

# A Comparative Evaluation of Herbal Efficacy Against *Candida Albicans* And *Streptococcus Mutans*

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**Abstract:** Dental caries or tooth decay is one of the most common chronic diseases in the world. *Streptococcus mutans* and *Candida albicans* species are the major etiological agent of caries. During the work we had extracted the plants extract in the presence solvents by the help of Soxhlet extraction method. By this we had obtained nearly 5 % of extract. From these extract we had moved for the isolation of pathogens from the samples collected from the patients, into which we had observed the pathogens similar to the *Streptococcus mutans* and *Candida albicans*. *Fenugreek* shows 3.6, *Curcuma Longa* shows good ZOI of 7.5, *Zingiber Officinale* shows 8.5 against chloroform, *Allium Sativum* shows ZOI of 6.5 against ethanol. When we perform their MIC it show that the best MIC were shown by *Syzygium aromaticum*, *Allium Cepa* and *Curcuma Longa* in the form of O.D. is 1.61, 1.55 and 1.33 respectively. Other extractant are also showing the better MIC as of *Zingiber Officinale*, *Fenugreek* and *Allium Sativum* shows 1.90, 2.10 and 2.22 respectively.

**Key words:** dental caries, chronic, solvents, patients.

## I. INTRODUCTION

Periodontal disease is one of the world's most prevalent chronic diseases, which has been considered as a possible risk factor in some systemic diseases; periodontal diseases seriously threaten people's quality of life. Periodontitis, a destructive gum disease, may progress irreversibly in breaking down supporting periodontal structures; results in loss of tooth and about 20% population of the world are affected by these diseases. An etiology of chronic periodontal disease remains unknown, although gram-negative anaerobic bacteria have been implicated in the disease<sup>2</sup>. It is a subgingival condition that has been linked and afforded a varied environment for the colonization of gram negative facultative or obligate anaerobes like *Porphyromonas gingivalis*, *Bacteroides* species, *Capnocytophaga* species, *Actinobacillus actinomycetemcomitans* and anaerobic gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus* species, *Prevotella* species and *Fusobacterium* species.<sup>1</sup> Fungal organisms are commonly seen to colonize the tongue, palate and buccal mucosa. If one turns around and check the prevalence of the occurrence of the number of fungal infections caused by *Candida* and related species, then we will find that there is dramatic and exponential increase in over the past several decades. *C.albicans* may play a vital role in the infrastructure of periodontal microbiota as well as on adherence of periodontal tissues *Candida* species have evolved as the most important opportunistic pathogens in immunocompromised hosts and may play important role in life threatening infections<sup>(3-7)</sup>.

## II. METHODOLOGY

### Collection Of Plant Material

All the samples were collected from dental clinic and hospitals near to REWA (M.P.) with proper media facilities.

S. No	Plants Name (Botanical Name )	Local name	Family	Parts used
1.	<i>Syzygium aromaticum</i>	Laung	Myrtaceae	Bud
2.	<i>Fenugreek</i>	Meethi	Fabaceae	Seeds
3.	<i>Curcuma Longa</i>	Haldi	Zingiberaceae	Rhizome
4.	<i>Allium Cepa</i>	Onion	Amaryllidaceae	Bulbs
5.	<i>Zingiber Officinale</i>	Ginger	Zingiberaceae	Rhizome
6.	<i>Allium Sativum</i>	Garlic	Amaryllidaceae	Rhizome

### Preparation Of Solvent Extractions

This method is convenient and widely used for extraction because of its continuous process, less time and solvent consumption compared to maceration and percolation. In this method, plant material is dried and powdered. The powdered plant sample is placed in Soxhlet apparatus which is on the top, a collecting flask beneath a reflux condenser. A suitable solvent such as methanol, ethanol, ethyl acetate and chloroform respectively is added to flask and the set up is heated under reflux. The steam of solvent dissolves the ingredients of plant and brings back to a flask. Several cycles are carried out for the collection of extracts. The solvent is evaporated by using rotary evaporator or at room temperature. Dried extract is then kept in freeze for further study<sup>(8-12)</sup>. Fill separately 25 g of shade dried powder of plant materials in the thimble and extract successively with 250 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 3-4 hours at maintaining the temperature of apparatus at 100°C. Concentrate all the extracts using rotary evaporator. After complete solvent evaporation, weigh each of these

solvent extract and preserve at 4°C in airtight bottles until further use. Dissolve 1 g of each solvent residue in 10 ml of respective solvents and use as the test extracts for antimicrobial activity test.

### Isolation Of The Dental Caries Samples

Sample was collected from mouth by swabbing across the gingival and sub gingival region as well as from the roof and floor of the buccal cavity. The samples were collected from several sites and were inoculated in Nutrient broth, Blood Agar and Sauboured Agar (HiMedia, India) and viable cells were enumerated. Severally colonies with visually distinguishable morphologies were randomly selected and isolated by directly streaking on Nutrient agar plates and incubated for another 24 hours. The isolated colonies were then re-streaked after incubation onto nutrient agar plates to obtain pure cultures. The viability of the isolated cultures was checked in nutrient agar and blood agar (HiMedia) broth and those found to be viable were screened for biofilm formation. For the conformation of *S. mutans* we had used blood agar medium and culture on it. For the growth of *Candida albicans* specifically we had prepared SDA medium<sup>(12-14)</sup>.

### Screening For Antibacterial Activity

Antibacterial activity of all plants extracts will be tested by Agar Well Diffusion method. The culture plates will be prepared by pouring 30ml of Mueller-Hinton agar medium into sterile petri plates. Then swab test bacteria then spread over the agar media using sterile cotton swabs to get uniform distribution of the bacterial cultures. Make 6 mm diameter wells using sterile cork borer. Then fill the wells with the sample extracts. The diameter of the zone of inhibition around each well will be taken as a measure of antibacterial activity.

