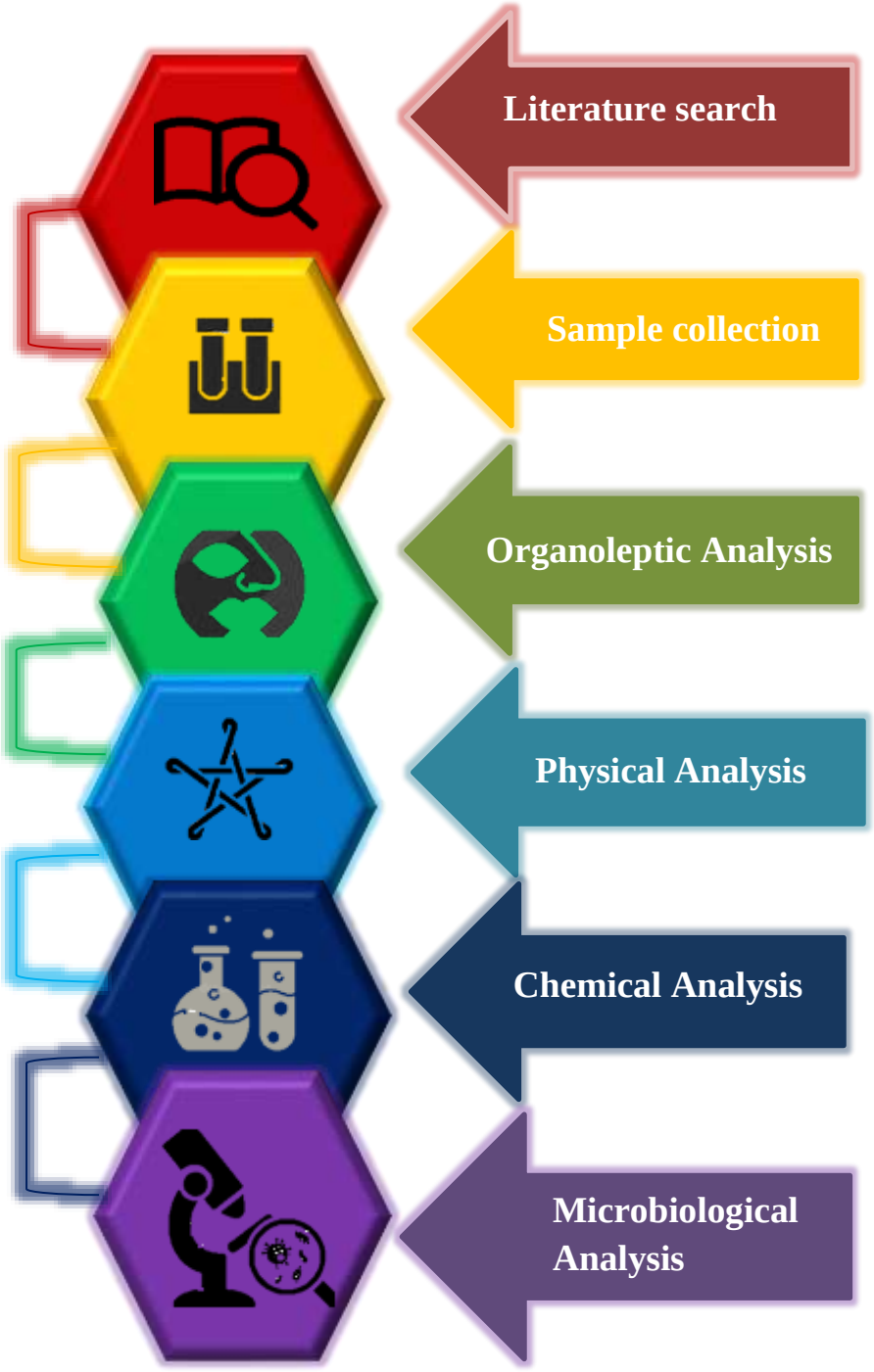



**Figure 50**:Flowchart of study

## 5.2: Materials: -

### 5.2.1: Sample:

There are six types of asafetida samples which has been used in it. While these sample has been imported from Afghanistan, Iran, Turkey, Uzbekistan, and Kazakhstan, where there were changes in the climate, temperature, variation in soil, etc. are mined as per their vernacular.

| Name of sample | Area of sample | Description of sample |
|---|---|---|
| **Peenaksir Brown** | Iran | Collected in raw and paste form. |
| **Peenaksir White** | Iran | Collected in raw and liquid form. |
| **Tazaki** | Tazakistan | Collected in raw and paste form. |
| **Mazari** | Kazakhstan | Collected in raw and paste form. |
| **Afghani Sheero** | Afghanistan | Collected in raw and solid form. |
| **Russian** | Uzbekistan | Collected in raw and paste form. |

**Table: 6-**Types of samples

## 5.2.2: Media:

Varieties of medium have been used for isolation of, coliforms, staphylococcus sp., enteric pathogen count. Media are the N. agar, Mac, EMB agar, DCA agar, SS agar respectively.

a) **Nutrient agar medium:**

   N. agar is basic growth medium for the isolation of non-fastidious bacteria.

b) **MacConkey agar medium:**

   It is selective as well as differential media. In MacConkey agar, bile salt and crystal violet play significant role by inhibiting the Gram +ve & some fastidious Gram -ve bacteria. As a sole source of carbohydrate, lactose has been utilized. Hence lactose fermenting bacteria produce pink colour colonies because it reacts with the neutral red dye while lactose non-fermenting bacteria produce white or pale-yellow colonies.

c) **Eosin Methylene Blue agar media (EMB):**

   EMB is differential as well selective media. In EMB, Aniline dye play significant role in inhibiting the Gram +ve & fastidious Gram -ve bacteria. EMB are used for isolation coliforms from samples. Typical coliforms are strong lactose fermenter as a result it shows greenish metallic sheen while atypical coliforms are weak lactose fermenter as a result it shows transparent colonies.

d) **Deoxycholate citrate agar media (DCA):**

   DCA agar is the differential media and isolates the Enterobacteriaceae from the mixed population. The concentration of bile salt in DCA is three times more than that the MacConkey agar media, hence due to the bile salt it isolates the species of Salmonella and Shigella. In DCA sodium and ferric citrate play significant role to slow down the growth of *E. coli*.

e) **Salmonella-Shigella agar media (SS agar):**

   SS agar is selective media as well as differential media. In SS agar, higher concentration of bile salt and citrate is presence; due to this it inhibits the growth of gram +ve bacteria & numerous gram -ve bacteria likes' coliforms.

## 5.2.3: Medium regarding Biochemical Test:

1. **MR – VP broth: -**

   MR – VP is mixed acid fermentation test. In this, bacteria have ability to split up the glucose from the glucose phosphate broth (GPB). GPB is strongly buffered. Due to this if little amount of acid produced then it will not allow to drop down the pH of media. And methyl red is the pH indicator, it will detect the acid.

2. **VP broth: -**

   Voges – Proskauer test detects the acetoin, which will be produced by bacteria. During the butanediol fermentation, acetoin is produced, and acetoin is the precursor of butanediol. Hence VP test is not mixed acid fermentation test. By adding the 40% KOH and 5% alcoholic α – naphthol into the media if acetoin is produced then it developed the pink colour in media.

3. **Indole production test: -**

   Indole production test is based on protein utilization. In this test tryptone broth is used, while tryptone are mostly recommended then peptone. Because tryptone consist high amount of tryptophan, and tryptophan is the precursor molecule for the formation of indole production. By adding Kovac's reagent in the media if indole is produced then form the pink ring on the surface of broth.

4. **Simmon's Citrate agar: -**

   Microorganisms utilize the citrate and changing in colour of simmon citrate agar has been observed after the inoculation and incubation of 24 hour.

5. **Gelatin Agar: -**

   Gelatinase is the proteolytic enzyme which hydrolyzed the gelatin, which create the liquification of the gelatin after the incubation of 24 – 72 hours.

6. **Nutrient sugar broth: -**

   Microorganism has ability to catabolize sugar through many different pathways and form the several acids (like pyruvate, lactate, succinate, etc) and gas.

## 5.3: Methodology:

Numerous diversities of spice are produced nationwide in divergent weather circumstances, which are definite to variety of spice cultivated. Spices are precious goods that bear the evaluation of numerous spices is done according to standard specifications like flavors, consistency, look and should secure for intake. Spices testing specification rely on various aspects like variation in the climate, or other adulterant in the complete operation of production to packaging, packaging which are generally influence the value of spices.

Hence for the asafetida, physico – chemical and microbial studied are done for that purpose of the asafetida as well.

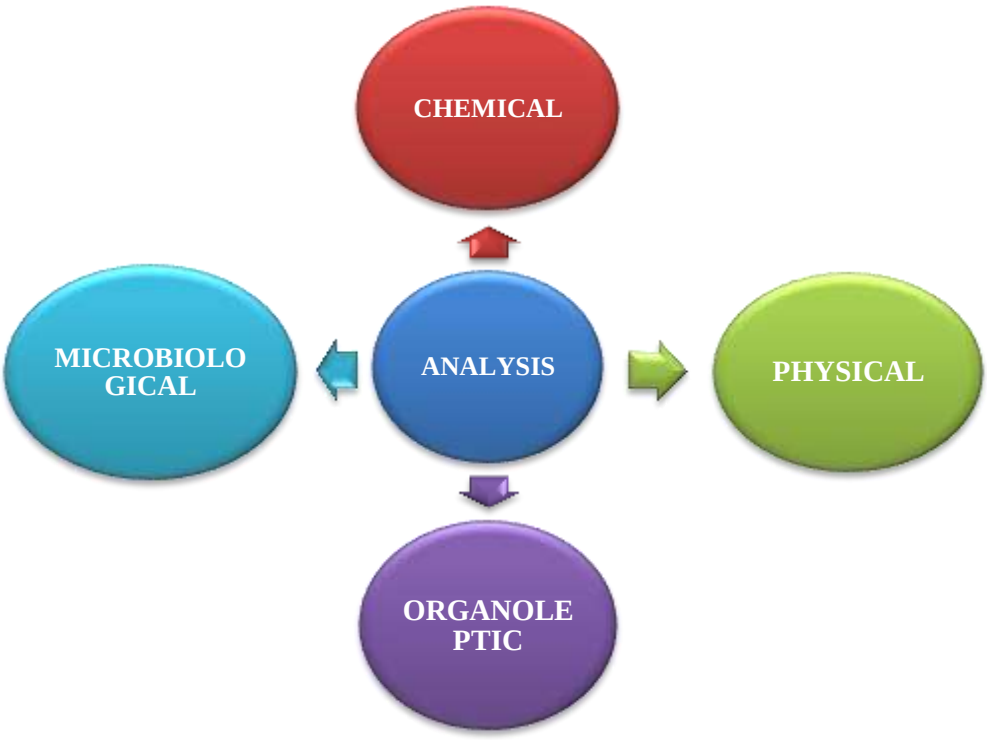



**Figure 51**:Methodology

## 5.3.1: SAMPLE COLLECTION

All six sample of the Asafetida has been imported from the different countries like Afghanistan, Uzbekistan, Iran, Kazakhstan, Tazakistan. Following below are the pictures of the six samples of Asafetida.

