

# Assessment of Antibacterial Efficacy of *Trychasperrum Ammi* Plant Against Oral Flora and Formulation of Ajwain Based Mouthwash

Damdhar Vaishnavi R<sup>1</sup>, Zodpe S. N<sup>2</sup>

<sup>1</sup>Student, P.G. Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola – 444003 (M.S.), India.

<sup>2</sup>Assistant Professor, P.G. Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola – 444003 (M.S.), India.

## ABSTRACT:

The current study was carried out to evaluate the antimicrobial efficiency of *Trychasperrum ammi* (Ajwain) leaf extract against oral flora and formulation of Ajwain based mouthwash. During the current study total 20 oral samples were collected from different dental clinics. The collected samples were further processed for isolation and identification. The predominant organism were found *Streptococcus mutans*. The Extraction of bioactive components in Ajwain extract by performing Qualitative phytochemical analysis and studying antimicrobial activity of Ajwain extract in different organic solvents such as Aqueous, Ethanol, Methanol is checked against *Streptococcus mutans*. Ethanol extracts shows excellent activity where as moderate activity was shown by methanol and aqueous extract. Similarly during the study Ajwain based mouthwash also prepared. Further studied was done to check the antimicrobial activity of prepared mouthwash and the result found was excellent.

**KEYWORDS :** *Trychasperrum ammi*, Antimicrobial efficacy, *S. mutans*, mouth wash.

## INTRODUCTION:

Medicinal plants constitute a large part of natural flora and are considered an important resource in various fields such as the flavor, pharmaceutical, fragrance, and perfumery industries. At present, more than 80% of the global population depends on traditional plant-based medications for treating various human health problems.

*Trachysperrum ammi* L is a Medicinal plant that belongs to family Apiacear comprising 270 genera and species. The name ajwain is derived from the Sanskrit words yavanaka or ajomoda. Ajwain tends grow in regions that are dry and barren. Ajwain is annual, aromatic and herbaceous plant. It is profusely branched with a height of 60-90 cm small, erect with soft fine hair. It has many branched leafy stems, feather-like leaves 2-3 pinnat divided, segments linear with flowers terminal and compound. T fruits are small, ovoid, muricate, around cremocarps, 2-3 mm long. *Trachysperrum* is a cross pollinated crop and has a somatic chromosome number of 2n=18 (Mostafavi Hossein *et al.*, 2015).

Ajwain plant include six major chemical compounds including 49% thymol, 30.8%  $\gamma$ -terpinene, 15.7% p-cymene, 2.1 b-pinene, 0.8% myrcene, and 0.7% limonene. The main component is thymol G5 (60%). A strong germicide, antispasmodic and fungicide agent However, sometimes  $\gamma$ -terpinene and p-cymene

exceed the thymol content. The active Constituent of these plants and herbs suggest the presence of phytochemicals, vitamins, minerals and anti-microbial Constituent in there tissues. (Okwu D.E., 2005).

Ajwain (*Trachyspermum ammi*) is plant with medicinally useful chemicals that can be Use for various therapeutic purpose. Medicinal properties of plants include antioxidant, antispasmodic, antimicrobial, antifungal. Oral cavity consists of various surfaces each coated with the enormous number of bacteria. Orel bacteria include a *Streptococci*, *Pseudomonas*, *Staphylococcus*, *Lactobacilli* and *Corynebacteria* with great number of anaerobic (Daniyan, 2011).

Lack of oral health leads to the formation of dental plaque which further leads to gingival inflammation and distruction of periodontics. The most common disease that can be seen in elderly population are xerostimia (Dharamis, *et al.*, 2009).The most common problems that are faced by youngsters is Halitosis or bad breath (Jaddoh *et al.*, 2017). *Streptococcus mutans* is a facultative anaerobic gram-positive coccus. The oral cavity was molt isolate the main *S. mutans* stains.

Mouthwashes are liquids with anti- inflammatory, antimicrobial. and analgesic properties. Mouthwash is a remedy that, is frequently used for its antiseptic, deodorizing, and refreshing qualities as well as for plaque control (Ozan and Sumer, 2007) Mouthwash can be chemical or herbal in nature. The highest quantity of ajwain leaf extract have highest antimicrobial activity due to the presence of bioactive substances. It is more potent against *Streptococcus mutan*.

