

# Phytochemical Screening, Antioxidant Activity and Antimicrobial Susceptibility of *Trachyspermum ammi* and *Trigonella foenum graceum* Against Dental Caries Causing Microbes

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**Abstract:** Use of herbs and spices for treating various health related problems are known and even practiced in our day to day life. Traditional medicinal system includes these herbs and spices for various dental problems like dental caries. The present microbiological study was aimed to evaluate the phytochemical screening and antimicrobial activity of two spices *Trachyspermum ammi* and *Trigonella foenum graceum* against microbes present in oral cavity (*Lactobacillus acidophilus* and *Streptococcus mutans*). The antimicrobial susceptibility of each plant extract was tested by using agar well diffusion method on Muller-Hinton Agar (MHA) media and zone of inhibition was measured in millimeters. Results showed that the plant extract of *Trigonella foenum graceum* has wide spectrum of antibacterial activity to be effective against *Lactobacillus acidophilus* and *Streptococcus mutans* as compared to *Trachyspermum ammi*.

**Keywords:** Dental Caries, *Trachyspermum ammi*, *Trigonella foenum graceum*, Oral Microbes, Antibacterial activity

## I. INTRODUCTION

Nature has provided us with lots of precious gifts to fulfill our daily basic needs one of the most important source is plants. Use of plants for medicinal purpose has been known from years ago. These traditional medicinal plants are used to treat several diseases and thus forms an integral part of complementary medicines. One such treasure of different plant species with numerous medicinal properties to be practiced is found in Indian subcontinent serving therapeutic values. Herbal medicines also play a major role for gums and oral problems. One of the most common oral disease which is primarily leading among the individuals of all age groups is "Dental Caries". Large number of population (about 80% or more) suffers from problem of dental caries once in their lifetime.

**Dental Caries:** also known as cavity or tooth decay, is a common oral disease caused by interaction of microorganisms present in oral habitat. These microorganisms causes demineralization of tooth affecting mineralized dental tissue (enamel, dentin) and resulting in tooth loss. Demineralization results due to production of acids by bacteria. Microorganisms reside on the surface of the teeth and adhere to form biofilm on tooth surface. On consumption of food rich in sugar content and starch microorganism present in mouth breakdown food debris and produce acid attacking the tooth surface. Repeating cycle of food consumption and acid formation causes dental caries.

Individual suffering from dental problem may not be aware of the disease at the first as the acid produced by the bacteria dissolves hard tissue of teeth and initially appears as small white lesions indicating demineralization of enamel. This demineralization continues turning lesion brown in color and thus causes cavitations. Wide variety of oral microbes habitat in our mouth like *Streptococcus mutans*, *Enterococcus faecalis*, *Lctobacillus acidophilus*, *Nisseria species*, *Candida albicans*, and other species.

Increasing use of synthetic drugs and development of bacterial resistance has showed varied side effects whereas use of medicinal plants for therapeutic purpose are relatively safe, easily available, affordable, have less adverse effect and pose minimum health hazard. Medicinal property of the plant is due to presence of many bioactive constituents like steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and glycosides which shows antimicrobial properties and serve well source of antioxidants.

Antioxidant molecule present in plants inhibits oxidation reaction which produces free radicals which in turn starts chain reaction causing damage or death of cell. Major natural compound present in possessing antioxidant activity is polyphenols (flavonoids) preventing free radical associated damage by various ways like nitrous oxide scavenging activity. Two such plants with effective traditional practices and potent antioxidant activity of interest are *Trachyspermum ammi* (Ajwain) and *Trigonella foenum graceum* (Fenugreek). The present microbiological study was aimed to study antimicrobial susceptibility of two medicinal plant *Trachyspermum ammi* (Ajwain) and *Trigonella foenum graceum* (Fenugreek) against dental caries causing microbes *Lactobacillus acidophilus* and *Streptococcus mutans* and to determine zone of inhibition at different concentration (25%, 50%, 75%, 100%).

Table 1: The ethanobotanical and phytochemical data of two medicinal plants:

Botanical name (family)	Common name	Parts used	Phytoconstituents	Traditional uses
<i>Trachyspermum ammi</i> (Apiaceae)	Ajwain	Seeds like fruit	Thymol, p-cymene, c-terpiene and $\alpha$ - and $\beta$ -pinene	Used in treating influenza, asthma, cough, cold, diarrhea, cholera, indigestion, rheumatism, abdominal pain
<i>Trigonella foenum graecum</i> (Fabaceae)	Fenugreek/Methi	Seeds	Saponins (disogenin), hydroxyisoleucine, flavonoids, lecithin	Used as preservatives in pickles, chutneys. Used in treatment of constipation, pneumonia, tuberculosis, reduce cholesterol, diabetes, aids digestion, prevent hair loss, increase lactation in breast feeding women.

## II. MATERIAL AND METHOD

**Collection of plant sample:** The seeds of *Trachyspermum ammi* and *Trigonella foenum graecum* were collected from grocery shop of Bhopal (M.P) during month of June.

**Preparation of aqueous extract (soxhletion):** Extraction of desired compound of plant part was done by using soxhlet apparatus. About 50 gram of *Trachyspermum ammi* and 78.62 gram of *Trigonella foenum graecum* was extracted with hydroalcoholic solvent using soxhlet apparatus for 5-6 days. The extract obtained was further concentrated at 60<sup>0</sup> C on a water bath to obtain semi solid mass and was preserved in closed vials for further use.

**Preliminary phytochemical analysis:** The extract obtained was subjected to preliminary phytochemical testing to detect presence of different compound. The extract was screened for presence of carbohydrates, non reducing polysaccharides, proteins, amino acids, steroids, glycosides, anthraquinone glycosides, flavonoides, alkaloids, tannins, phenolic compound, coumarine glycosides, organic and inorganic acids.

**Thin layer chromatography:** The plant extract of *Trachyspermum ammi* and *Trigonella foenum graecum* was purified by TLC. TLC on silica gel plate was done by using Chloroform: Methanol: Formic acid (9:1:2) and Water: Acetic acid: Methanol: n- butanol (1:1:5:4) as mobile phase for *Trachyspermum ammi* and *Trigonella foenum graecum* respectively which was observed in UV light (366nm) and retention factor ( $R_f$  value) was measured.