**Figure 57**:Peenakxir White Hing Vernacular from Iran

**Figure 56**:Peenakxir Brown Hing Vernacular of Iran

**Figure 55**:Mazari Hing Vernacular from Uzbekistan

**Figure 54**:Tazaki Hing Vernacular of Tazakistan

**Figure 53**:Afghani Sheero Hing Vernacular of Afghanistan

**Figure 52**:Russian Hing Vernacular of Uzbekistan

## 5.3.2: Physical Analysis

In Physical analysis, purity of asafetida will be check in different manner. Determination of extraneous matter in asafetida by two distinct ways

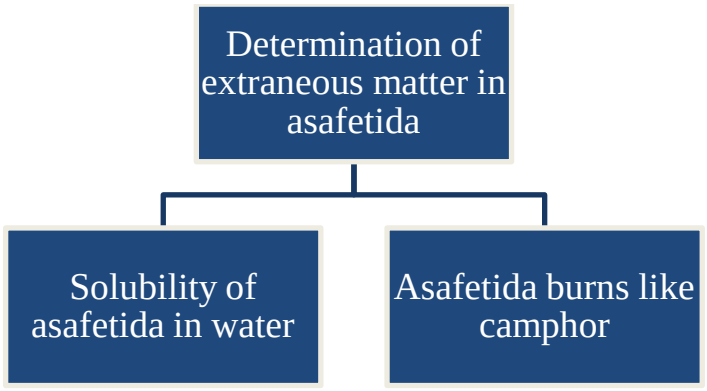



**Figure 58**: physical Analysis

**1) Solubility of asafetida in water:**

Take the small amount of asafetida and dissolve it in the water. If the asafetida is completely soluble in water, then it is concluded as that asafetida is in pure form. When the asafetida is insoluble and settle down at the bottom of glass, then it is concluded as the asafetida is adulterate.

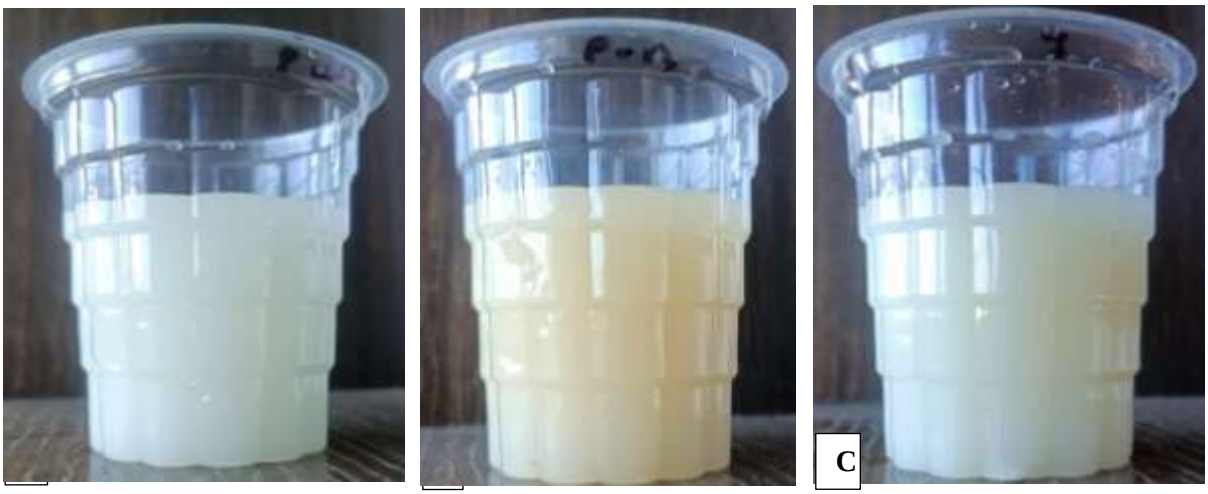



**Figure 59**: Solubility of (A)Peenakxir white, (B)Peenakxir Brown, (C)Tazaki asafetida in water

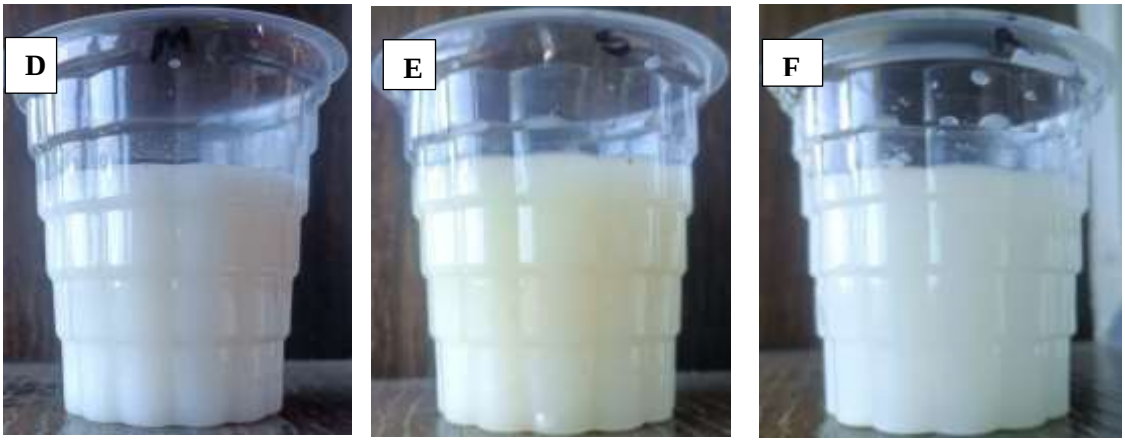



**Figure 60**:Solubility of (D)Mazari, (E)Sheero, & (F)Russian asafetida in water

**2) Asafetida burns like Camphor: -**

Take small amount of asafetida at the tip of spoon. And flame it over the candle. When the asafetida burns like the camphor and it produce the bright flame, then it is concluded as the asafetida is in pure form. But when the asafetida is in impure form then it does not produce camphor like flame.

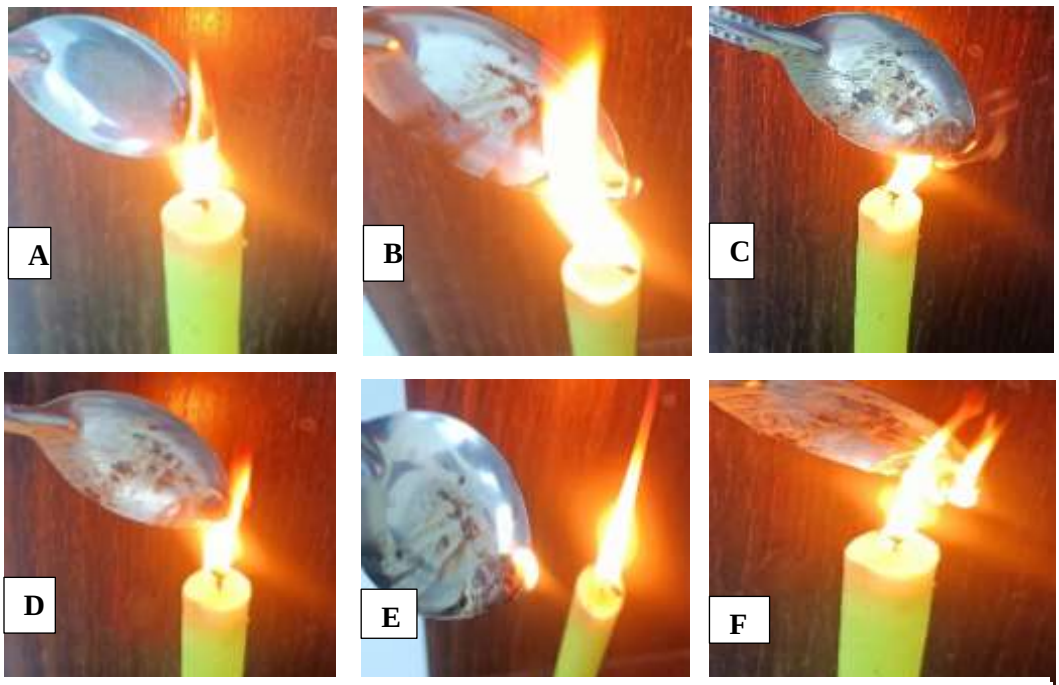



**Figure 61**: (A)Peenakxir Brown, (B)Peenakxir white, (C)Tazaki, (D)Mazari, (E)Sheero, & (F)Russian asafetida are burns like camphor.

### 5.3.3: Organoleptic Analysis

Organoleptic analysis of asafetida has been accomplished by incidents via the senses - including taste, smell, sight, and touch. The organoleptic analysis includes determining.