## MATERIALS AND METHOD

### Collection of Ajwain plant :

The Ajwain plant, *Trachyspermum ammi*, is gathered from Achalapur city and grown in the Microbiology Department's Terrace Garden at Shri Shivaji College in Akola. Locally, leaves of *T. ammi* were harvested from the Indian state of Maharashtra's Akola city. The leaves were thoroughly cleaned and allowed to dry at room temperature for four to five days in the shade. Next, an electrical grinder was used to grind the dried leaves into a fine powder. The powder was kept for later usage in an air-packed container.

### Preparation of plant extract (Chaudhury *et al.*, 2013) :

- Take Ajwain leaf powder of about 5gm in 50ml ethanol, methanol and water in a separate container and kept at room temperature for 3 days.
- Keep the suspension on rotary shaker for 5-6 hours.
- The extract was filtered through Whatman No. 1 filter paper followed by muslin cloth.
- The extract was concentrated further in a hot water bath at 40°C by heating and evaporating the solvent.
- The dried extract were transferred to separate amber glass jars and 2ml of DMSO (Dimethyl sulphoxide) is added and stored until further use.

### Phytochemical Screening :

The phytochemical tests were carried out for the above mentioned plant extract using the standard procedures to identify the components mentioned at the below (Shaha *et al.*, 2018)

- **Test for Carbohydrates:** 1 ml of Molisch's reagent was added to 2 ml of Ajwain extract after which a few drops of concentrated sulphuric acid was added. A purple colouration depicts the presence of carbohydrates.
- **Test for Tannins:** 2 ml of 5% ferric chloride was added to 1 ml of Ajwain extract. A greenish black colouration depicts that tannins are present.

- **Test for Saponins:** 2 ml of distilled water was added to 2 ml of Ajwain extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.
- **Test for Flavonoids:** 5 ml of dilute NH<sub>3</sub> solution was added to 1 ml of Ajwain extract prior to the addition of concentrated sulphuric acid. A yellow colouration depicts that flavonoids are present.
- **Test for Alkaloids:** 2 ml of concentrated HCl was added to 2 ml of Ajwain extract before a few drops of Mayer's reagent were added. A greenish colouration depicts that alkaloids are present.
- **Test for Glycosides:** 2 ml of glacial acetic acid and a few drops of 5% ferric chloride was added to 0.5 ml of Ajwain extract before 1 ml of concentrated sulphuric acid was added to the mixture. A brown ring formation at the interface depicts that glycosides are present.
- **Test for Terpenoids:** 2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added to 0.5 ml of Ajwain extract. A red brown colouration at the interface depicts that terpenoids are present. **Test for Phenols:** 2 ml of distilled water and a few drops of 10% ferric chloride was added to 1 ml of oil extract. A green colouration depicts that phenols are present.
- **Test for phenol :** 0.5 ml of plant extract few drop of 10% lead acetate solution was added white precipitate indicated presence of phenolic compound. (Mostafavi et al 2015)
- **Test for protein :** 0.5 ml of Ajwain extract 4% NaOH solution and few drop of 1%. CuSO<sub>4</sub> solution were added violet color appear indicate the presence of protein (Mostafavi *et al.*, 2015).
- **Test for coumarins :** 0.5 ml of plant extract, the solution of 10% NaOH was added the appearance of yellow colour indicated the presence of coumarins. (Mostafavi *et al.*, 2015).
- **Test for steroids:** 3 drops of concentrated sulphuric acid was added into 5 mg extract. The formation of red colour indicated presence of steroids (Panchal *et al.*, 2019).
- **Samples Collection:** Sterilized cotton swabs were used to preserve aseptic conditions throughout the collection of oral infection samples, including tooth decay, gingival infections, periodontal illnesses, mix types of infections, and odontogenic infections, from MSB Dental Hospital in Akola.

#### **Isolation and Identification:**

- Without much delay, every sample that was inoculated using the swabbing method on nutrient agar plates. After 24 hours of incubation at 37°C, gram staining was carried out. Under a microscope, the morphological characters of *S. mutans* was observed and then recaptured on specific media, such as Mitis salivarius agar, where watery colonies are observed.
- The isolates were identified on the basis of cultural, morphological and biochemical and were confirmed on the basis of Bergey's Manual of Determinative Bacteriology.