**Determination of total phenolic content:** TPC of extract obtained from *Trachyspermum ammi* and *Trigonella foenum graecum* seeds was determined spectrophotometrically using Folin- Ciocalteu's method. Total phenolic content was measured from calibration curve drawn using Gallic acid as standard solution.

**Determination of total flavonoid content:** TFC of both plant extract *Trachyspermum ammi* and *Trigonella foenum graecum* was determined spectrophotometrically. Appropriate dilute sample of extract was taken in a test tube followed by addition of 150 $\mu$ l of 10% AlCl<sub>3</sub>, 6 ml of NaNO<sub>2</sub> and 1ml of 1M sodium hydroxide. Total flavonoid content was calculated by using Quercetin as standard solution.

**Determination of total alkaloid content:** Plant extract of *Trachyspermum ammi* and *Trigonella foenum graecum* at different concentration was taken in a tube followed by adding 1ml 2N HCl, 5ml phosphate buffer, 1ml chloroform and 5ml bromocresolgreen. The tubes were incubated for 10 mins and absorbance was measured at 470nm against blank. Colchicine was used as standard and total alkaloid content was determined.

**Total antioxidant capacity assay:** To different concentration of plant extract of *Trachyspermum ammi* and *Trigonella foenum graecum* added 1ml of reagent solution (0.6 M sulphuric acid, 28mM Sodium phosphate and 4mM ammonium molybdate).

**Nitric oxide radical scavenging activity:** Sodium nitroprusside in aqueous solution generates free radical interacting with oxygen producing nitrite ion which is estimated by using Griess reagent. Reaction mixture was prepared containing 5mM Sodium nitroprusside in phosphate buffer saline with plant extract at different concentration was incubated at 29<sup>0</sup> C for 3 hours. The incubated sample was diluted with 2.5ml of Griess reagent. The absorbance of chromophore (purple azo dye) developed as a result

of diazotization of nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediaminehydro-chloride was measured at 550nm. Ascorbic acid was used as standard.

**Collection of bacterial sample:** Bacterial samples were collected from children's of different age group from Mahak public school Nehru Nagar Bhopal M.P. For isolation of desired bacterium *Lactobacillus acidophilus* and *Streptococcus mutans*, bacterial samples were inoculated in MRS agar media and LB agar media respectively for 24 hrs. Colony traits of isolated colonies were recorded and Gram staining was performed.

**Biochemical characterization:** Characterization of desired bacteria was done by biochemical testing.

**Antimicrobial susceptibility of *Trachyspermum ammi* and *Trigonella foenum graceum* against oral microbes:** Antimicrobial activity of both plant extract were tested against *L.acidophilus* and *S.mutans* by Well diffusion method on Muller-Hinton agar(MHA) media. MHA agar plates were inoculated with bacterial strains under aseptic condition and wells were filled with 50 $\mu$ l test samples and incubated at 37<sup>0</sup> C for 24hrs and zone of inhibition was measured in millimeters.

### III. RESULTS

Extraction yield for *Trachyspermum ammi* and *Trigonella foenum graceum* was found to be moderate for aqueous extract. The total % yield for Soxhlet extraction of *Trachyspermum ammi* is 12.32% and *Trigonella foenum graceum* is 6.41%

**Table 2:- Total % yield of *Trachyspermum ammi* and *Trigonella foenum graceum*:**

S.No.	Soxhlet extraction with aqueous solvent	% yield
1.	<i>Trachyspermum ammi</i>	12.32%
2.	<i>Trigonella foenum graceum</i>	6.41%

**Table 3:- Phytochemical investigation:**

S.No.	Test	<i>Trachyspermum ammi</i>	<i>Trigonella foenum graceum</i>
1.	Carbohydrate	+ve	+ve
2.	Steroid	+ve	-ve
3.	Glycosides	+ve	-ve
4.	Flavonoids	+ve	+ve
5.	Alkaloids	+ve	+ve
6.	Phenolic compound	+ve	+ve

**Qualitative analysis by TLC:** Rf value of produced content in crude of *Trachyspermum ammi* is 0.25 & 0.75 and Rf value of produced content in crude of *Trigonella foenum graceum* is 0.16, 0.61 & 0.84.



(a)



(b)

Fig 1:- TLC of (a) *Trachyspermum ammi* and (b) *Trigonella foenum graceum* observed under U.V. Light

**Total phenolic content (TPC) assay:**

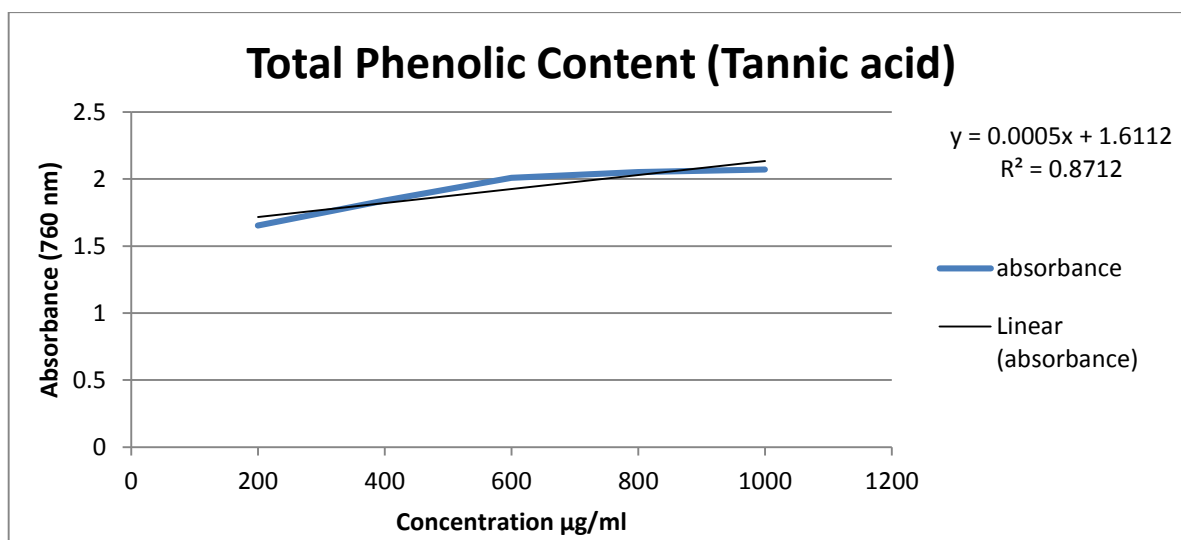


Fig 2: Standard curve of Tannic acid

Table 4:- Total Phenol Levels of *Trachyspermum ammi* and *Trigonella foenum graecum*