### Screening For Antifungal Activity

To evaluate the antifungal activity, sterile agar plates will be used according to disc diffusion assay. Impregnate the Sterile filter paper discs with leaf and rhizome extracts. Then place the discs in fungal seeded plates and incubate at 30°C for 48-72 hours. For the fungal sensitivity test we had used 5 mm sterile filter paper discs were purchased and sterilized. These were placed and inoculated on dried SDA plates. 30µl of the extraction was placed on the disc. These plates were incubated at 30°C. Zone of inhibition was noted around the disc at 48-72 hrs. All the results were obtained between 48-72 hours of incubation. This agent was dissolved in 95% of solvent. For testing of fungal activity, all the solvents are taken in powder form and eventually dissolved in the 95% of solvents. 4 gm of solvent powder is dissolved and kept for 4 hours in cold condition (4-8°C) undisturbed. After they were taken to perform the FST test and observation were taken after 48 hours of inoculation<sup>(15-16)</sup>

## III. RESULTS

### Enumeration Of Viable Cell Count

Total viable count was determined from selected plates having 30 to 500 colonies (Table 3.1. 1).

**THB = No. of colonies × Dilution factor / Inoculum size CFU/ml**

**Table: 3.1.1. VIABLE CELL COUNT**

S. No.	Number of bacterial Colonies	Dilution factor	THB(CFU/ml)
Control	Above 500	$10^{-0}$	$10.00 \times 10^5$
1	465	$10^{-1}$	$4.65 \times 10^5$
2	445	$10^{-2}$	$4.45 \times 10^5$
3	445	$10^{-3}$	$4.45 \times 10^5$
4	442	$10^{-4}$	$4.42 \times 10^5$
5	400	$10^{-5}$	$4.00 \times 10^5$
6	380	$10^{-6}$	$3.80 \times 10^5$
7	366	$10^{-7}$	$3.66 \times 10^5$
8	362	$10^{-8}$	$3.62 \times 10^5$
9	288	$10^{-9}$	$2.88 \times 10^5$
10	150	$10^{-10}$	$1.50 \times 10^5$

10 bacterial strains with observable difference in colony morphology were randomly selected from initial spread plate and re-streaked



Fig.A



Fig.B



Fig.C



Fig.D

Fig.E

**FIG.3. 1:** Fig. A and B: Showing the mixed culture of collected samples.  
Fig. : Showing the *Candida albicans* culture in SDA medium  
Fig. D and E showing the *S.Mutans* culture in blood Agar

### Biochemical Identification Of Microbes

Biochemical identification of the selected strains was performed by biochemical characterization

Table no: 3.2 showing biochemical identification of isolated species from oral culture.

<b>Biochemical tests:</b>														
Sample No.	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5	B6	B7	B8
Grams Staining	+	-	+	-	+	+	+	+	-	+	+	+	+	+
Catalase Activity	-	+	-	+	+	-	-	+	+	+	-	-	+	-
Oxidase Test	+	-	+	+	+	+	+	+	-	+	+	+	-	+
MR-VP Test	+	-	+	-	+	+	+	+	-	+	+	+	+	+
Citrate-Utilization Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole Test	+	-	+	-	+	+	+	+	-	+	+	+	+	+
Motility Test	-	+	-	-	-	-	+	+	+	+	-	-	-	+
(M.S.)medium	+	-	+	-	-	+	+	-	+	-	+	+	+	+

Where: + shows positive and -ve shows negative results in test

### Screening For Antibacterial Activity

S.NO.	Solvents used	CONC. (µl)	<i>Syzygium aromaticum</i> extract	(ZOI)
1	DMSO(control)	100	50	00.00
2	METHANOL	100	50	1.2
3	ETHANOL	100	50	0.2
4	CHLOROFORM	100	50	0.2
5	ETHYL ACETATE	100	50	0.4

Table 3.1. showing activity of *Syzygium aromaticum* against selected solvents

s.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI
1	<i>Syzygium aromaticum</i>	DW(control)	<i>Streptococcus Mutans sp.</i>	0.00
2	<i>Syzygium aromaticum</i>	200	<i>Streptococcus Mutans sp.</i>	0.6
3	<i>Syzygium aromaticum</i>	150	<i>Streptococcus Mutans sp.</i>	0.2
4	<i>Syzygium aromaticum</i>	100	<i>Streptococcus Mutans sp.</i>	0.5

Table 3.2. showing activity of extract of *Syzygium aromaticum* against *Streptococcus Mutans sp.*

S.NO.	Solvents used	CONC. (µl)	Fenugreek	(ZOI)
1	DMSO	100(control)	50	0.00
2	METHANOL	100	50	1.3
3	ETHANOL	100	50	0.6
4	CHLOROFORM	100	50	0.9
5	ETHYL ACETATE	100	50	0.8

Table 3.3. showing activity of solvents used against extract of Fenugreek

S.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI
1	Fenugreek	DW(control)	<i>Streptococcus Mutans sp</i>	0.00
2	Fenugreek	200	<i>Streptococcus Mutans sp</i>	1.50
3	Fenugreek	150	<i>Streptococcus Mutans sp</i>	0.70
4	Fenugreek	100	<i>Streptococcus Mutans sp</i>	0.80

Table 3.4 showing activity of extract of Fenugreek against *Streptococcus Mutans sp.*

S.NO.	Solvents used	CONC. (µl)	Curcuma Longa extract	(ZOI in cm)
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1	DMSO	100(control)	50	0.00
2	METHANOL	100	50	1.2
3	ETHANOL	100	50	0.5
4	CHLOROFORM	100	50	0.3
5	ETHYL ACETATE	100	50	1.2