#### **Antimicrobial Activity (Deshmukh *et al.*, 2022)**

Antibacterial activity using plant extract was evaluated by Agar Well Diffusion Method on Muller Hinton Agar or nutrient agar

Each isolate of *S. mutans* was uniformly swabbed onto the individual plates using sterile cotton swabs. Wells for different extract were made by using sterile borer. A micropipette was used to pour the extract solution into each well on all plates. After incubation at 30° C for 24 hours the zone of inhibition was measured in millimetres.

#### **Preparation of Mouthwash (Chowdhury *et al.*, 2013)**

##### **Procedure:**

The Ajwain (*Trychasperrum ammi*) leaf powder is boiled in sterilized water for 15 min by keeping it in

the water bath (10 g/100 mL). The liquid thus obtained is filtered using a Whatman paper (40 ml of each extract). Clove oil, Badam oil and Neem oil (each 2 mL) are added finally to the extract mixture. The edible food color is mixed in it. The sample thus prepared is now kept in two volumetric flasks at two varied temperatures, one at ambient temperature and the other at low temperature.

#### **Mouthwash evaluation by considering different parameters :**

- **Physical evaluation:** Visual inspection was used to assess physical parameters such color, taste, and consistency.
- **pH:** Using pH strips, the pH of the herbal mouthwash was determined.
- **Viscosity:** A digital viscometer is used to test viscosity.
- **Microbial Assay:** The zones of inhibition (measured in millimeters) were used to assess the antibacterial activity.
- **Stability studies:** At room temperature and 40°C, physical characteristics such as color, odor, consistency, and PH were measured.
- **Taste:** Throughout the week, the test is bitter and unchanged.
- **Flavor:** The flavor is essentially the same and has a wonderful Ajwain and clove aroma.
- **Texture :** Sample remain clear after one week, it become turbid.

#### **RESULTS AND DISCUSSION :**

Ajwain (*Trachyspermum ammi*) is a plant with medicinally useful chemicals that can be used for various therapeutic purposes. Medicinal properties of the plant include Antioxidant, Antispasmodic, Antimicrobial and Antifungal. This study has helped in understanding medicinal properties of herbal extract of Ajwain against oral isolates such preparation can be used for the formulation of herbal mouth wash.

In the current study total 20 samples were collected using sterile oral swab under aseptic conditions from MSB dental clinic, Akola and the samples were categorized as male and female. There were ten female and ten male samples. Fifty percent male and fifty percent female are found in the frequency distribution of the sex ratio of the sample collection (Table and Graph).

The collected sample were further processed for isolation and identification of bacteria on various selective media. The frequency obtained isolated were 40 % isolates were *S. mutans*, 16% isolates were *E. coli*, 12 % isolates for *Lactobacillus* and *S. aureus*, whereas 10 % isolates were *S. salavarius* and *S. oralis* (Table and Graph).

On the basis of cultural and morphological characteristics the isolate obtained were tentatively confirmed as *S. Mutans*, which is Gram Positive, short rods, non motile, 0.5 – 1 mm in size white in colour Convex or raised elevation, opaque having entire margine and pigment formation is variable (Graph).

Phytochemical studies was described in Table Qualitative phytochemical investigation discovered presence of saponins compounds (foam formation); flavonoids (The yellow colour formation), white precipitate indicated the presence phenols and brown ring indicated presence of Glycosides. It show presence absence of steroids (Red colour formation). It showed negative result in case of Carbohydrates, Tannins, Alkaloids, Terpenoids and Proteins. According to Chatterjee *et al.*, (2012) Alkaloids, Steroids, Saponins, and tannins found in extracts are Known to have curative activity against several pathogens. Antimicrobial Activity of Ajwain Plant Extract against *S. mutans*, was checked by Agar Well Diffusion Method. It was carried out for 3 different solvent such as Methanol extract, Ethanol Extract and Aqueous

Extract. The highest zone of inhibition was found in Ethanol extract for Isolate No. S3, (28mm) followed by S1 (27 mm). The methanol extract showed height activity for isolated S17 (20) followed S10 (10 mm). The highest activity for Aqueous extract was shown by isolate No. S7 (17mm) followed by S6 (15mm). All the results of Ethanol extract were showed good to moderate antimicrobial activity as compare to Methanol and Aqueous extracts (Table and Graph).

Nag *et al.*, (2023) also study that leaf extract of *Trachyspermum ammi*, exhibit a wide range of antimicrobial and antioxidant activity and can potentially be used as natural biopreservative. Hassanshabian *et al.*, (2014) also study the antimicrobial activity of *Trachyspermum ammi* against different kinds of microorganisms Rao *et al.*, (2023) also study the antimicrobial activity of solar dried Ajwain leaves.