Quantitative analysis	<i>Trachyspermum ammi</i>	<i>Trigonella foenum graecum</i>
Total phenols (mg of TAE/ serving)	788.231	779.90

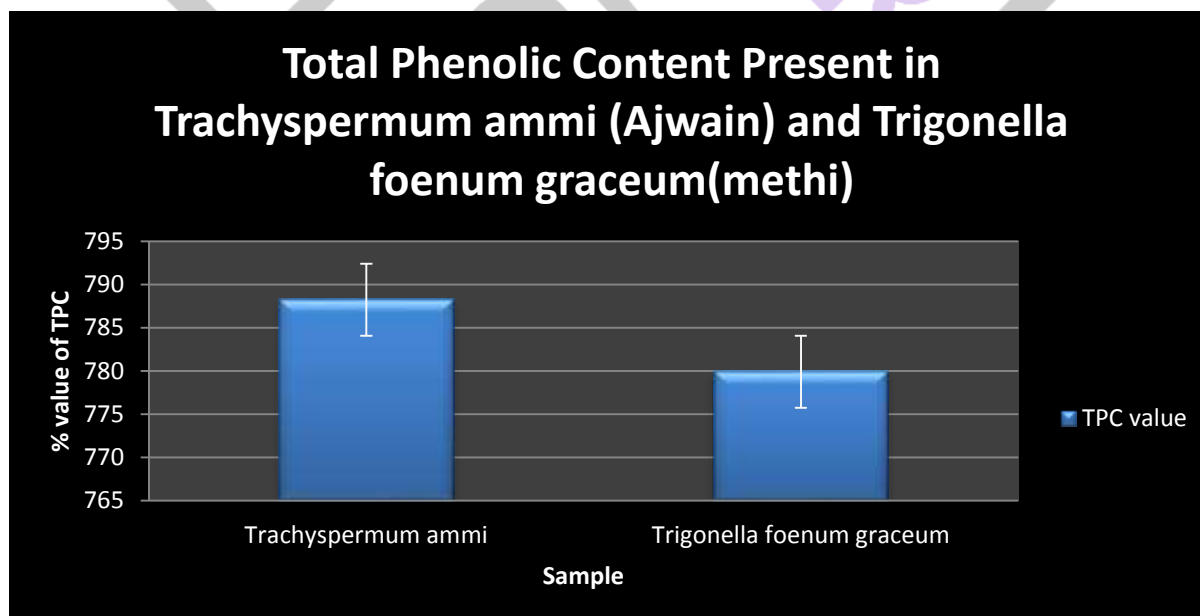


Fig 3 : Total Phenolic Content Present in *Trachyspermum ammi* and *Trigonella foenum graecum*

**Total Flavonoid concentration (TFC) assay:**

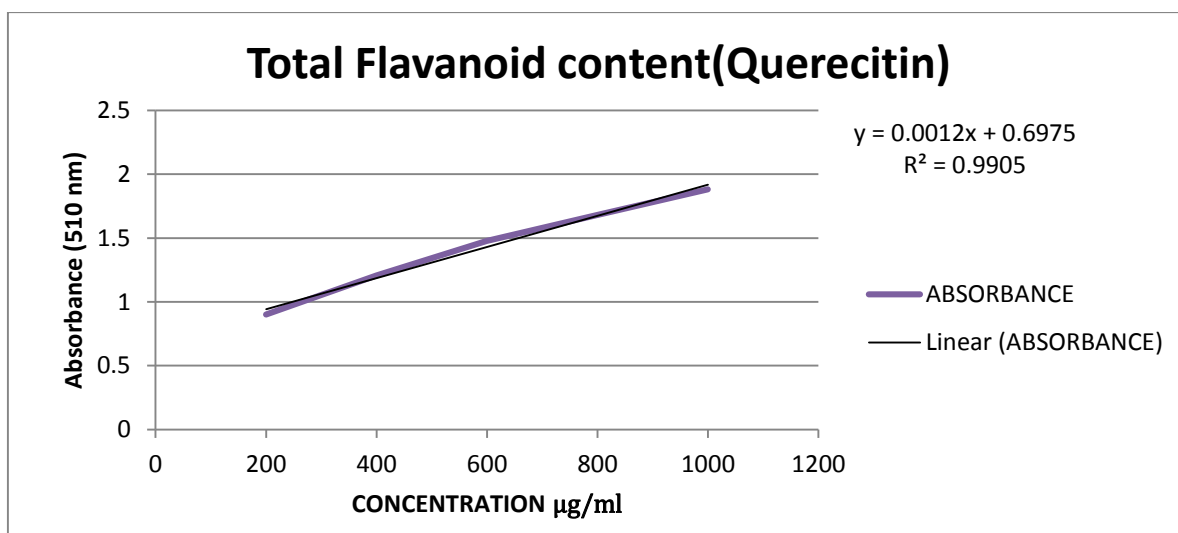


Fig 4: Standard curve of Querecitin

Table 5: Total Flavonoid Levels of *Trachyspermum ammi* and *Trigonella foenum graecum*

Quantitative analysis	<i>Trachyspermum ammi</i>	<i>Trigonella foenum graecum</i>
Total Flavonoid (mg of QE/ serving)	413.50	608.27