Table 3.5. showing activity of solvents used against extract of *Curcuma Longa*

S.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI(cm)
1	<i>Curcuma Longa</i>	DW(control)	<i>Streptococcus Mutans sp.</i>	0.00
2	<i>Curcuma Longa</i>	200	<i>Streptococcus Mutans sp.</i>	1.3
3	<i>Curcuma Longa</i>	150	<i>Streptococcus Mutans sp.</i>	0.7
4	<i>Curcuma Longa</i>	100	<i>Streptococcus Mutans sp.</i>	0.2

Table 3.6. showing activity of extract of *Curcuma Longa* against *Streptococcus Mutans sp.*

S.NO.	Solvents used	CONC. (µl)	<i>Allium Cepa</i> extract	(ZOI)
1	DMSO	100(control)	50	0.00
2	METHANOL	100	50	0.9
3	ETHANOL	100	50	0.1
4	CHLOROFORM	100	50	0.1
5	ETHYL ACETATE	100	50	0.2

Table 3.7. showing activity of solvents used against extract of *Allium Cepa* and *Streptococcus Mutans*

s.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI
1	<i>Allium Cepa</i>	DW(control)	<i>Streptococcus Mutans sp.</i>	0.00
2	<i>Allium Cepa</i>	200	<i>Streptococcus Mutans sp.</i>	0.8
3	<i>Allium Cepa</i>	150	<i>Streptococcus Mutans sp.</i>	1.1
4	<i>Allium Cepa</i>	100	<i>Streptococcus Mutans sp.</i>	0.8

Table 3.8 showing activity of extract of *Allium Cepa* against *Streptococcus Mutans sp.*

S.NO.	Solvents used	CONC. (µl)	<i>Zingiber Officinale</i> extract	(ZOI)
1	DMSO	100(control)	50	0.0
2	METHANOL	100	50	1.0
3	ETHANOL	100	50	0.4
4	CHLOROFORM	100	50	0.6
5	ETHYL ACETATE	100	50	1.4

Table 3.9 showing activity of solvents used against extract of *Zingiber Officinale* and *Streptococcus Mutans*

s.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI
1	<i>Zingiber Officinale</i>	DW(control)	<i>Streptococcus Mutans sp.</i>	0.00
2	<i>Zingiber Officinale</i>	200	<i>Streptococcus Mutans sp.</i>	0.00
3	<i>Zingiber Officinale</i>	150	<i>Streptococcus Mutans sp.</i>	0.6
4	<i>Zingiber Officinale</i>	100	<i>Streptococcus Mutans sp.</i>	2.3

Table 3.10 showing activity of extract of *Zingiber Officinale* against *Streptococcus Mutans sp.*

S.NO.	Solvents used	CONC. (µl)	Amount of Extract	(ZOI)
1	DMSO	100(control)	50	0.0
2	METHANOL	100	50	1.1
3	ETHANOL	100	50	0.5
4	CHLOROFORM	100	50	0.3
5	ETHYL ACETATE	100	50	1.2

Table 3.11. Showing activity of solvents used against extract of *Allium Sativum* and *Streptococcus Mutans*

s.no.	Plant name	Amount of extract used (µl)	Bacterial species	ZOI
1	<i>Allium Sativum</i>	DW(control)	<i>Streptococcus Mutans sp.</i>	0.00
2	<i>Allium Sativum</i>	200	<i>Streptococcus Mutans sp.</i>	3.5
3	<i>Allium Sativum</i>	150	<i>Streptococcus Mutans sp.</i>	3.2
4	<i>Allium Sativum</i>	100	<i>Streptococcus Mutans sp.</i>	2.1

Table 3.12. Showing activity of extract of *Allium Sativum* against *Streptococcus Mutans sp.***Screening For Antifungal Sensitivity Activity (FST)**

The tests were performed by adding 4 gm of powder of plant material and addition of 95% of solvents *Candida albicans*.

Table 3.5.1 showing the Fungal Sensitivity Test against *Candida albicans* against *Syzygium aromaticum*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0.	<i>Syzygium aromaticum</i> (4gm)	DW(control)	<i>Candida albicans</i>	0.0
1	<i>Syzygium aromaticum</i> (4gm)	Methanol	<i>Candida albicans</i>	5.6
2	<i>Syzygium aromaticum</i> (4gm)	Ethanol	<i>Candida albicans</i>	4.3
3	<i>Syzygium aromaticum</i> (4gm)	Chloroform	<i>Candida albicans</i>	2.1
4.	<i>Syzygium aromaticum</i> (4gm)	Ethyl Acetate	<i>Candida albicans</i>	3.6

Table 3.5.2 showing the Fungal Sensitivity Test against *Candida albicans* against *Fenugreek*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0.	<i>Fenugreek</i> (4gm)	DW(control)	<i>Candida albicans</i>	0.0
1	<i>Fenugreek</i> (4gm)	Methanol	<i>Candida albicans</i>	3.6
2	<i>Fenugreek</i> (4gm)	Ethanol	<i>Candida albicans</i>	2.3
3	<i>Fenugreek</i> (4gm)	Chloroform	<i>Candida albicans</i>	0.0
4	<i>Fenugreek</i> (4gm)	Ethyl Acetate	<i>Candida albicans</i>	2.4

Table 3.5.3 .showing the Fungal Sensitivity Test against *Candida albicans* against *Curcuma Longa*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0.	<i>Curcuma Longa</i> (4 gm)	DW(control)	<i>Candida albicans</i>	0.0
1	<i>Curcuma Longa</i> (4 gm)	Methanol	<i>Candida albicans</i>	4.9
2	<i>Curcuma Longa</i> (4 gm)	Ethanol	<i>Candida albicans</i>	3.2
3	<i>Curcuma Longa</i> (4 gm)	Chloroform	<i>Candida albicans</i>	1.6
4	<i>Curcuma Longa</i> (4 gm)	Ethyl Acetate	<i>Candida albicans</i>	7.5