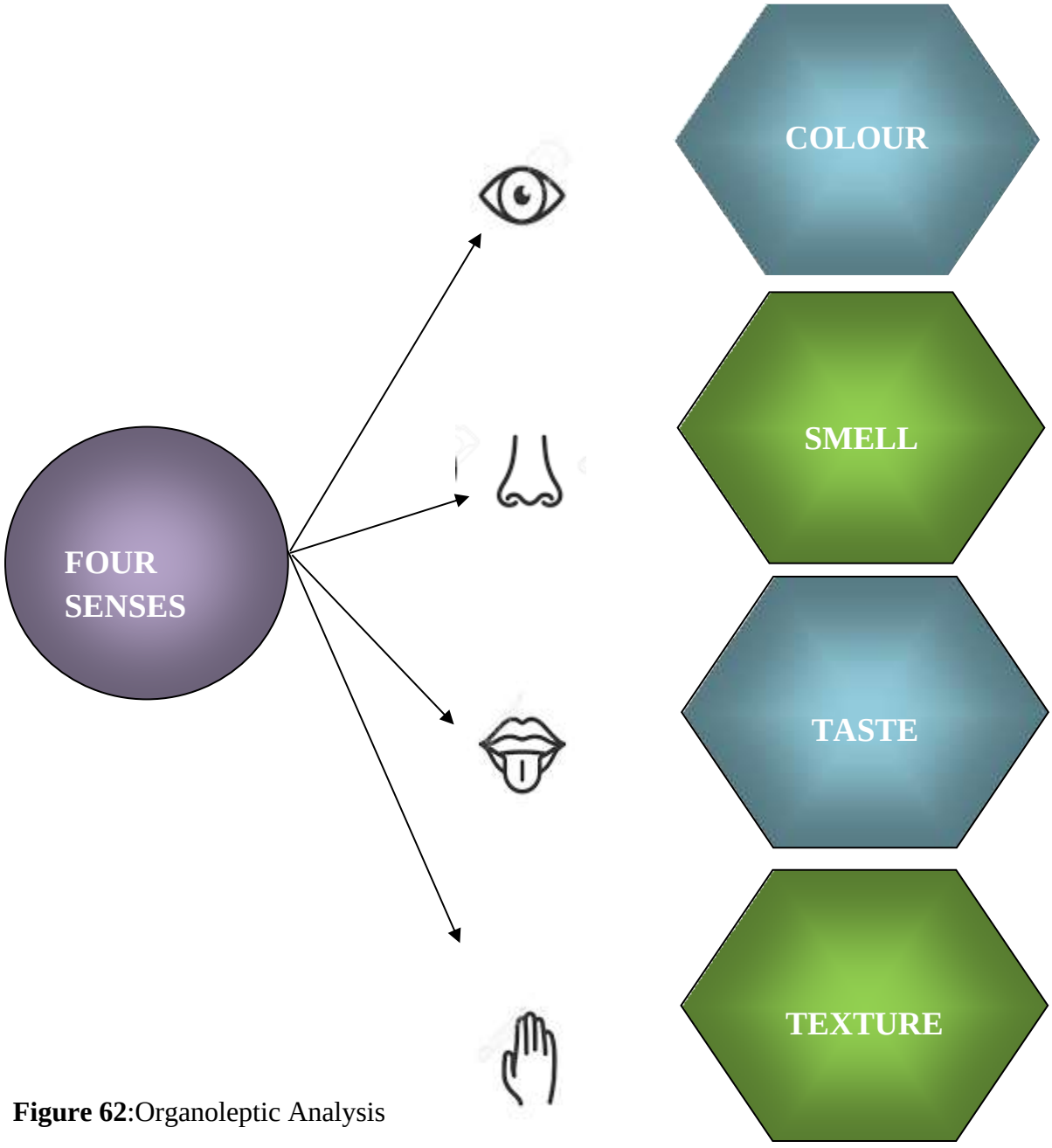



**Figure 62**:Organoleptic Analysis

### 5.3.4: chemical Analysis

Testing of spices involve the evaluation of chemical residue which analyze the wield of injurious insecticide on spices. In addition to chemical analysis toxins examination is involved that analyze toxins adulterants like Aflatoxins and Ochratoxins in asafetida. Chemical analysis also involved screening of metal, that examine for existence of heavy metals like Heavy metals in the spices. Moreover, Chemical analysis determinate moisture content, quantity of carbon-free ash, and quantity of acid insoluble ash.

TEST: - Several tests are performing on regular basis to check the quality of food sample.

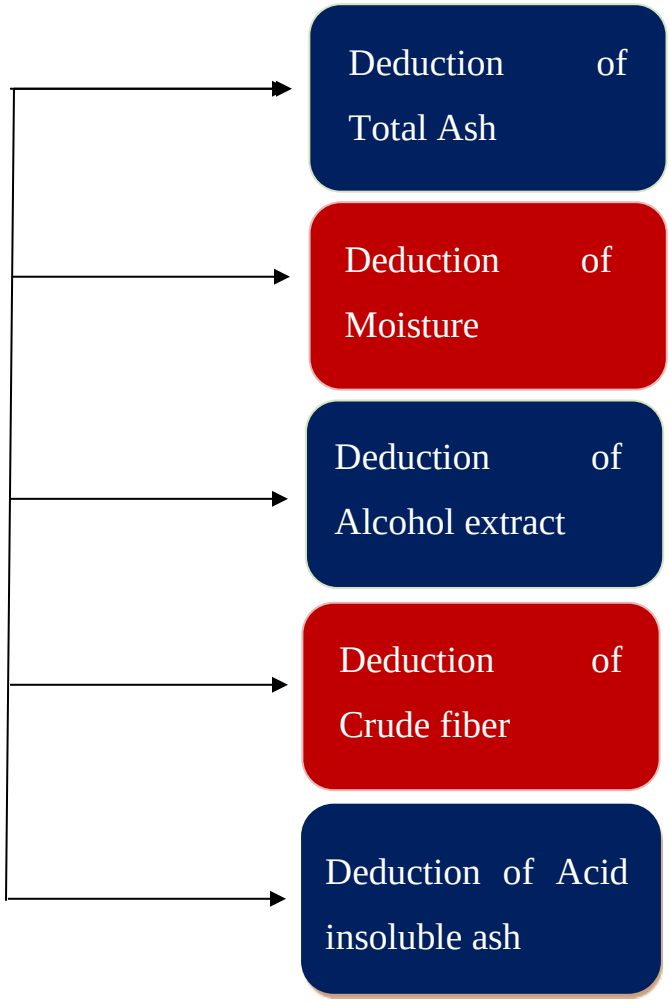



**Figure 63**:Test includes in Chemical Analysis

## 5.3.4.1: DETERMINATION OF MOISTURE: -

**Principle: -**

Examine the quantity of water by filtering the asafetida along - with an organic liquid not mixed with liquid and assemble the purified liquid into the tube.

**Procedure: -**

- Accurately weight the 0.01 gram of asafetida sample and transfer it into the distilling flask. Add enough quantity of toluene in the flask enough cover to the sample.
- Fill toluene in the receiving tube till top of condenser. Now apply cotton plug on the top of condenser to avoid the condensation in the tube due to atmospheric moisture.
- Add few pumice stone to prevent it from bouncing. Start the boiling and slowly distillation is also being start. At initial step distillation is quite slow, after few minutes the process become fast.
- With the help of wire loop remove the water from the condenser and rinse it with the help of toluene. Continue distillation for 3 -5 minutes.
- Allow it to stand for cooling by water or air cooling. Now the two clear layer of solvent and water has been separating out. And note down the reading and calculate it.

**Calculation: -**

$$\text{Content of Moisture (\%)} = \frac{100 + V}{M}$$

Where, V = vol. of water collection (ml)

M = Wt. of specimen

## 5.3.4.2: DETERMINATION OF TOTAL ASH: -

**Principle:**

Dismantling the organic matter by ignite the sample into the muffle furnace at the constant higher temperature 550°C for a constant mass.

**Procedure:**

- Note the wt. of vacant crucible. Precisely wt. a 2 grams asafetida specimen in the crucible. Add 2ml of ethanol on sample.
- When the complete elimination of ethanol is obtained then heat the crucible above the flame to char the material. Put this crucible containing sample in the muffle furnace with respect to 550°C for 240 minutes.
- Cool & soak the ash by dropping the water over it and then carefully evaporate the water to dryness. Again, ignite sample into the muffle furnace for 1 hour. On the next day, note the weight of the crucible + residue.

**Calculations:**

Total Ash: - Indicate in % by mass applying the subsequent equation.

$$\text{Total ash on \% by weight} = \frac{(W_2 - W1) \times 100}{W_1 - W} \times \frac{100}{100 - M}$$

Where, W = wt. of vacant crucible

$W_1$ = wt. of crucible + specimen

$W_2$ = wt. of crucible + total ash

M= content of moisture

## 5.3.4.3: DETERMINATION OF ASH INSOLUBLE IN DILUTE HCL: -

**Principle:**

Treat the total ash with the dilute HCL, filtration, incineration, and as a result, weight the residue.

**Procedure:**

- Take a 5 grams of asafetida sample in flask; add 25 ml of dilute HCL in it. Heating for 10 mins for complete dissolving.
- After heating, solution is filter with Whatman filter paper (I). Wash filter paper with lukewarm water at twice for complete removal of HCL from the paper. Keep washing until the removal of HCL from the sample.
- As a result, residue of the sample has been present in the filter paper. Hence the filter paper folds gently and put into the crucible.
- Note the wt. of the vacant crucible & note wt. of crucible with paper. Now this crucible is ignited in the muffle furnace at 550°C temperature for 2 hours. After 2 hours take the weight of the crucible.

**Calculations:**

Total Ash: - Indicate in % by mass applying the subsequent equation.

$$\text{Acid insoluble Ash} = \frac{(W_2 - W) \times 100}{(W_1 - W)} \times \frac{100}{100 - M}$$

Where, W = wt. of vacant crucible

$W_1$ = wt. of specimen + crucible

$W_2$ = wt. of residue

M/C = content of moisture

## 5.3.4.4: DETERMINATION OF CRUDE FIBERE: -

**Principle:**

Consecutive dissolution of sample with sulphuric acid and sodium hydroxide itemized mass to degenerate the macromolecule. By the process of filtration segregation of residue is obtained and afterward drying, and ashing of followed sample. As a result, loss of weight is occurred due to the ashing of component.