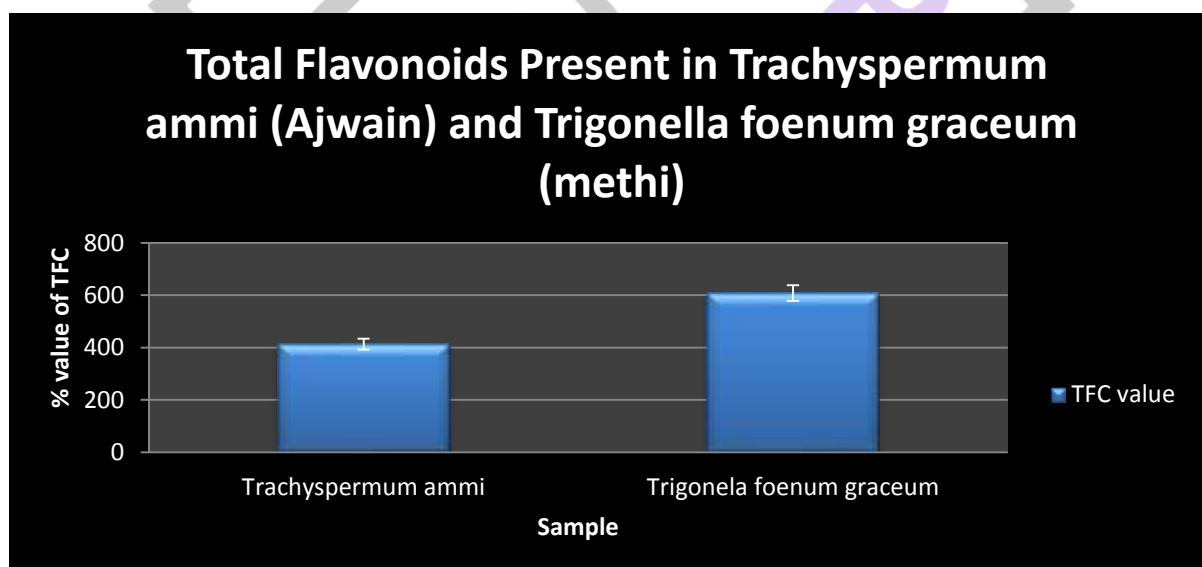


Fig 5: Total Flavonoid Present in *Trachyspermum ammi* and *Trigonella foenum graecum*

**Total Antioxidant Activity:**Table 6 : Total Antioxidant activity of *Trachyspermum ammi* and *Trigonella foenum graecum*

S.No.	Concentration	Standard Ascorbic Acid	<i>Trachyspermum ammi</i>	<i>Trigonella foenum graecum</i>
1.	200µg/ml	2.498	94.30	86.39
2.	400µg/ml	4.207	80.02	57.54
3.	600µg/ml	10.649	69.15	42.30
4.	800µg/ml	12.577	54.36	21.96
5.	1000µg/ml	17.528	46	4.43

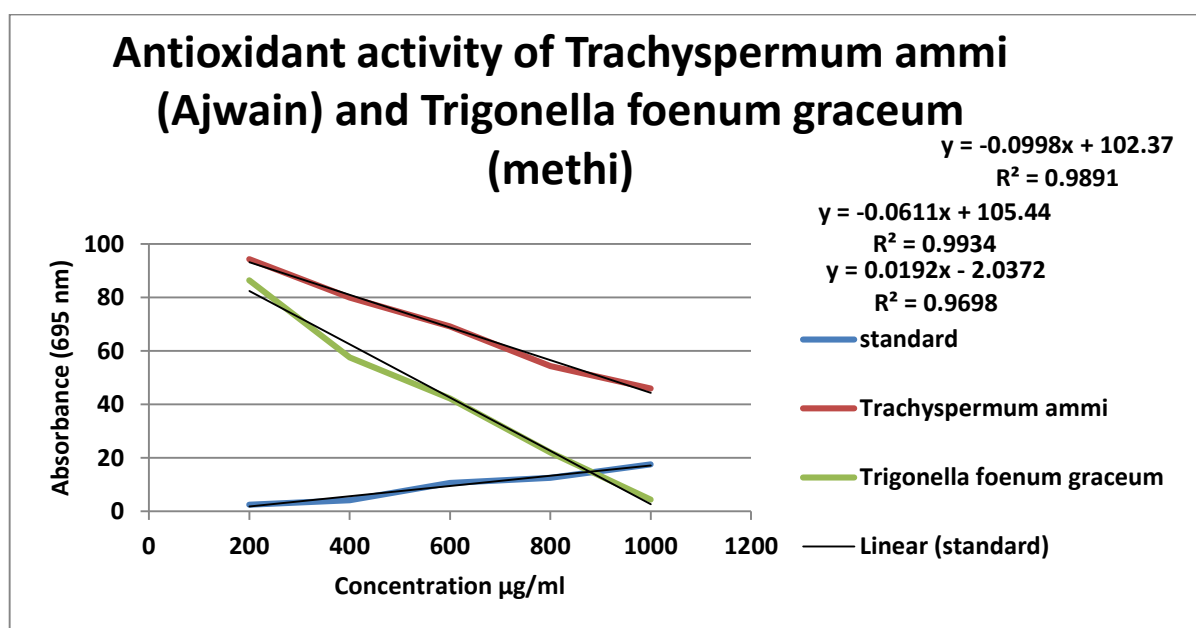


Fig 6: Antioxidant activity assay

**Nitrous Oxide Scavenging Activity:**Table 7 : Nitrous Oxide Scavenging Activity of *Trachyspermum ammi* and *Trigonella foenum graecum*

S.No.	Concentration	Standard (Ascorbic acid)	<i>Trachyspermum ammi</i>	<i>Trigonella foenum graecum</i>
1.	200	22.300	54.63	59.74
2.	400	15.718	53.35	44.40
3.	600	16.230	53.35	36.74
4.	800	17.316	49.52	30.67
5	1000	16.805	53.35	27.15