Table 3.5.4.showing the Fungal Sensitivity Test against *Candida albicans* against *Allium Cepa*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0	<i>Allium Cepa</i> (4 gm)	DW(control)	<i>Candida albicans</i>	0.0
1	<i>Allium Cepa</i> (4 gm)	Methanol	<i>Candida albicans</i>	3.1
2	<i>Allium Cepa</i> (4 gm)	Ethanol	<i>Candida albicans</i>	2.1
3	<i>Allium Cepa</i> (4 gm)	Chloroform	<i>Candida albicans</i>	1.9
4	<i>Allium Cepa</i> (4 gm)	Ethyl Acetate	<i>Candida albicans</i>	6.0

Table 3.5.5. showing the Fungal Sensitivity Test against *Candida albicans* against *Zingiber Officinale*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0.	<i>Zingiber Officinale</i> (4 gm)	DW(control)	<i>Candida albicans</i>	0.0
1	<i>Zingiber Officinale</i> (4 gm)	Methanol	<i>Candida albicans</i>	7.7
2	<i>Zingiber Officinale</i> (4 gm)	Ethanol	<i>Candida albicans</i>	6.9
3	<i>Zingiber Officinale</i> (4 gm)	Chloroform	<i>Candida albicans</i>	8.5
4	<i>Zingiber Officinale</i> (4 gm)	Ethyl Acetate	<i>Candida albicans</i>	3.7

Table 3.5.6 showing the Fungal Sensitivity Test against *Candida albicans* against *Allium Sativum*

S.No.	Powder of plants (gm)	Solvent used (95 % )	Used species	ZOI (mm)
0.	<i>Allium Sativum</i> (4 gm)	DW (control)	<i>Candida albicans</i>	0.0
1	<i>Allium Sativum</i> (4 gm)	Methanol	<i>Candida albicans</i>	3.2
2	<i>Allium Sativum</i> (4 gm)	Ethanol	<i>Candida albicans</i>	4.5
3	<i>Allium Sativum</i> (4 gm)	Chloroform	<i>Candida albicans</i>	2.8
4	<i>Allium Sativum</i> (4 gm)	Ethyl Acetate	<i>Candida albicans</i>	5.0

**Minimum Inhibitory Concentration (MIC)**

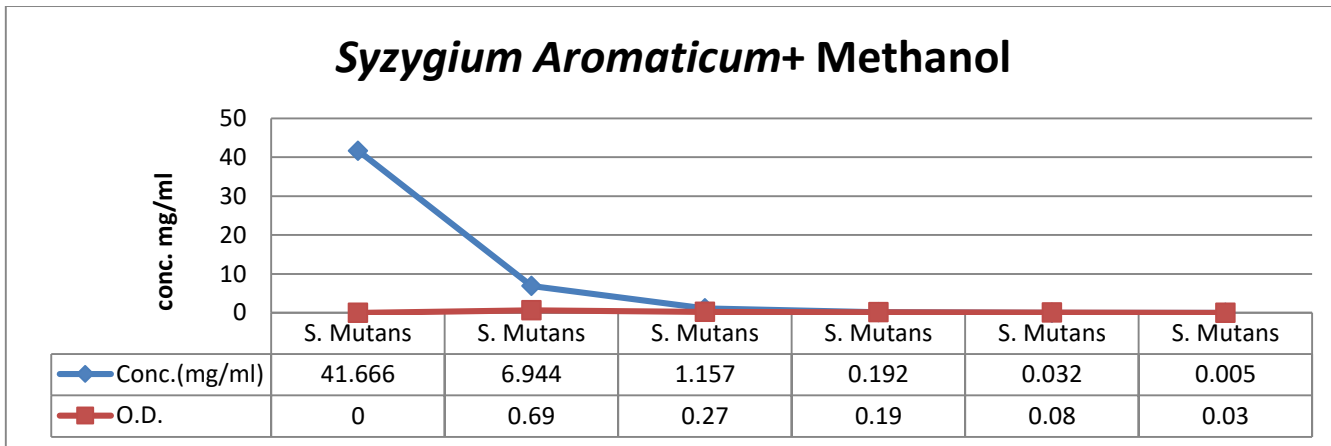


Chart no.1 showing the MIC results of *Syzygium aromaticum* in the presence of methanol against *Streptococcus Mutans*