**Procedure:**

- Take a 200 ml of $H_2SO_4$ in flask, add the 2 g of sample in flask containing acid solution & stir the solution completely. Put it on heating plate for 30 mins.
- After 30 minutes, filter the solution with linen cloth. After filtration, wash the filter cake with hot water for complete removal of acid particle from it.
- Take this filter cake and add into the flask containing 200 ml of NaOH solution, heat for 30 mins again, filter with linen cloth & clean the filter cake with lukewarm water. Wash filter cake with methanol, for the complete removal of water from the cake.
- Take the filter cake and place in crucible and put this crucible in the hot air oven for 3 hours at 105℃ temperature.
- After this measure the weight of crucible and place this crucible in electric muffle furnace for 2 hours, after this, measure the weight of crucible. And determine the loss of weight of sample.

**Calculations:**

$$\text{Crude fiber \% by weight} = 100 \times \frac{(W_1 - W_2)}{W} \times \frac{100}{100-M}$$

Where, $W_1$ = wt. of crucible + matter + asbestos prior to ashing

$W_2$ = wt. of crucible + ash and asbestos after ashing

W = wt. of specimens

M = moisture content

## 5.3.4.5: DETERMINATION OF ALCOHOL SOLUBLE: -

**Principle: -**

The sample of asafetida has been excreted with the help of alcohol and carried out the insoluble residue and it's been determined by gravimetrically.

**Procedure:**

- Take the extracted thimble and note down the weight of empty thimble. Take an accurately 2 grams of asafetida sample in to tared extracted thimble.
- Place this thimble in the Soxhlet apparatus for the extraction of the asafetida for the 3 hours. Now the extraction has been carried out with the help of 90% alcohol.
- After 3 hours, put this thimble containing insoluble residue in the hot air oven for drying. At last note down the dry weight of insoluble residue.

**Calculations: -**

Alcohol soluble extract = 100 – (A +B)
% by weight

Where, A = % of dry insoluble residue
B = % of moisture

## 5.3.5: Microbiological Analysis

### 5.3.5.1: Sample Preparation: -

Take the 1 gram of semi solid and paste form asafetida sample add aseptically into the 9ml of disinfected water tube & prepare the sample solution. Allow vortex for few seconds for complete dissolved of asafetida in water.

### 5.3.5.2: Plating of sample: -

Take the sample and prepare the dilution of each of the six sample and plated on duplex plates of individual agar medium includes N. Agar, Mac agar, EMB agar, DCA agar, SS agar respectively. Allow it to incubation for overnight.

Also inoculate six different asafetida sample into the MacConkey broth. And incubate it for overnight for 24 – 48 hours.

### 5.3.5.3: Observation: -

After the 24 – 48 hours of incubation, growth of microbes is observed and count the CFU in individual agar plates.

### 5.3.5.4: Biochemical Test: -

In microorganism specific enzyme is present, and it can be illustrated by assimilating a certain substrate in media and as a result determine the product form or else fading of substrate from media. This biochemical test recruit by several media, by inoculated with specific species of bacteria, which pursue a particular metabolic pathway to hydrolyze the substrate present in media. Some of specific biochemical test which will performed routinely is, Gram staining, IMVIC test, sugar utilization test, catalase test, etc.

- **Gram Staining**

  Prepared the bacterial smear on the microscopic glass slide and serially applied Gram's - CV, Gram's iodine, decolorizer, Gram's Safranin on the dry slide and examine below the Microscope to discriminate bacteria in huge category.

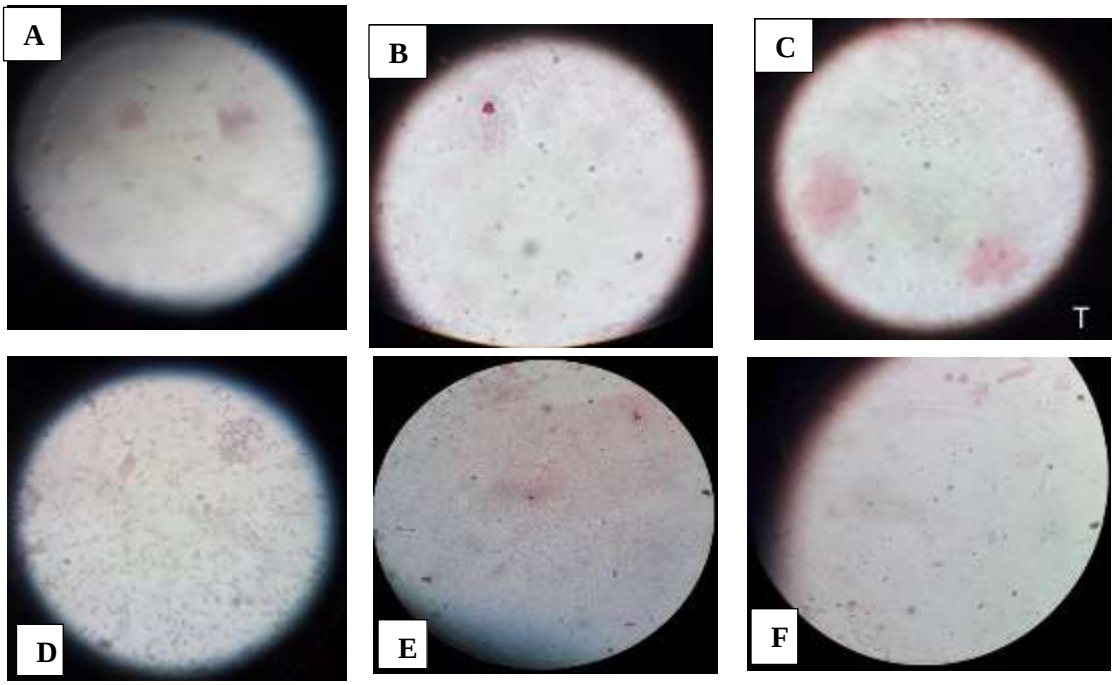



**Figure 64**:Gram Staining of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Catalase Test**

  I. Place a drop of 3% $H_2O_2$ (Catalase reagent) on microscopic glass slide.

  II. Transfer small number of bacterial colonies from N. agar to glass slide by using the sterile wire loop and mixed it with Hydrogen peroxide.

  III. A positive result gave the immediate production of the gas bubbles or effervescence.

  IV. And negative result shows no effervescence.

  V. While some bacteria consist of enzyme apart from catalase that degrade Hydrogen peroxide, as a result it shows little minute gas bubble, but this result is consider as negative result.

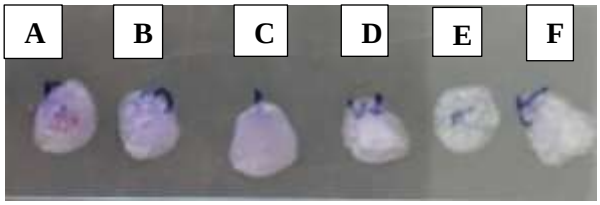



**Figure 65**: Catalase reaction of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Indole Production Test**

I. Take the loopful of young culture with the help of sterile wire loop.

II. Inoculate into the 1% tryptone broth & incubate at 37°C for overnight.

III. Afterward of incubation, add 1ml of Kovac's regent on the surface of broth within 10 sec it gave results.

IV. Positive result gave the pink to red color ring on the top of broth.

V. While negative result yellow color on the top of broth.

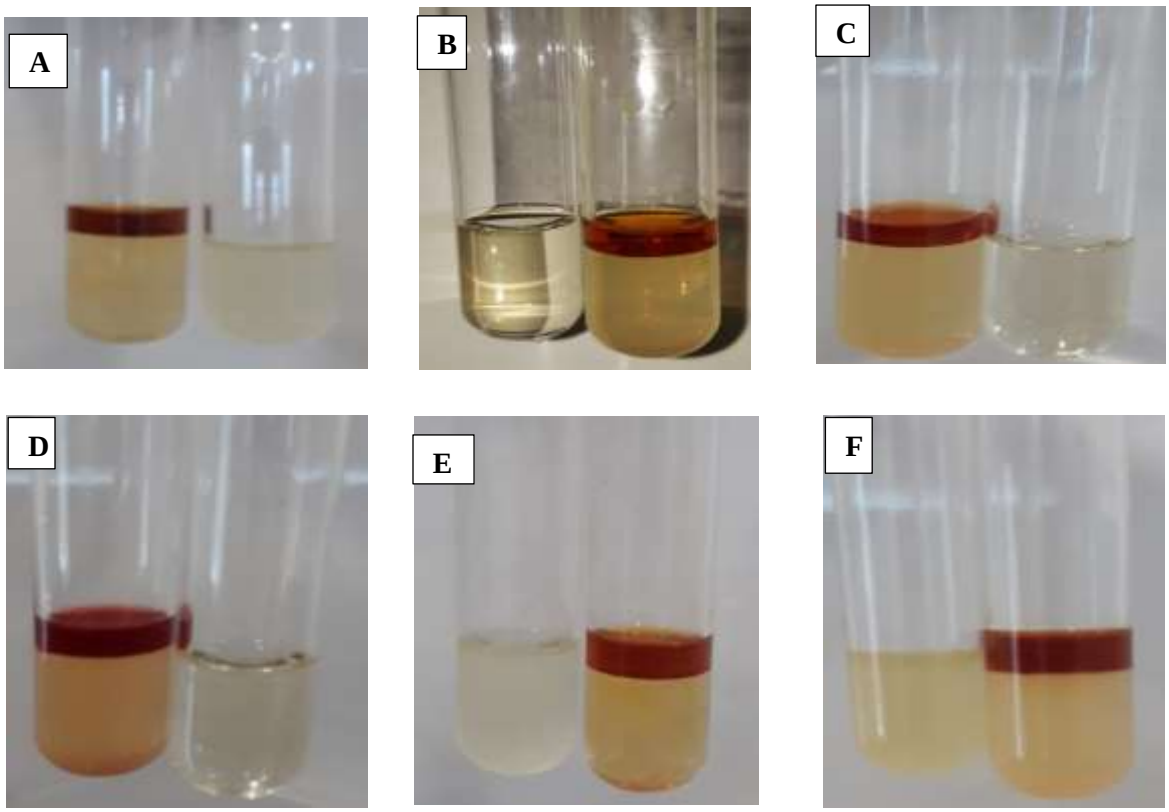



**Figure 66**:Indole test of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Citrate Utilization Test**

I. Pick up the colorless bacterial colonies from agar plate and streak heavily on SC slant & incubate at 37°C for overnight.