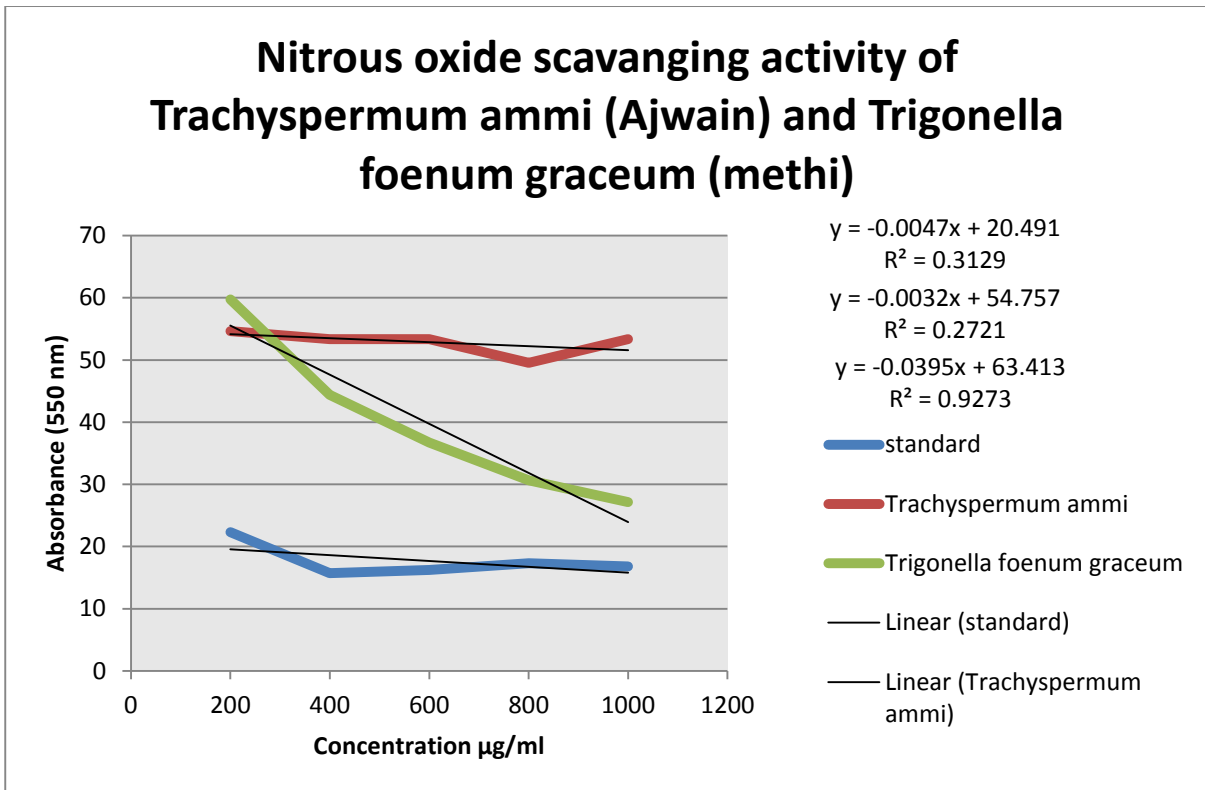


Fig 7: Nitrous Oxide Scavenging Activity

**Total Alkaloid Content:**

Table no. 8 : Total alkaloid content of standard Colchisine

S.No.	Concentration	Standard (Colchisine)
1.	200	0.194
2.	400	0.398
3.	600	0.63
4.	800	0.895
5.	1000	1.196

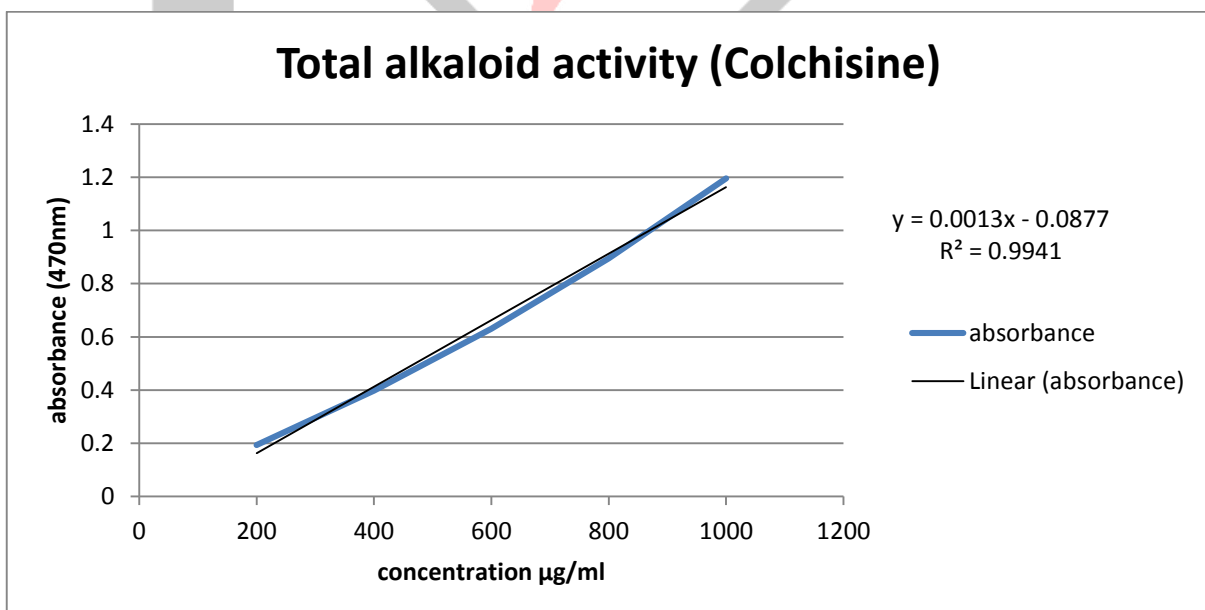


Fig 8: Total Alkaloid Content of standard (Colchisine)



Table No. 9 : Total Alkaloid Level of *Trachyspermum ammi*

Quantitative analysis	<i>Trachyspermum ammi</i>
Total alkaloid content	1120.24

**MICROBIAL ANALYSIS:**

Bacterial sample were collected from saliva of children's of age group 5-10 from Mahak Public School Nehru Nagar Bhopal (M.P.). 7 samples of *Lactobacillus* and 3 samples of *Streptococcus mutans* were taken into consideration for Gram's Staining and Biochemical identification. (L stands for *Lactobacillus* and S stands for *Streptococcus mutans*)

Table No. 10: Results of Gram's staining of isolated bacterial species

S.No.	Culture	Staining
1.	L1	Gram positive
2.	L2	Gram positive
3.	L3	Gram positive
4.	L4	Gram positive
5.	L5	Gram positive
6.	L6	Gram positive
7.	L7	Gram positive
8.	S3	Gram positive
9.	S5	Gram positive
10.	S7	Gram positive