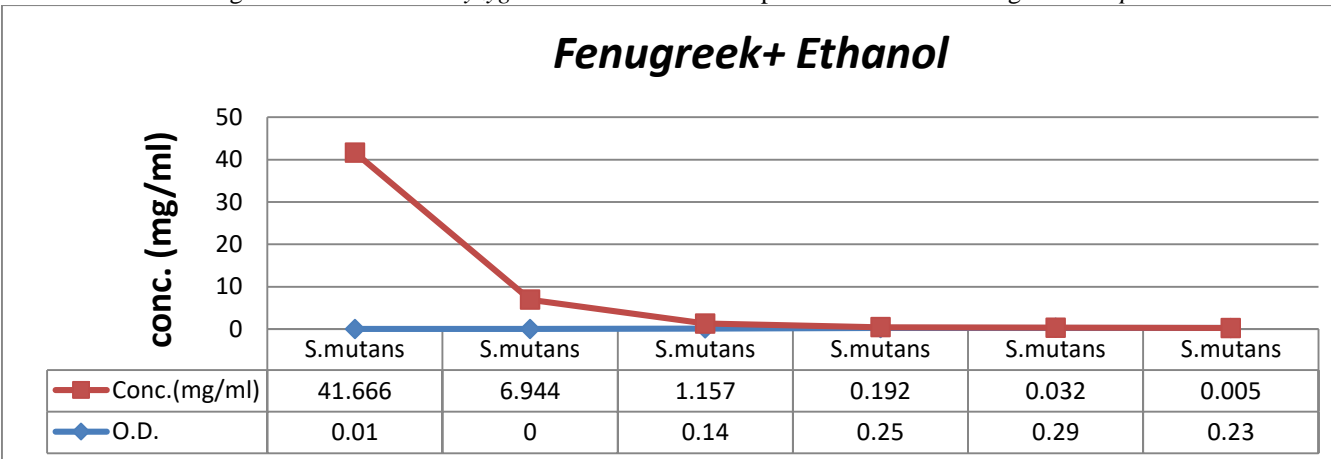


Chart no.2 showing MIC results of *Fenugreek* against *Streptococcus Mutans*

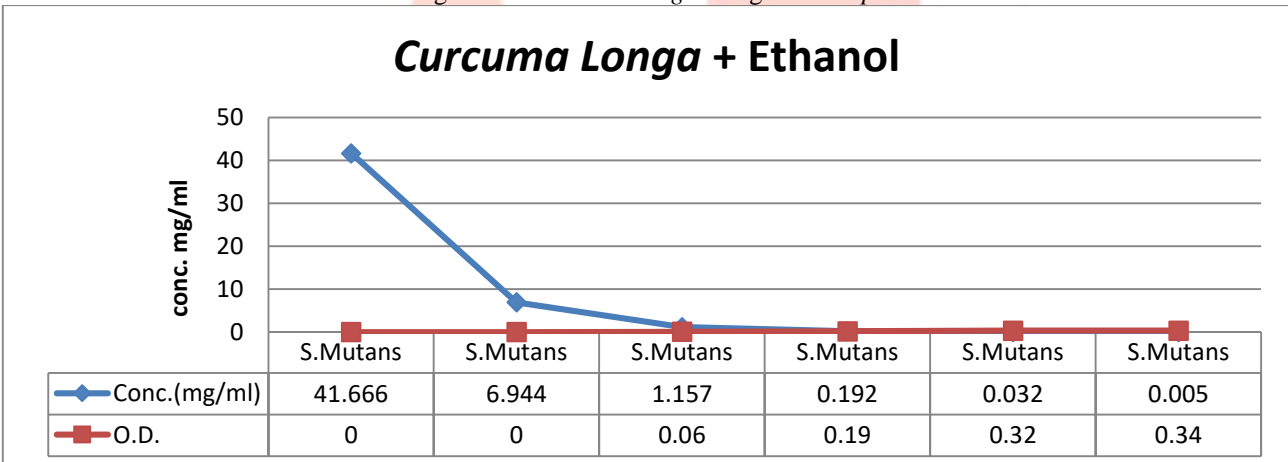


Chart no..3 showing the MIC results of *Curcuma Long* against *Streptococcus Mutans*

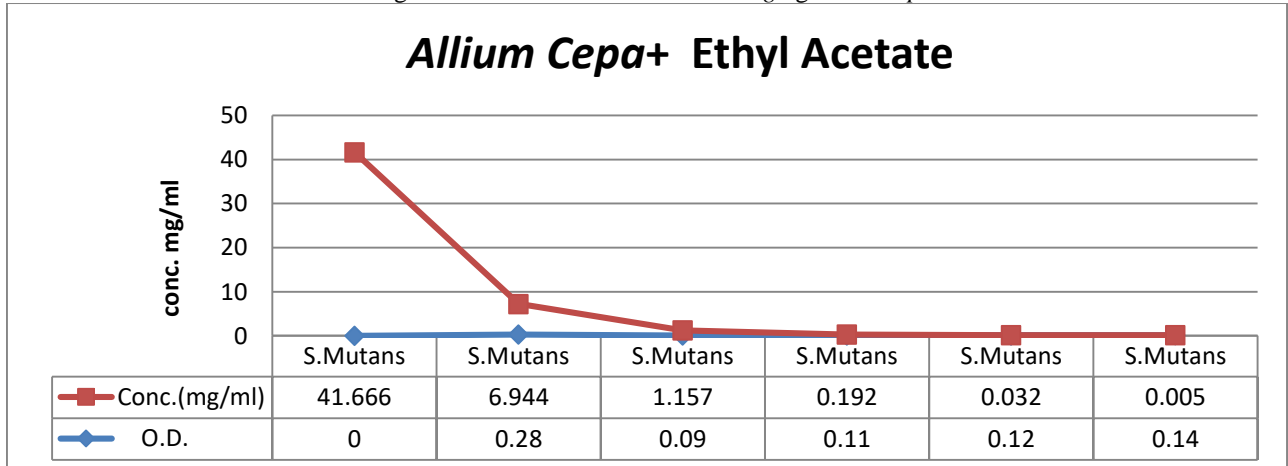


Chart no.4 showing the MIC results of *Alleum Ceba.* against *Streptococcus Mutans*