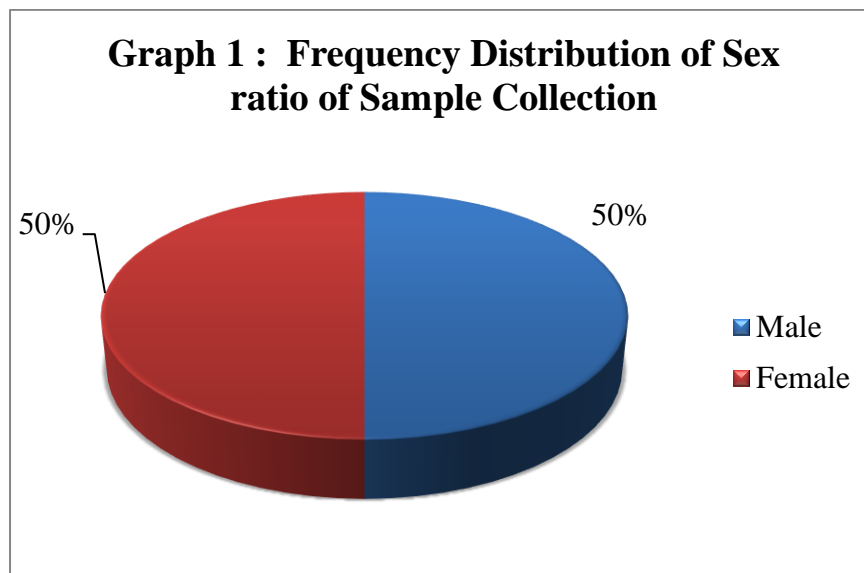
On the basis of complete study done on prepared Ajwain based mouthwash, it showed excellent results in terms of Colour, Flavour, Taste and Texture indicating the mouthwash is excellent in controlling oral flora. The formulation was found to be free from microbes as they have not produce any microbial growth when they inoculated on Agar medium.

Antimicrobial activity of the prepared mouthwash also was checked at Refrigeration i.e. 4°C and at room temperature. The zone of inhibition was found 21.0 to 21.2 mm in both the temperature. The results were found very excellent by our Ajwain based mouth wash preparation.

The similar work was done by Chowdhury *et al.*, (2013) as our study they also studied physiochemical characteristics like pH and Antimicrobial Activity was also studied. Deshmukh, *et al.*, (2022) prepared polyherbal mouthwash and evaluate its antimicrobial activity and anti-inflammatory efficiency against commercially available herbal mouthwash.

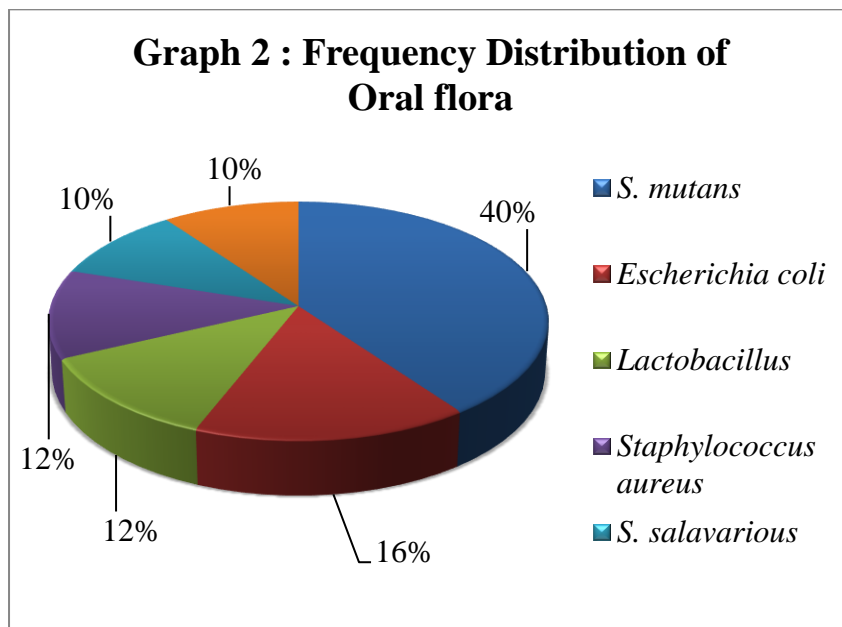
**Table : Frequency Distribution of Sex ratio of Sample Collection**