Fig 9: Gram's staining of bacterial species

Table No. 11: Results of Biochemical Test of bacterial species

S.No.	Biochemical test	Test Organism									
		L1	L2	L3	L4	L5	L6	L7	S3	S5	S7
1.	Amylase test	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
2.	Cellulase test	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	Pectin test	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
4.	Gelatinase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5.	Casein test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
6.	Urease test	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
7.	H <sub>2</sub> S production test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve



8.	Citrate utilization test	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
9.	VP test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10.	MR test	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
11.	Litmus milk test										
	Acid pH	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
	Alkaline pH	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Reduction	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
	Acidic curd	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Rennet curd	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Gas production	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
12.	Indole test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
13.	Carbohydrate fermentation test										
	Glucose	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve
	Sucrose	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
	Mannitol	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
	Mannose	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve		
	Fructose	+ve	+ve	+ve	+ve	+ve	+ve	+ve		-ve	+ve
	Rhamanose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve		
	Inositol	+ve	+ve	+ve	+ve	+ve	-ve	+ve			
	Lactose	+ve	+ve	+ve	+ve	-ve	-ve	+ve		-ve	+ve
14.	Nitrate test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
15.	Motility test	+ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
16.	Carbohydrate catabolism	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
17.	Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve

Table No. 12: Bacterial identification using PIBWIN software

S.No.	Bacterial ID	Bacterial species
1.	L2	<i>L.acidophilus</i>
2.	L7	<i>L.acidophilus</i>
3.	S5	<i>Streptococcus mutans</i>

**Antimicrobial Activity:**Table no. 13 : Antimicrobial activity of *Trachyspermum ammi*

S.no.	Sample ID	25 mg/ml	50mg/ml	75mg/ml	100mg/ml
1.	L1	10 mm	0mm	0mm	19 mm
2.	L2	13 mm	12 mm	11 mm	10 mm
3.	L3	0 mm	10 mm	0 mm	10 mm
4.	L4	0 mm	0 mm	0 mm	0 mm
5.	L5	0 mm	0 mm	0 mm	0 mm
6.	L6	0 mm	0 mm	0 mm	0 mm
7.	L7	0 mm	0 mm	10 mm	10 mm
8.	S3	0 mm	0 mm	0 mm	0 mm
9.	S5	0 mm	0 mm	0 mm	0 mm
10.	S7	0 mm	0 mm	0 mm	0 mm

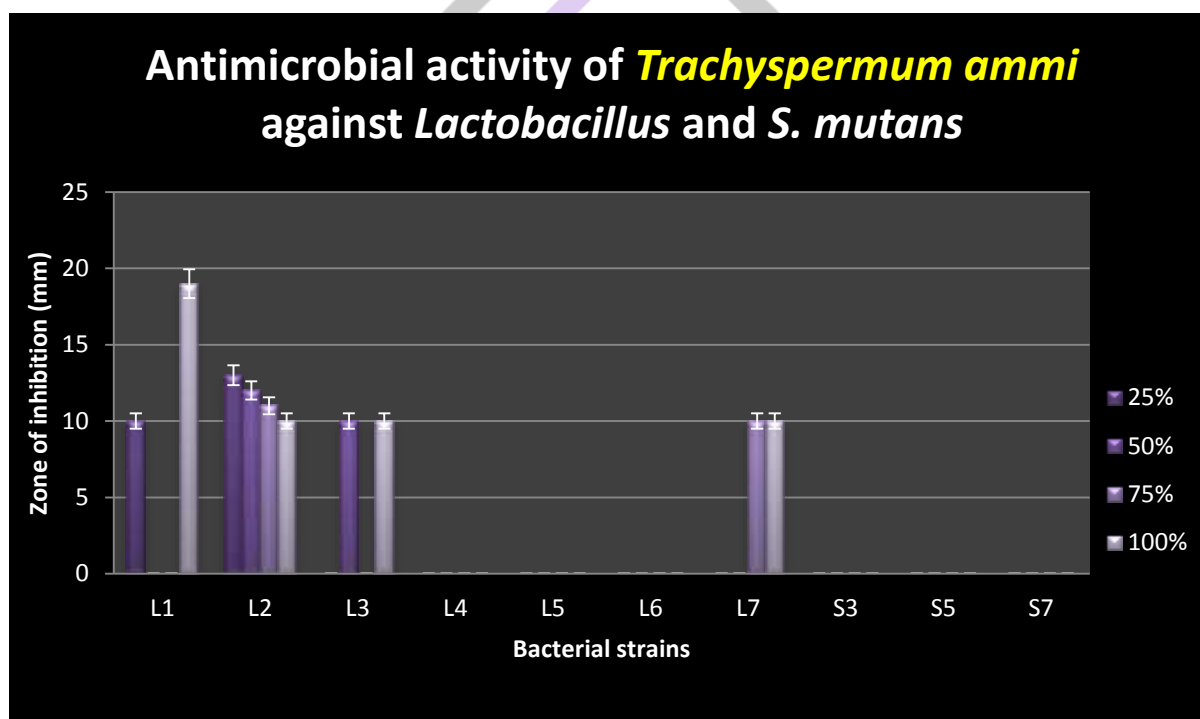
Fig No. 10: Antimicrobial activity of *Trachyspermum ammi*

Table no. 14: Antimicrobial activity of *Trigonella foenum graecum*

S.No.	Sample ID	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1.	L1	13 mm	14 mm	14 mm	10 mm
2.	L2	17 mm	15 mm	16 mm	16 mm
3.	L3	11 mm	14 mm	13 mm	0 mm
4.	L4	0 mm	0 mm	0 mm	0 mm
5.	L5	13 mm	14 mm	0 mm	17 mm
6.	L6	0 mm	0 mm	0 mm	0 mm
7.	L7	11 mm	0 mm	13 mm	13 mm
8.	S3	0 mm	0 mm	0 mm	0 mm
9.	S5	0 mm	0 mm	0 mm	0 mm
10.	S7	0 mm	0 mm	0 mm	0 mm