II. If organism has capability to use citrate from media, then color of slant will change from green to blue then it is considering as positive result.

III. While negative result is, there is no conversion of slant color from green to blue due to organism does not able to utilize citrate.

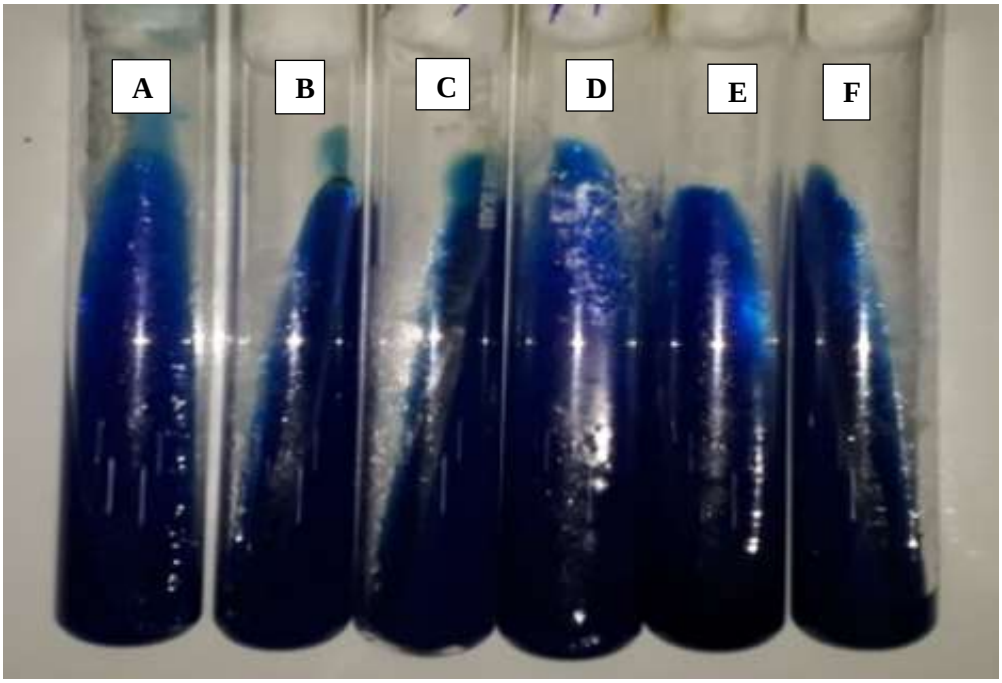



**Figure 67**:Citrate Utilization of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Sugar Utilization Test**

I. Microorganism has ability to catabolized different sugar through different metabolic pathway.

II. Inoculate young culture into the nutrient sugar broth containing different sugars like glucose, mannitol, and sucrose & incubate it for 37°C for overnight.

III. By changing the color of media and gas production in media it is consider as the positive result and it is due to fermentation. While there is no gas production and no change in color then it is considering as negative result.

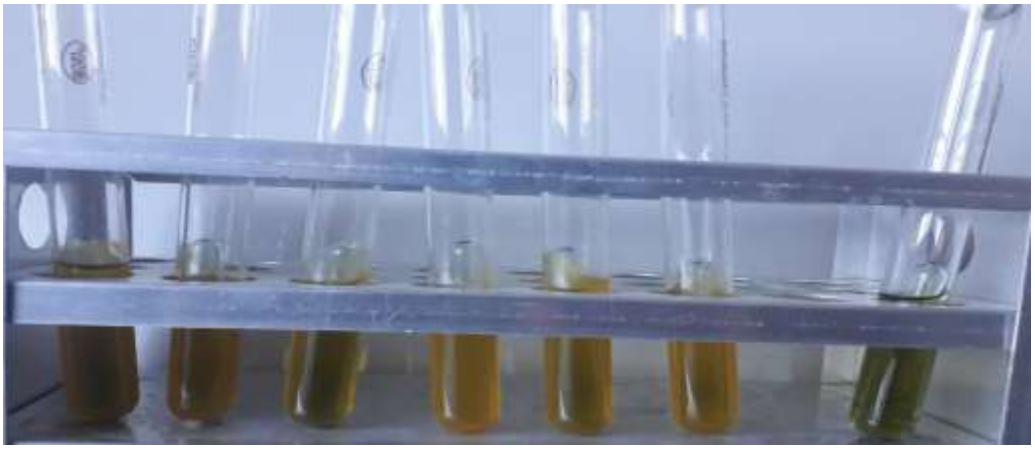


**Figure 68**:Sugar (Glucose) Utilization of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

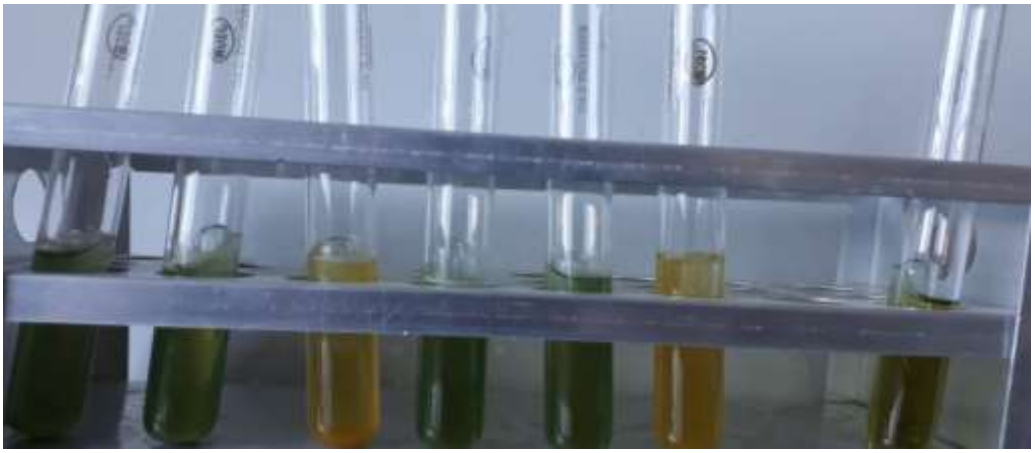


**Figure 69**:Sugar (Mannitol) Utilization of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

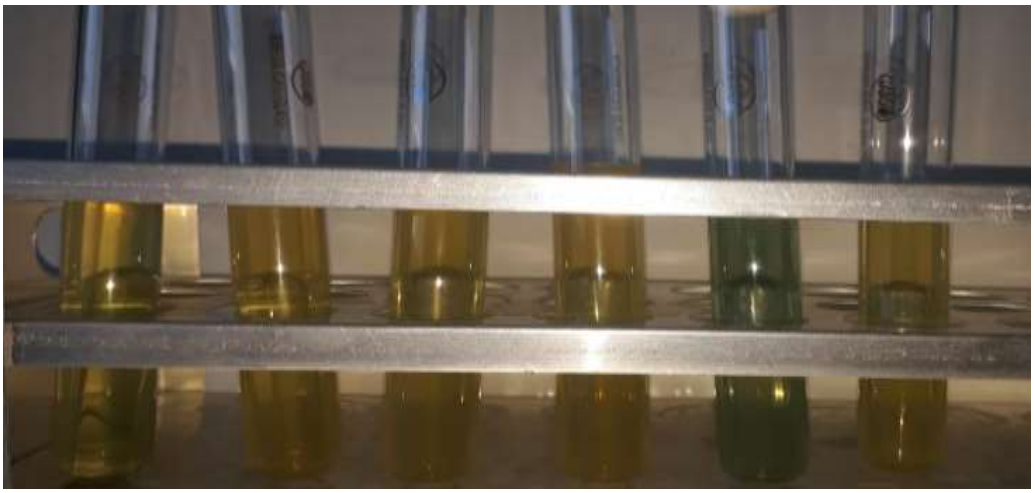


**Figure 70**:Sugar (Sucrose) Utilization of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Methyl Red Test**

I. Inoculate loopful culture into the GPB & incubate it for 37°C for 1-2 days.

II. Afterward of incubation, add 5 ml methyl red indicator.

III. Observe the forming of pink colour on the broth within few seconds.

IV. While negative result develops the fades of yellow color.

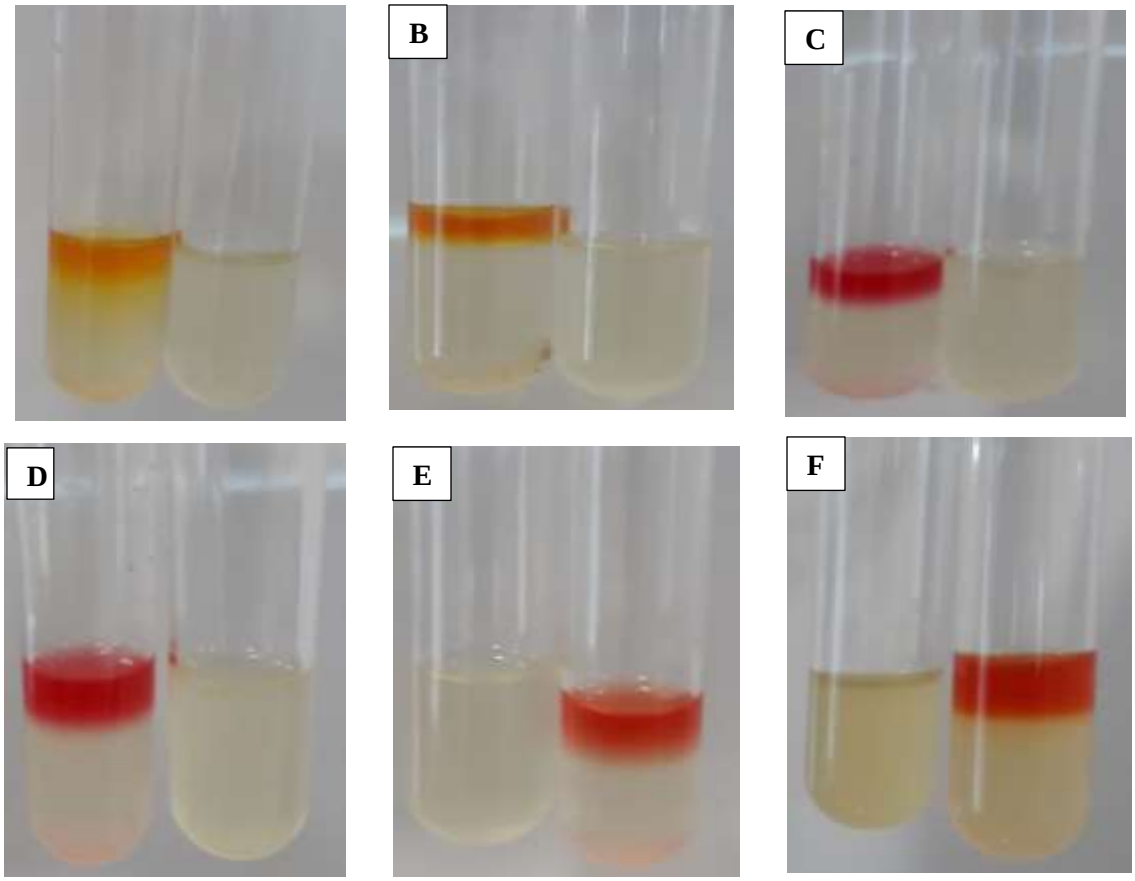



**Figure 71**:MR test of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Voges – Proskauer test**

I. Inoculate loopful culture into GPB & incubate at 37°C for 2-3 days.

II. After incubation, add dropwise 0.6 ml 5% alcoholic α – naphthol (no gas) + 0.2 ml 40% KOH solution.

III. Shake the broth after addition of reagent and tilt the tube to allow maximum aeration. Note the result after 15 – 60 minutes.