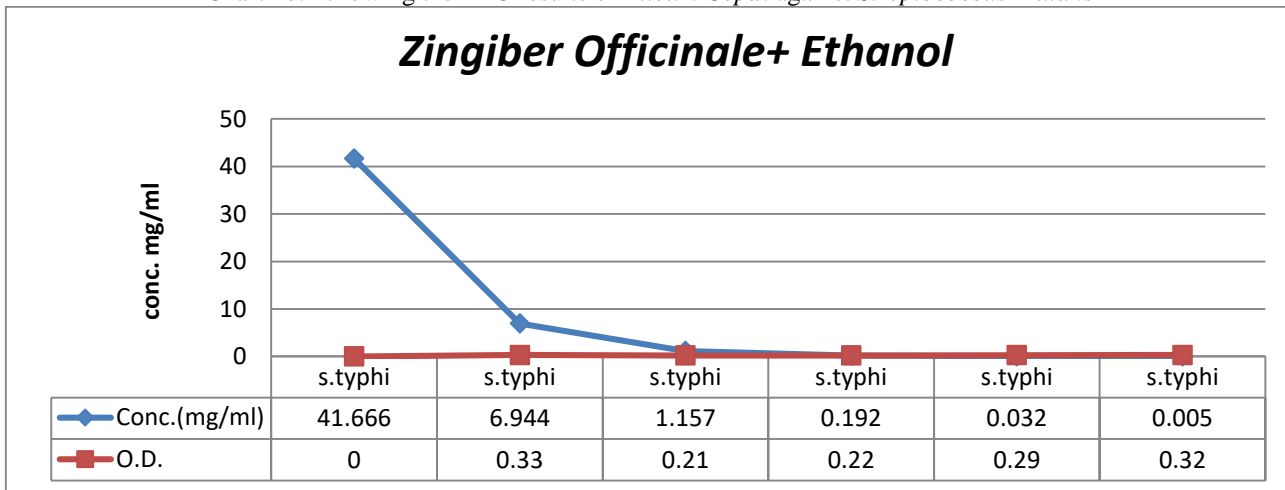


Chart no.5 showing the MIC results of *Zingiber Officinale* against *Streptococcus Mutans*

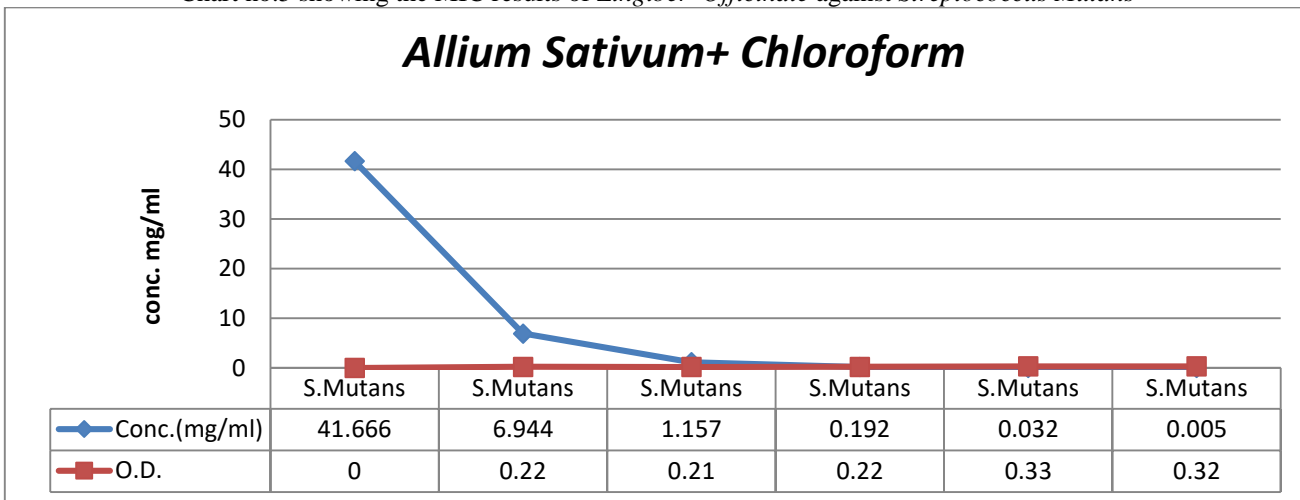


Chart no. 6 showing the MIC results of *Allium Sativum* against *Streptococcus Mutans*