Sr. No.	Sex	Number	Percentage
1	Male	10	50
2	Female	10	50
	Total	20	100



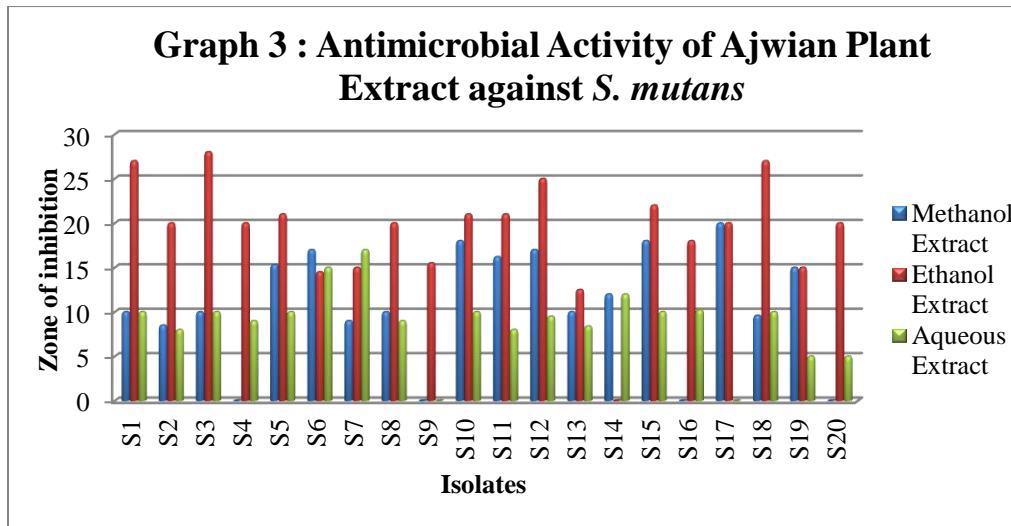
**Table : Frequency Distribution of Oral Flora**

Sr.No.	Name of organisms	No. of Isolates (out of 50)	Percentage
1	<i>S. mutans</i>	20	40
2	<i>Escherichia coli</i>	8	16
3	<i>Lactobacillus</i>	6	12
4	<i>Staphylococcus aureus</i>	6	12
5	<i>S. salavarius</i>	5	10
6	<i>S. oralis</i>	5	10



**Table : Preliminary Phytochemical Analysis of *T. ammi* (Ajwain) plant leaves extract**

Sr. No.	Phytoconstituents	Observation	Inference
1	Carbohydrate	No purple colouration	Negative
2	Tannins	No greenish black colouration	Negative
3	Saponins	Foam formation	Positive
4	Flavonoids	Yellow colour formation	Positive
5	Alkaloids	No greenish colouration	Negative
6	Glycosides	Brown ring	Positive
7	Terpenoids	No green colouration	Negative
8	Phenols	White Precipitate	Positive
9	Protein	No violet colour	Negative
10.	Coumarins	Yellow colour formation	Positive
11.	Steroids	Red colour formation	Positive