IV. Positive result develops the red colour in media after addition of reagent with 15 minutes or more.

V. While negative result appears pale yellow colour of broth.

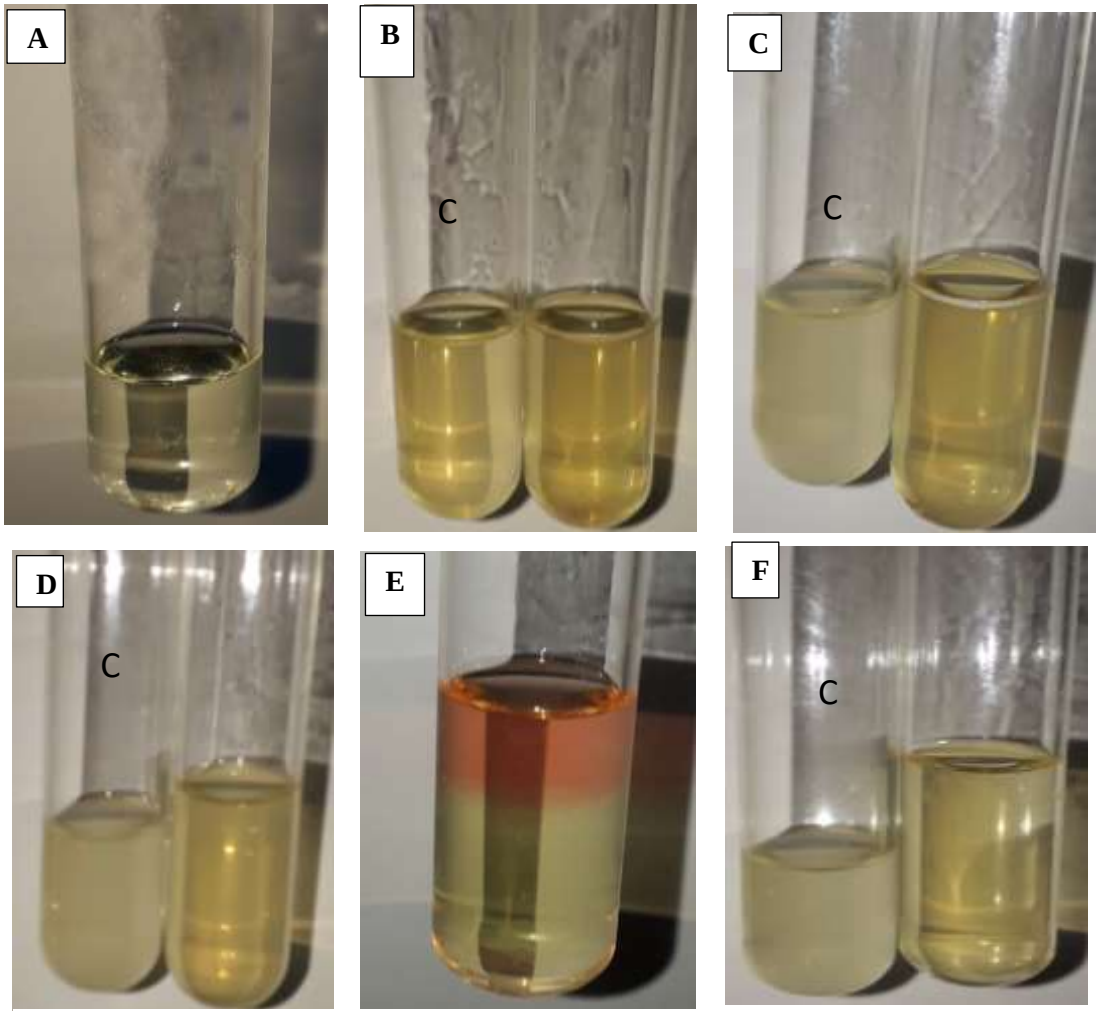



**Figure 72**:VP test of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Phenylalanine Deamination test**

I. Streak the Phenylalanine agar slant with loopful of the young culture & incubate at 37°C for overnight.

II. After incubation, add 4-6 drops of FeCl3 solution on surface of growth.

III. Positive result allow for formation of the intense aquamarine colour after addition of ferric chloride solution.

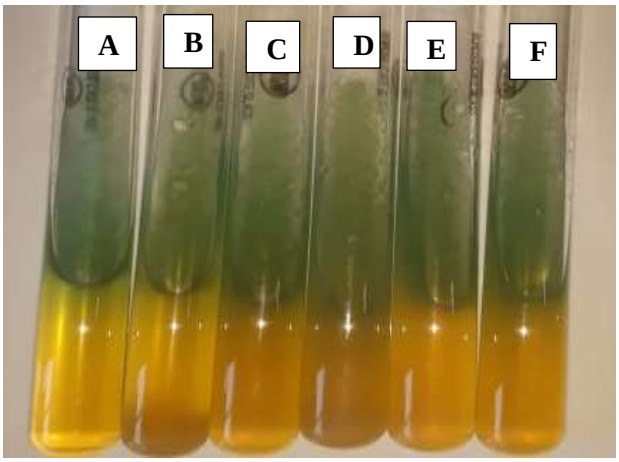



**Figure 73**:Phenylalanine test (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Ammonia production test**

I. Inoculate loopful of young culture into the peptone nitrate broth.

II. Place the red litmus paper ¼ position inside the test tube and ½ position outside to test tube & incubate broth at 37°C for 1 day.

III. Afterward of incubation observe the change in colour of red litmus paper.

IV. If it is giving change in colour from red to blue or purple, then it is considering as positive result.

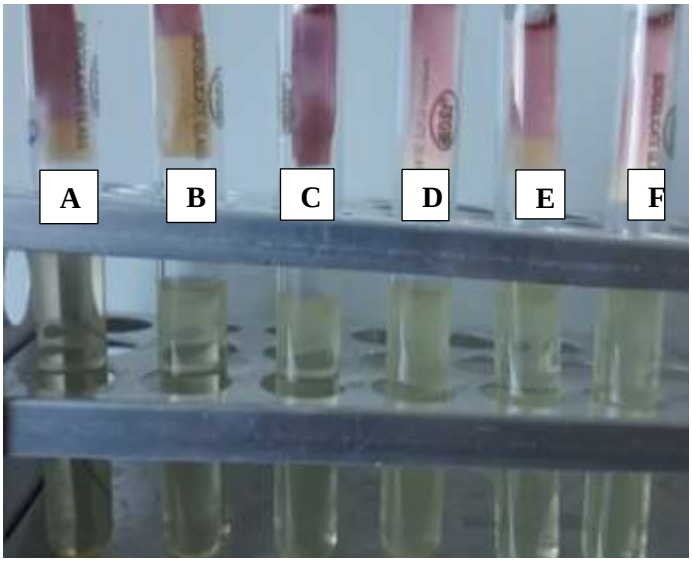



**Figure 74**:Ammonia Production test of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

- **Gelatin Hydrolysis Test**

I. Streak a loopful of culture on Nutrient gelatin agar plate in a plus (+) manner.

II. Incubate at 37°C for 1 – 2 days.

III. Afterward of incubation, add the Frazier's reagent in the plate.

IV. After addition of reagent, plate shows a clear zone around to growth and surrounding by cloudy white precipitates.

V. Development of clear zone around the colony is due to production of gelatinase by microorganism.

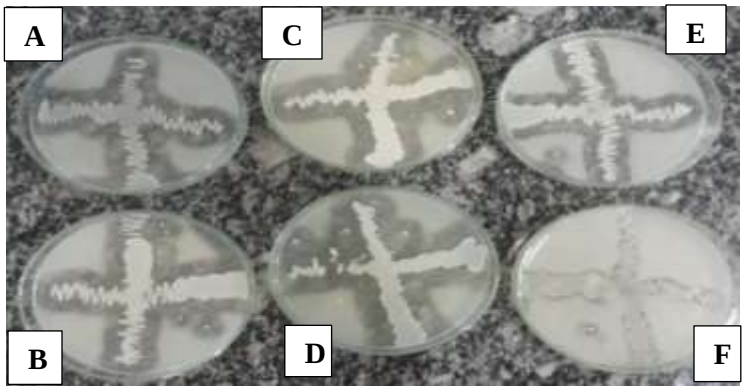



**Figure 75**:Gelatin Hydrolysis Stest of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian asafetida

**Dehydrogenase Test**

i. Inoculate 0.5 – 1ml of dense culture into nutrient broth and add 1ml of sterile methylene blue. Incubate it at 37°C for overnight.

ii. Afterward of incubation, observe the disappearance of blue colour of methylene blue from broth hence, it is considered as the positive result.