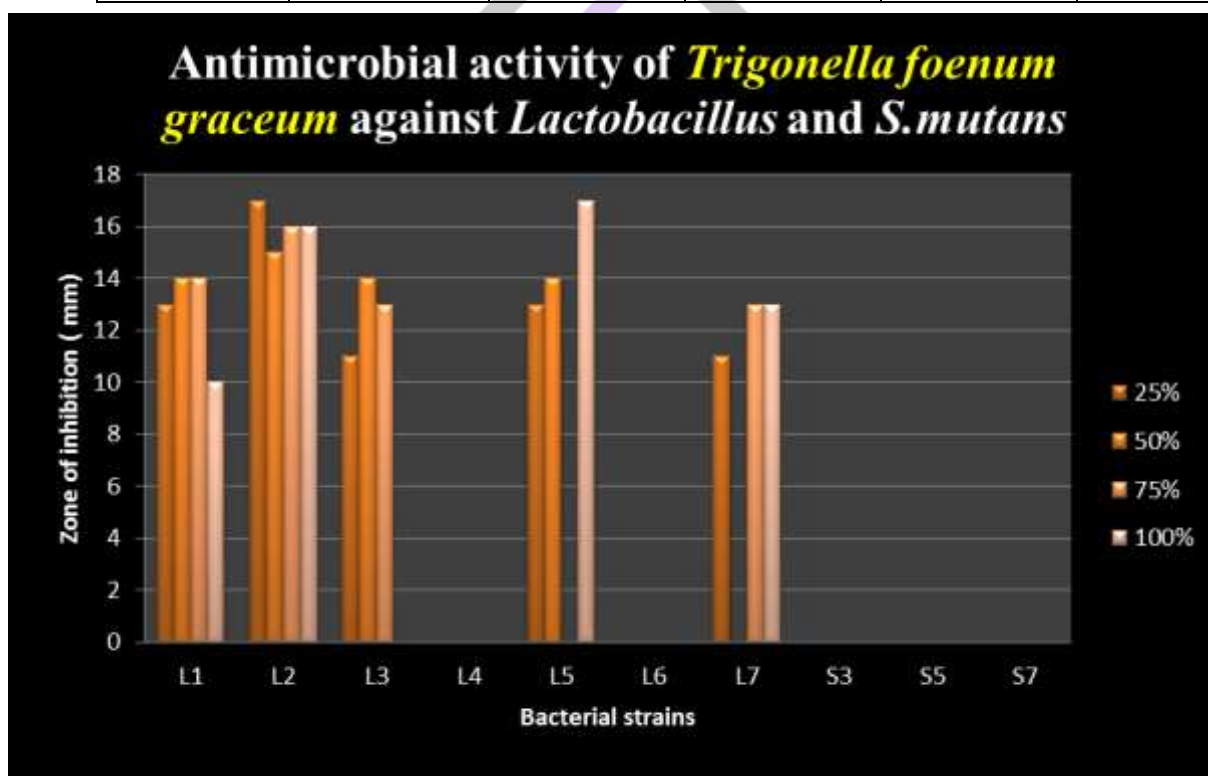


Fig No. 11: Antimicrobial activity of *Trigonella foenum graecum*

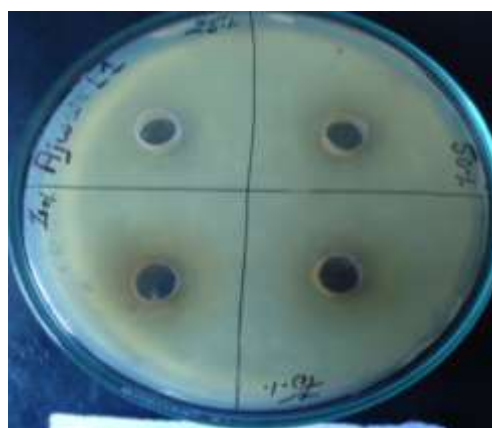
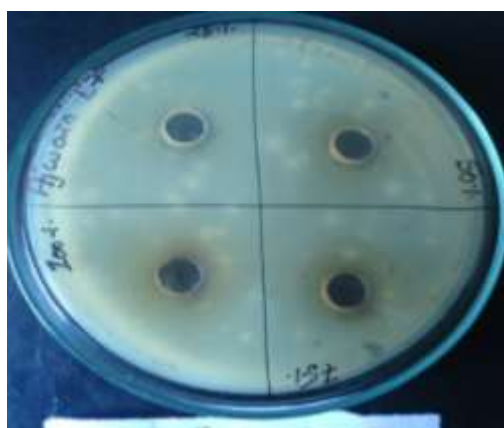
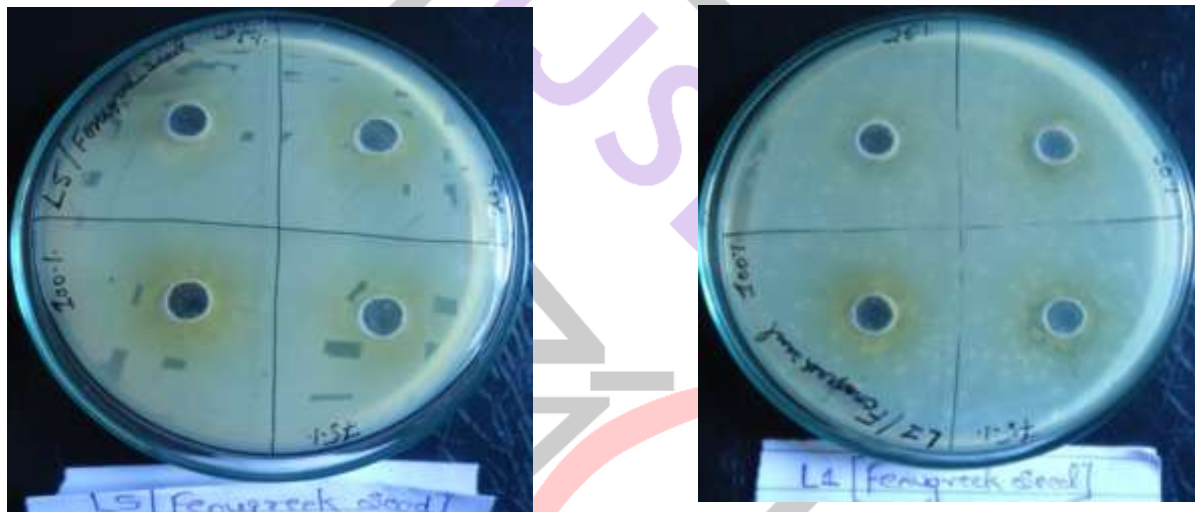
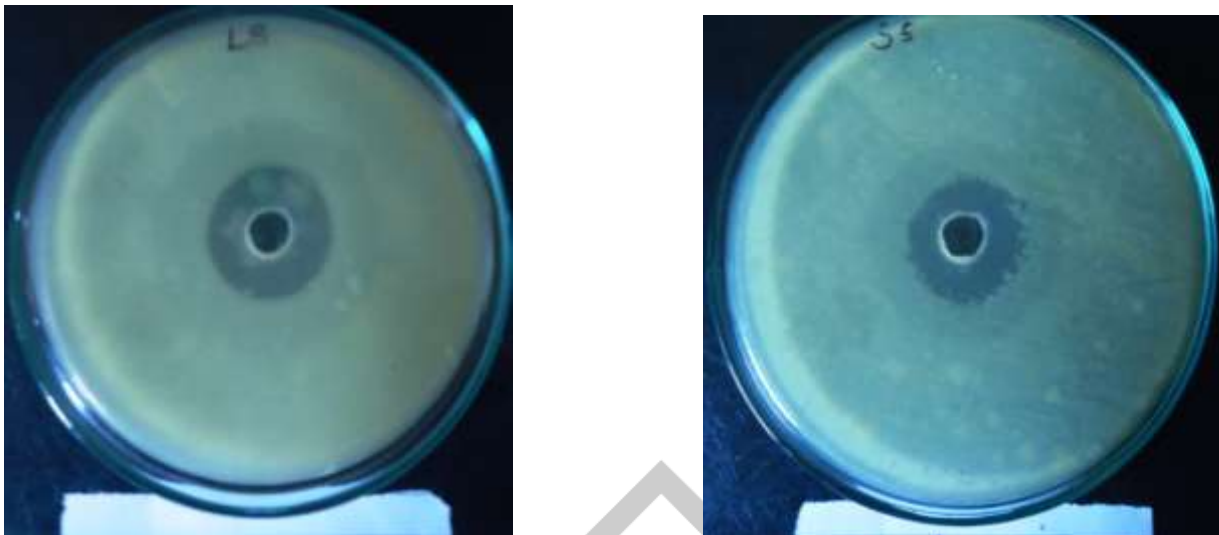