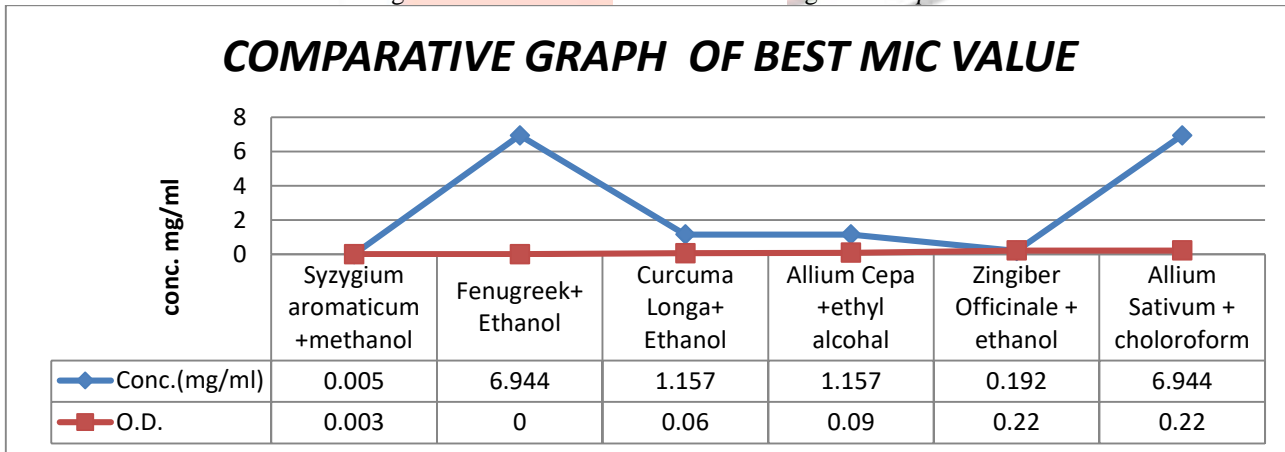
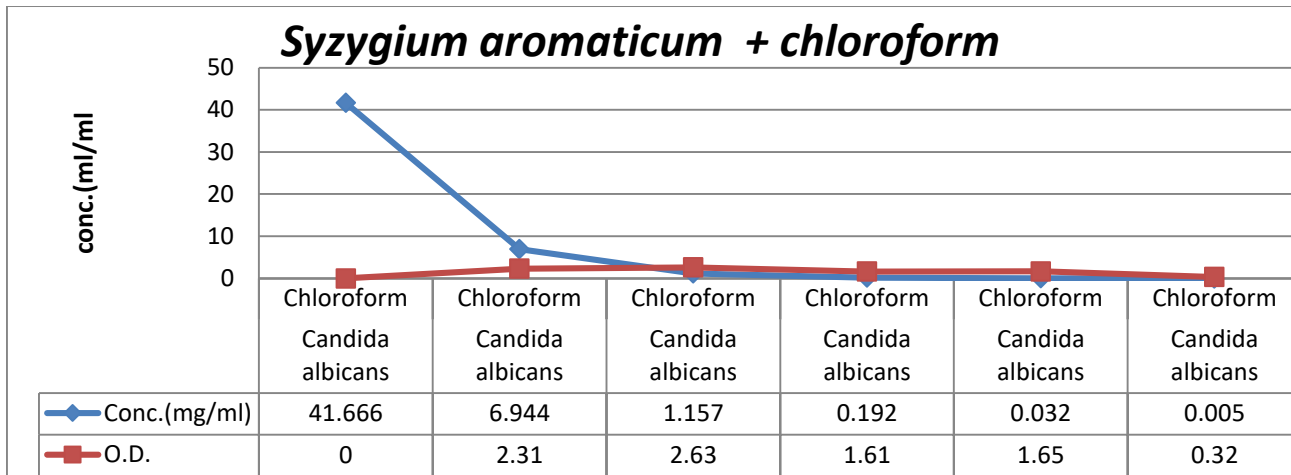
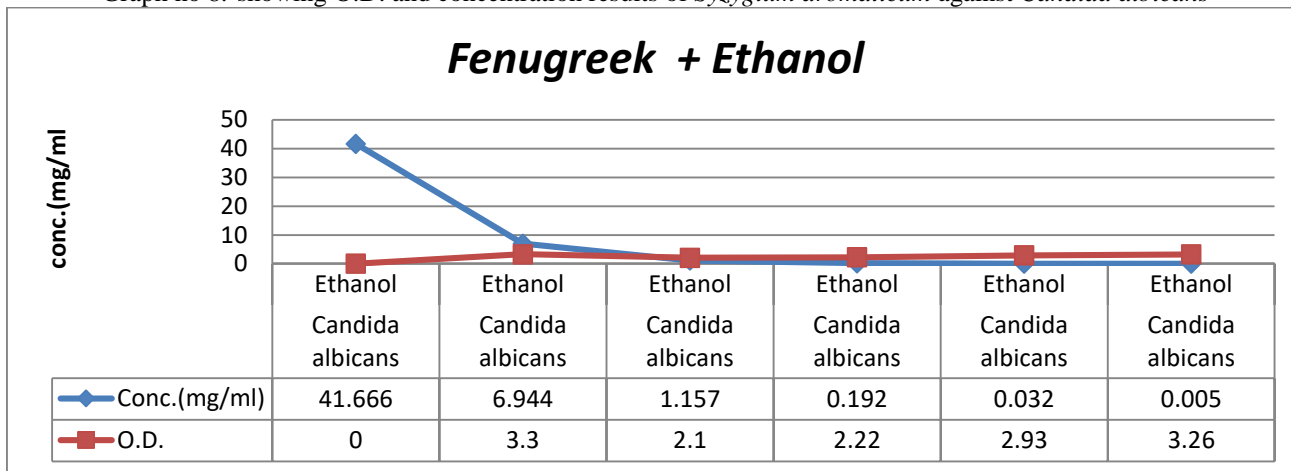


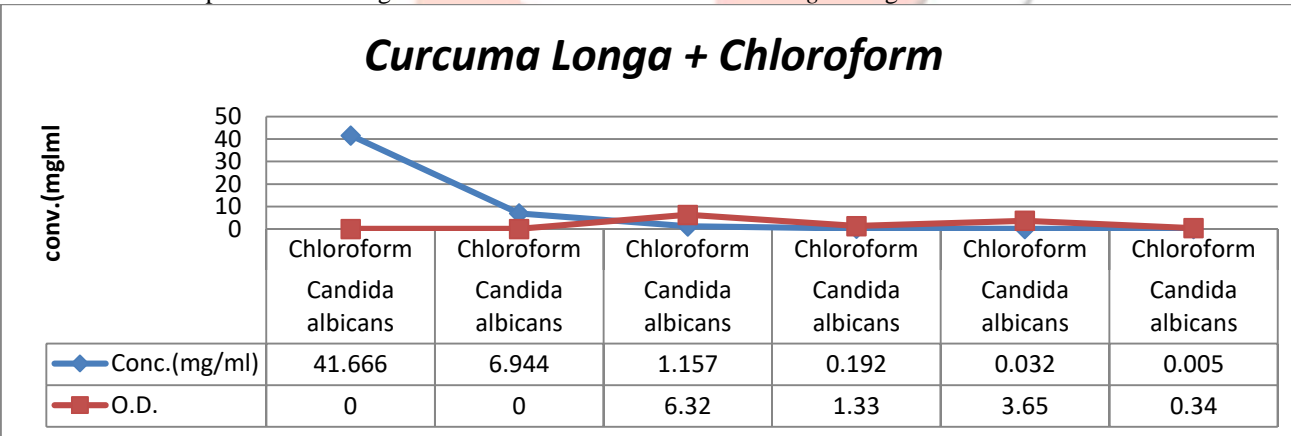
Chart no.7 showing the comparative MIC results against *Streptococcus Mutans*