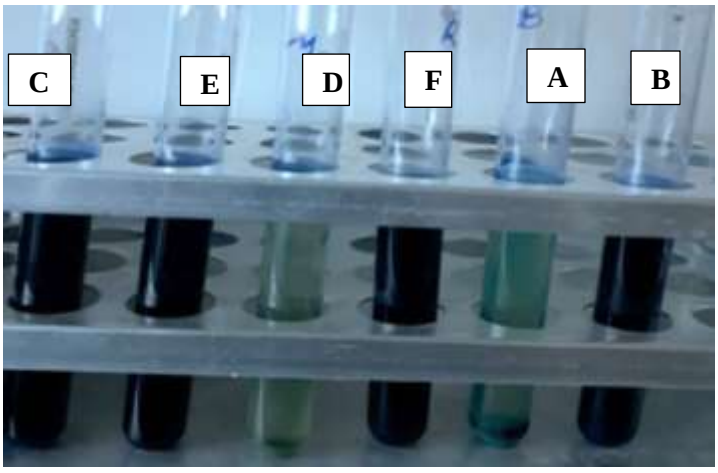



**Figure 76**:Dehydrogenase test of (A) Peenakxir white, (B) Peenakxir Brown, (C)Tazaki, (D)Mazari, (E)Sheera, & (F)Russian

### 5.2.4.5: Antibiogram test:

- Firstly, extraction of asafetida is carried out with the help of Soxhlet apparatus for 3 hours, as a result extracted matter is collected on the surface of round bottom flask.
- This extracted matter has used to check the antimicrobial activity against microbes present in tap water, fertile soil, rooted soil, etc.
- Prepare the 25 ml molten or soften agar per plate.
- Take the sample (tap water, fertile soil, rooted soil) and inoculate 0.1 µl of sample into the molten nutrient agar tube.
- After adding the sample, rub the tube for few seconds & floods within sterile petri plate and permit it for solidification of plate.
- After solidifying the plate, Take the forceps and deep into the methanol and flame it for the sterilization and with the help of this forceps take the paper disc and deep the paper disc into different extracted matter of asafetida and put into the agar plate & press the paper disc for complete attachment with nutrient agar.
- Put this plate in refrigerator at 5-10°C for 30 minutes and allow it for diffusion. And incubate it at 37°C for overnight.
- Positive result gave the zone of inhibition surrounding to paper disc and note the result.

**Figure 77**:Effect of antibacterial activity of asafetida

# CHAPTERS – 6: RESULTS

## 6.1: Results of Organoleptic Analysis: -

The organoleptic analysis of asafetida has been carried out by the taste, smell, texture, and flavor of asafetida. Hence this been done by four senses of body.

| Name | Colour | Taste | Texture | Flavors |
|---|---|---|---|---|
| Peenakxir Brown | Dark Brown to Blackish | Hot | Paste | Mordantly Pungent |
| Peenakxir white | Dull White | Hot | Thick Liquid | Highly Pungent |
| Tazaki | Dark cream to pinkish | Mild | Paste | Pleasant |
| Mazari | Pinkish to light purple | Hottest | Paste | Mild pungent |
| Russian | Pink to light yellow | Mild | Paste | Pleasant |
| Afghani Sheera | Dark cream | Hotter | Paste | Mild pungent |

**Table: 7**-Result table of Organoleptic analysis of asafetida

## 6.2: Results of Physical Analysis: -

The results for physical analysis of asafetida are all the six types of asafetida is soluble in water and burns like camphor. This parameter has been standardized by fssai.

| Name | Water solubility of Asafetida | Asafetida is burn like camphor or not |
|---|---|---|
| Peenakxir Brown | Soluble | Burn like camphor |
| Peenakxir white | Soluble | Burn like camphor |
| 0-Tazaki | Soluble | Burn like camphor |
| Mazari | Soluble | Burn like camphor |
| Russian | Soluble | Burn like camphor |
| Afghani Sheera | Soluble | Burn like camphor |
| Pictures |  |  |

**Table: 8**-Result table of Physical analysis of asafetida

## 6.3: Results of Chemical Analysis: -

In chemical analysis, different variety of asafetida gave the result of several test like moisture content in asafetida, total ash, ash insoluble in acid, crude fiber, pH, alcohol extract. Hence the value of each chemical test for the asafetida has been decided by FSSaI.

| **Name** | **Moisture (%)** | **Total Ash (%)** | **Ash insoluble acid (%)** | **Crude Fiber (%)** | **Alcohol Extract (%)** | **pH** |
|---|---|---|---|---|---|---|
| Peenakxir Brown | 0.79 | 3.9 | 0.36 | 2.78 | 67.76 | 5.57 |
| Peenakxir white | 2.45 | 2.7 | 0.43 | 0.93 | 57.41 | 4.90 |
| Tazaki | 1.21 | 6.8 | 3.44 | 4.71 | 48.29 | 6.12 |
| Mazari | 0.18 | 3.2 | 1.41 | 0.91 | 46.92 | 5.66 |
| Russian | 0.63 | 1.6 | 0.27 | 0.81 | 66.68 | 5.05 |
| Afghani Sheera | 0.28 | 4.6 | 2.50 | 5.58 | 62.39 | 4.98 |

Table: 9-Result table of Chemical analysis of asafetida

## 6.4: Results of Microbiological Analysis: -

### 6.4.1: Isolate colonies from variety of asafetida:

From six samples of asafetida different types of microorganism has been isolated by plating on different types of agar media including, MacConkey agar, EMB agar, N. agar, DCA agar, SS agar. Hence different microorganism produces different types of pigment on respective agar plate and there is difference in Morphological characteristic, cultural characteristics, biochemical test, as well as antibiogram test.

#### 6.4.1.2: Bacterial colonies on different agar plate:

| **Different media** | **Peenakxir Brown & Peenakxir White** | **Tazaki & Mazari** | **Afghani Sheera & Russian** |
|---|---|---|---|
| **Nutrient Agar** |  |  |  |
| **Eosin Methylene blue agar** |  |  |  |

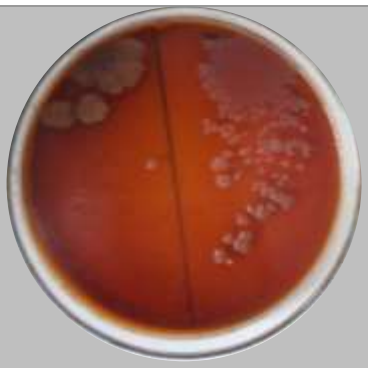
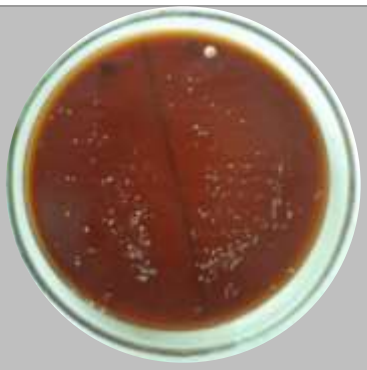
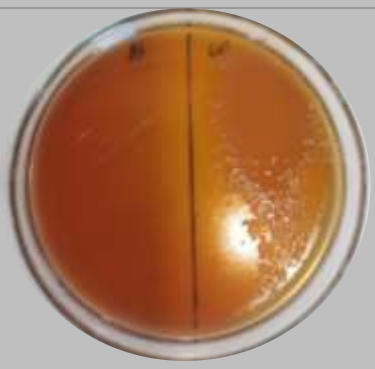
| | | | |
|---|---|---|---|
| **Deoxycholate Citrate agar** |  |  |  |
| **Salmonella shigella agar** |  |  |  |
| **MacConkey Agar** |  |  |  |

**Table: 10**-Microbiological result: Bacterial colonies on different agar plate

**6.4.1.2: Pigmentation of microorganism on different media:**

| Different media | Peenakxir Brown | Peenakxir White | Tazaki | Mazari | Afghani Sheera | Russian |
|---|---|---|---|---|---|---|
| **Nutrient Agar** | Off white | Off white | Off white | Off white | Off white | Off white |
| **MacConkey Agar** | Pink | Pink | Pink | Pink | Pink | Pink |
| **Eosin Methylene blue agar** | Pink to purple | Pink to Purple | Light pink | Light pink | Light pink To purple | Light pink to purple |
| **Deoxycholate Citrate agar** | Off white - cream | Off white – cream | pinkish | pinkish | Off white | Off white |
| **Salmonella - Shigella agar** | - | Transparent | Light pink | Light pink | Transparent | Transparent |

**Table: 11**-Microbiological result: Pigmentation of microorganism on different media

### 6.4.2: Cultural Characteristics:

| Characteristics | Peenakxir Brown | Peenakxir White | Tazaki | Mazari | Afghani Sheera | Russian |
|---|---|---|---|---|---|---|
| **Shape** | Punctiform | Round | Round | Round | Punctiform | Round |
| **Edges** | Even | Wavy | Even | Even | Even | Even |
| **Elevation** | Convex | Umbilicate | Flat | Flat | Flat | Slight convex |
| **Surface** | Smooth | Smooth | Smooth | Smooth | Smooth | Smooth |
| **Consistency** | Moist | Moist | Moist | Moist | Viscous | Viscous |

**Table: 12**-Microbiological result: Cultural Characteristics

### 6.4.3: Morphological Characteristics:

| Characteristics | Peenakxir Brown | Peenakxir White | Tazaki | Mazari | Afghani Sheera | Russian |
|---|---|---|---|---|---|---|
| **Gram's Nature** | Gram negative | Gram negative | Gram negative | Gram negative | Gram negative | Gram negative |
| **Size** | Small | Small | Large | Large | Small | Large |
| **shape** | cocci | cocci | Rod | Rod | cocci | Rod |

**Table: 13**-Microbiological result: Morphological Characteristics

**6.4.4: Results of Biochemical test:**

| Name of Sample | Peenakxir Brown | Peenakxir White | Tazaki | Mazari | Afghani Sheera | Russian |
|---|---|---|---|---|---|---|
| MR | - | - | + | + | + | + |
| VP | - | - | - | - | + | - |
| Indole | - | - | - | + | - | - |
| Simmon Citrate | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + |
| Ammonia production | - | - | - | - | - | - |
| Phenylalanine | + | + | + | + | + | + |
| Dehydrogenase | + | - | - | + | - | - |
| Gelatin Hydrolysis | + | + | + | + | + | + |
| Suspected Microorganism | | | | | | |

**Table: 14**-Microbiological result: Biochemical test

### 6.4.5: Results of Antibiogram susceptibility test:

By extraction of the six types of asafetida, the extracted matter has been used for the determination of antimicrobial activity. Hence these six types of different extracted matter are used to against various sample like microbes of tap water, fertile soil, rooted soil, parts of processing machine. Following fig;6 (A) shows the result of antibiogram test.