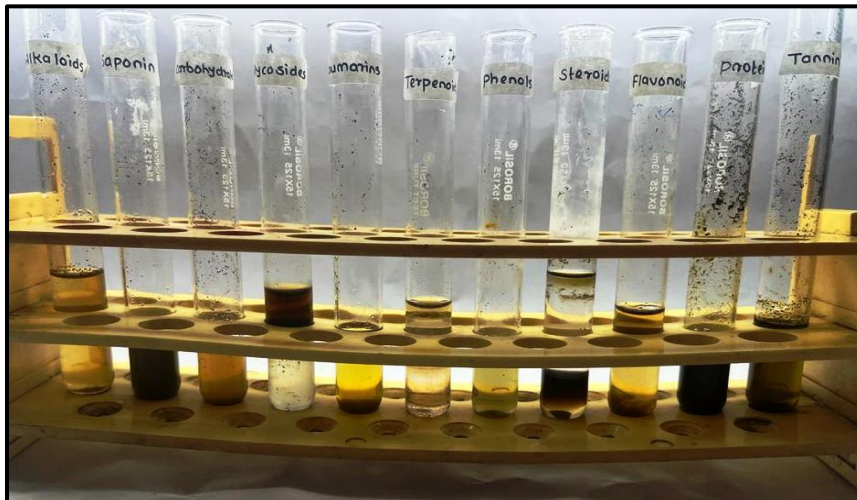
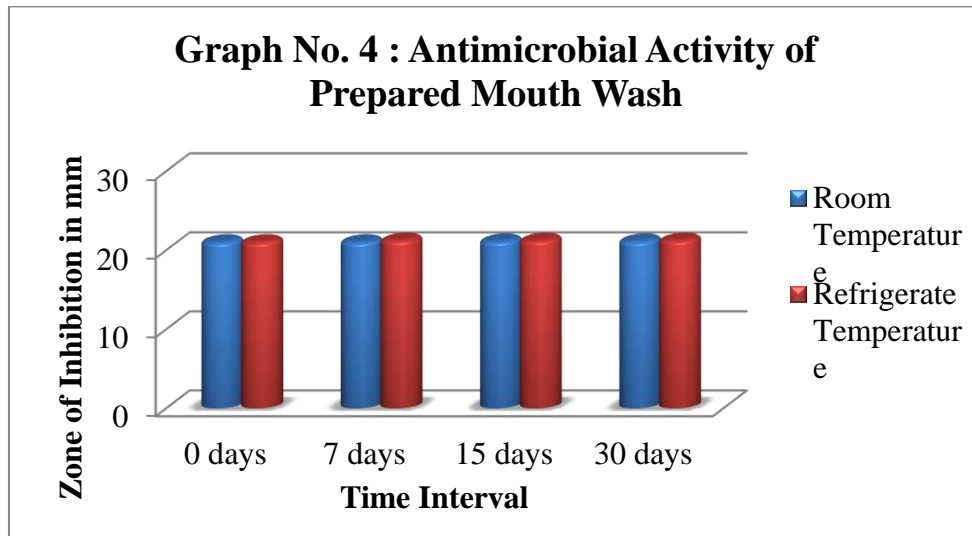


**Table : Characteristics of Prepared Mouth Wash**

Days after preparation of mouth wash	Temperature	Colour	Flavour	Taste	Texture
0 days	Room Temp.	Brownish Colour	Fragrance of Clove and Ajwain	Bitter	Clear Liquid
0 days	Refrigerate Temp.	Brownish Colour	Fragrance of Clove and Ajwain	Bitter	Clear Liquid
7 days	Room Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid
7 days	Refrigerate Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid
15 days	Room Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid
15 days	Refrigerate Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid
1 Month	Room Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid
1 Month	Refrigerate Temp.	Brownish Colour	Fragrance some what lost	Bitter	Turbid

**Table : Antimicrobial Activity of Prepared Mouth Wash**

Time Interval	Zone of Inhibition in mm	
	Room Temperature	Refrigerate Temperature
0 days	21.0	21.0
7 days	21.0	21.2
15 days	21.1	21.2
30 days	21.1	21.2



**Phytochemical Test for Ajwain Plant**



***S. mutan* on Mitis Salavarius Agar**

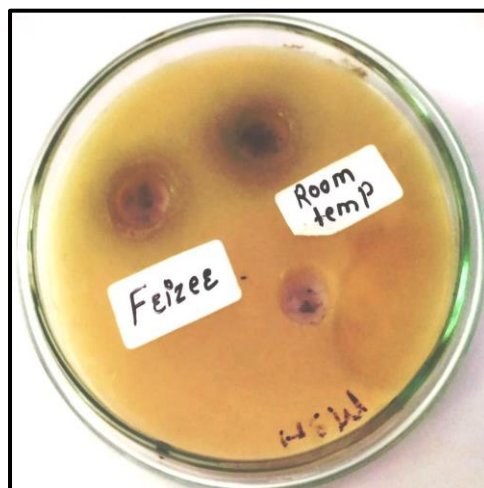




**Antimicrobial Activity of Ajwain extract in different solvent against *S. mutans***



**Formulated Ajwain based Mouthwash**



**Antimicrobial Activity of Prepared Mouthwash**

## CONCLUSION

Ajwain plant shows high antimicrobial activity against oral pathogens due to the presence of many bioactive components. The study shows that mouth wash formulation by using Ajwain was found to be excellent alternative to chemical mouthwash with less side effects. Even today in the modern world people have changed their focus from chemical drugs to the use of natural products to cure quite a number of diseases, dental problems being one of them. It can be a cost-effective measures of maintaining the oral health which are innocent of any untoward Side effects, it should help in overcoming some common dental problems.

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