Fig No.12 : Results of Antimicrobial activity of *Trachyspermum ammi* against isolated oral microbes.Fig No. 13: Results of Antibacterial activity of *Trigonella foenum graecum* against isolated oral microbes.

#### IV. DISCUSSION

Table No. 1: Represents ethanobotanical and phytochemical data of two spices with medicinal properties. Table No.2: showing % yield of plant extract of *Trachyspermum ammi* and *Trigonella foenum graecum* obtained from soxhlet extraction. Table No.3: Phytochemical analysis of *Trachyspermum ammi* and *Trigonella foenum graecum* showing presence of bioactive compounds. Fig No.3: Qualitative analysis of *Trachyspermum ammi* and *Trigonella foenum graecum* by TLC. Fig No.2: showing standard curve of Tannic acid. Table No. 4/ Fig No. 3:shows that high phenolic content is present in *Trigonella foenum graecum*. Fig No.4: showing standard curve of Quercetin. Table No. 5/ Fig No.5; shows high flavonoid content in *Trigonella foenum graecum*. Table No. 6/ Fig No. 6: Total antioxidant activity. Table No.7/Fig No. 7: shows nitrous oxide scavenging activity of *Trachyspermum ammi* and *Trigonella foenum graecum*. Table No.8: shows alkaloid content. Fig No.8:shows standard curve of Colchicine. Table No. 9: represents alkaloid content of *Trachyspermum ammi* whereas it is found to be negative for *Trigonella foenum graecum* in phytochemical analysis. Table No.10/ Fig No. 9: shows results of Gram's Staining of isolated oral microbes (Bacterial ID L represents *Lactobacillus acidophilus* and S represents *Streptococcus mutans*). Table No.11: Results of biochemical identification of isolated microbial species. Table No. 12: Bacterial identification using PIBWIN software (strains L2 and L7 is *Lactobacillus acidophilus* and S5 *Streptococcus mutans*). Table No. 13/Fig No.10: shows Antibacterial activity of *Trachyspermum ammi* at different concentrations (25%,50%, 75%,100%). Table No.14/Fig No. 11: shows Antibacterial activity of *Trigonella foenum graecum* at concentration (25%, 50%, 75%, 100%). Fig No. 12: shows results of antibacterial activity of *Trachyspermum ammi* against oral microbes. Fig No.13; shows results of antibacterial activity of *Trigonella foenum graecum*. *Trigonella foenum graecum* shows maximum inhibition against oral microbes and zone of inhibition increases with increase in concentration. The antibacterial activity was found to be maximum due to presence of flavonoid and phenolic content.

## V. CONCLUSION

Dental caries is the most infectious microbial disease in the world which is nowadays affecting people of all age group. It is one of the most costly disease and needed to be treated as soon as possible. The bacteria and fungi that in habitat in oral microbes are Lactobacillus, S.mutans, S. sorbinus, Candida etc which causes tooth decay, cavities day by day with the increase in daily life style of the peoples and thus effect the human health.

As the treatment with the allopathic medicine is also getting increased with lots of side effects. There has been a shift from allopathic medicine to Ayurveda with no or minimum side effects. Two such plants to be used against dental caries causing microbes were Trachyspermum ammi (Ajwain) and Trigonella foenum graceum (Methi). Isolation of active compounds from the Ajwain and Methi seeds and their role in inhibition of dental caries causing microbes were studied.

With all the wide spectrum of Antimicrobial properties, Fenugreek i.e. Methi extract can be considered effective Antimicrobial agent against Lactobacillus as compared to Ajwain extract whereas no such effect was observed against S.mutans. The study support scientifically the pharmacological uses of the herbal plants as an antimicrobial and antifungal agent and could account for some of the variation observed in pharmaceutical preparation methods. Therefore, the use of these plants as antimicrobial agent is validated by the result obtained in the work. Further studies are ongoing to identify the chemical compound of these antimicrobial extract. It was found that the oral microbes Lactobacillus and S.mutans were not showing resistance against penicillin whereas it was found to be resistance against Amoxicillin. Though, the results were found to be positive there is a need to shift from allopathic medicines to Ayurveda due to increasing side effects and cost factors.

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