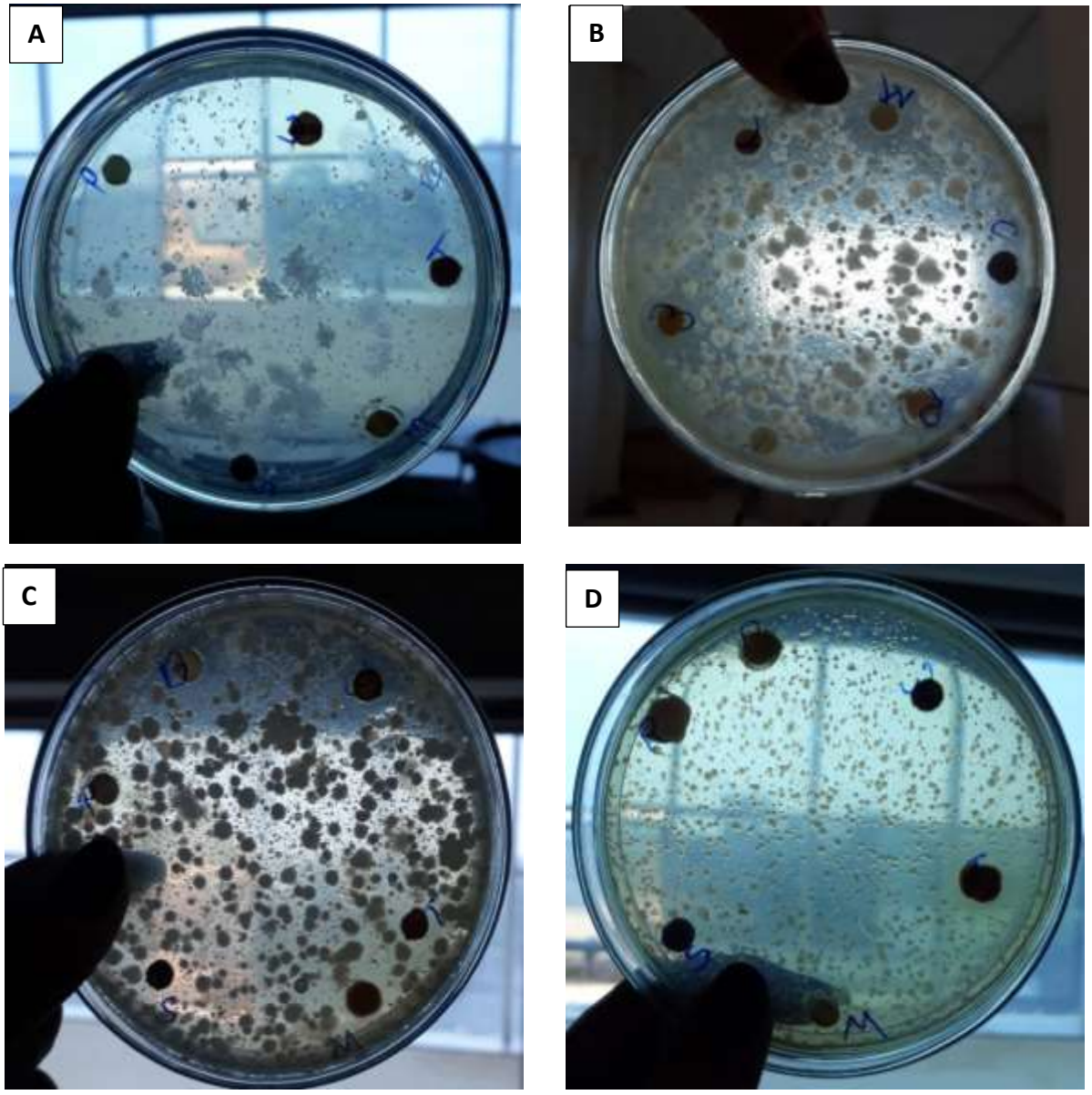



**Figure 78**:Antimicrobial activity of asafetida against (A) part of processing machine, (B) Fertile soil, (C) Rooted soil, (D) Tap water.


**Figure 79**:Antimicrobial activity of asafetida against equipment(H1)

# CHAPTER-7: PROCESS FLOW OF ASAFETIDA

## 7.1: processing of Asafetida:

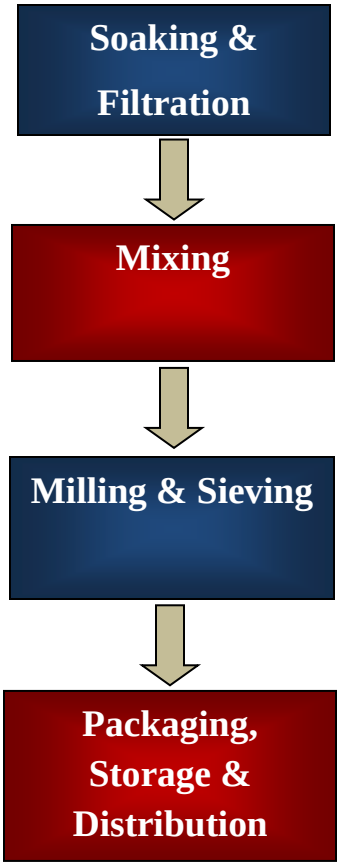
There are multiple verities of persistent asafetida and are belonging to the territorial between Mediterranean to central Asia chiefly at Iran, and Afghanistan. In India there are two types of asafetida are widely accepted: Hing and Hingra. Different verities of asafetida are obtained based on location, climate, overall environment, etc. Traditionally resin like gum has been excreted through the root and creeping rootstalk of ferula plant, then this gum has been crushed in between two stone. Hence this traditional method required more manpower. Hence the raw form of asafetida is not directly consumed because it causes burning in stomach, irritation on lips and many other side effects are there, hence it needs to be diluted. There are two different processes which has been widely used to dilute the asafetida. Hence diluted form of the asafetida is safe to consume.

## 7.2: Manufacturing Process: -

Dried form of asafetida has been obtained through the stem and roots of asafetida plants. Production of the asafetida take approx. 5 years on the plant, after one time incision. Just before March -April, incision should be made on the stem on the upper side to the crown. Hence by make the cut once on the plant then it produces only 500 grams of the dried latex of asafetida. Due to this reason, asafetida is much expensive.

### 7.2.1: Process - 1:

During this time all process of asafetida has been done with the help of machinery. There are four steps for the asafetida processing. Given below is the flowchart for the processing of asafetida.




**Steps-**

1) Soaking & Filtration: -

   The paste of asafetida has been soaking into the 10% water for filtration. Filter it and sieve the sample of asafetida for the removal of extraneous matters like stone, slit, wooden chips, etc.

2) Mixing: -

   Add the ingredients like wheat flour or rice flour in specific amount through mixer then add the soaking paste of asafetida in it and mixed it properly.

3) Milling & Sieving: -

   By using the milling machine, compound form of asafetida and other material are converted into the powder form of asafetida.

4) Packaging Storage & Distribution: -

   After completing the whole process of asafetida, lastly the processing form of asafetida is pack in the container like polyethylene bags, PET jars etc. Jars filled with asafetida powder have been sealed by sealing machine to maintain adequate moisture of asafetida.

   A powder form of asafetida consists, 30% of asafetida resin, along with wheat flour or rice flour, and Arabic gum.

### 7.1.2: Process – 2:

In second process, dough is kneaded with water containing dissolved Asafoetida and placed in tray to make equal sized chunks. Followed by prolonged (at least 20 – 25 days) shadow drying of these chunks grinding process to form powder has been done.

1) Cleaning:

   Raw form of asafetida is dissolved in excess amount of water and, after complete dissolving of asafetida, as a result slurry of asafetida will get.

2) Dough Making:

   Make dough with wheat flour or rice flour with help of slurry of asafetida and put it into tray to obtain equal sized chunks. Allow these chunks for shadow drying for 20 – 25 days.

3) Grinding:

   In the third step, dried chunks of asafetida milled to make fine or granular powder.

4) Packaging:

   After completing the whole process of asafetida, lastly the processing form of asafetida is pack in the container like polyethylene bags, PET jars etc. Jars filled with asafetida powder has been sealed by packaging machine, hence it will maintain the exact moisture of asafetida.

**Figure 80**: Processed asafetida

# CHAPTER – 8: CONCLUSION

The present research study has been concluded that, based on results of the Organoleptic, Physical, Chemical, and Microbiological analysis; the pre – eminent types of asafetida are **Mazari**, **Peenakxir Brown**, and **Tazaki.** This three asafetida has passed all the criteria very well. In organoleptic analysis, it is concluded that by using these three types of asafetida or their combination with each other results in highly aromatic liking flavor and taste with preeminent physico – chemical, organoleptic and microbial properties, hence can be used to form compounded Asafoetida powder of one's choice.

These three varieties of asafetida show desirable results in context of smell, flavor, and appearance. While Physical analysis approve these variants of asafetida based on the pure form by testing the solubility of asafetida in water and asafetida burns like camphor.

Chemical studies of Mazari, Peenakxir Brown, and Tazaki gave the excellent result of the various test parameters like moisture, total ash, ash insoluble in acid, alcoholic extract, and crude fiber. While in Microbiological analysis, it is concluded that Mazari, Peenakxir Brown, and Tazaki gave the higher zone of inhibition against to the fertile soil, rooted soil, Tap water, Parts of the processing machine.

As all varieties of asafetida shows antibacterial activity in different extent, but from the six samples of our studies (Peenakxir Brown, Peenakxir White, Tazaki, Mazari, Afghani Sheero, and Russian), Mazari, Peenakxir Brown, and Tazaki shows the highest zone of inhibition. Hence these three varieties of asafetida Mazari, Peenakxir Brown, and Tazaki have been selected for commercial production.

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