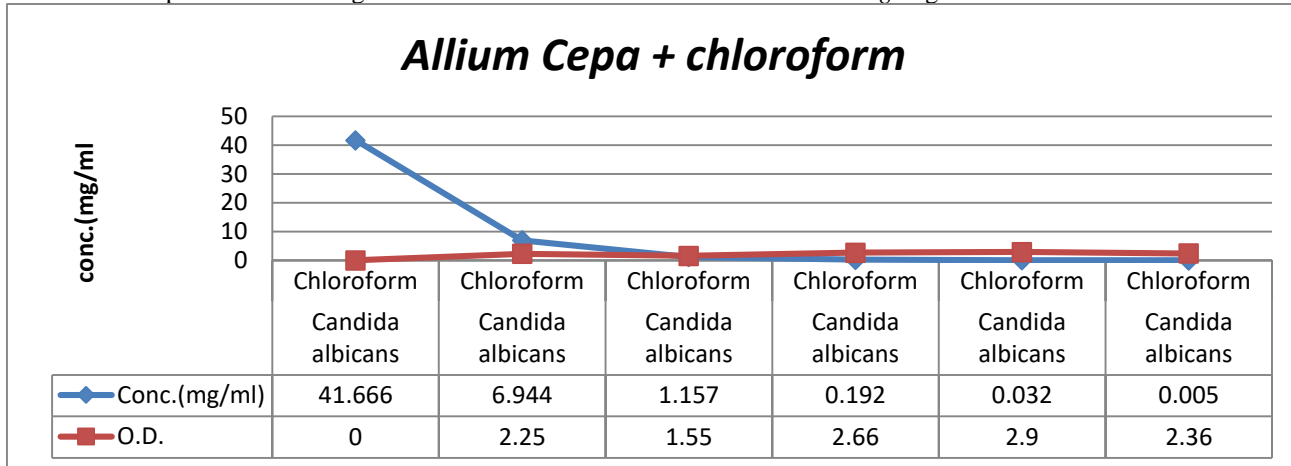
Graph no 8: showing O.D. and concentration results of *Syzygium aromaticum* against *Candida albicans*



Graph no 9: showing O.D. and concentration results of *Fenugreek* against *Candida albicans*

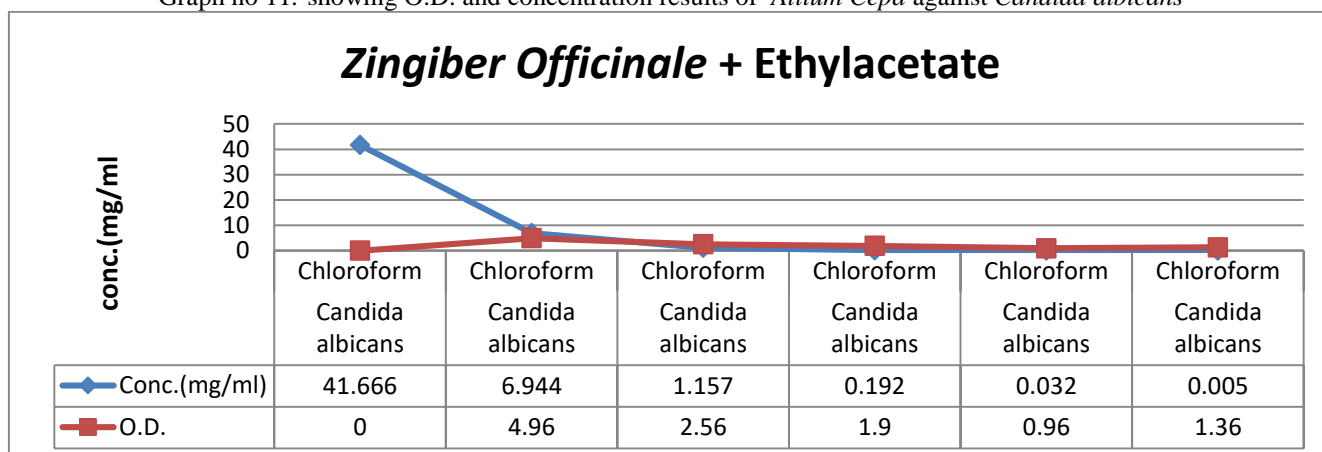


Graph no 10: showing O.D. and concentration results of *Curcuma Longa* against *Candida albicans*

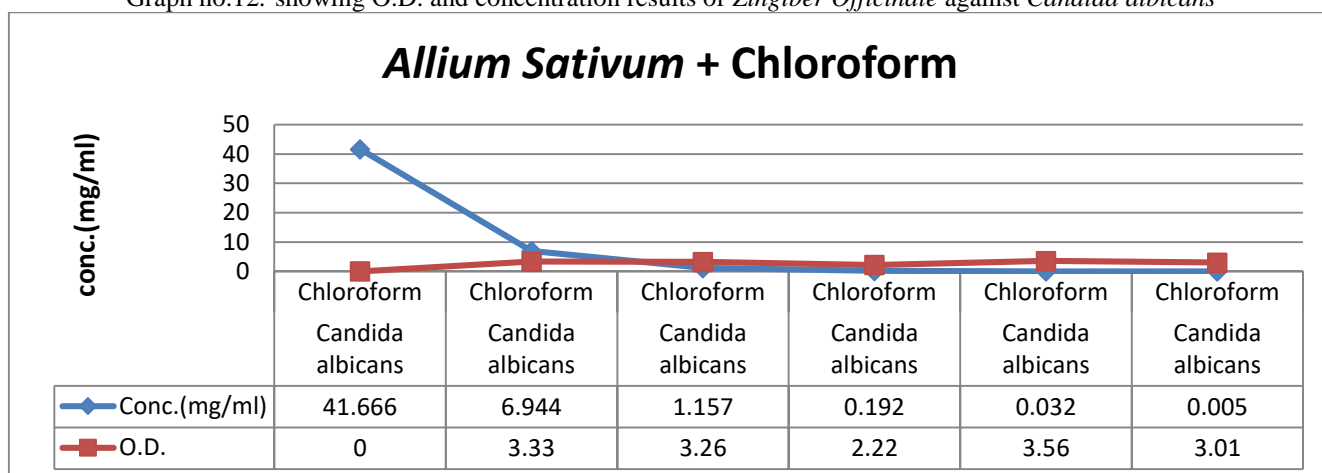




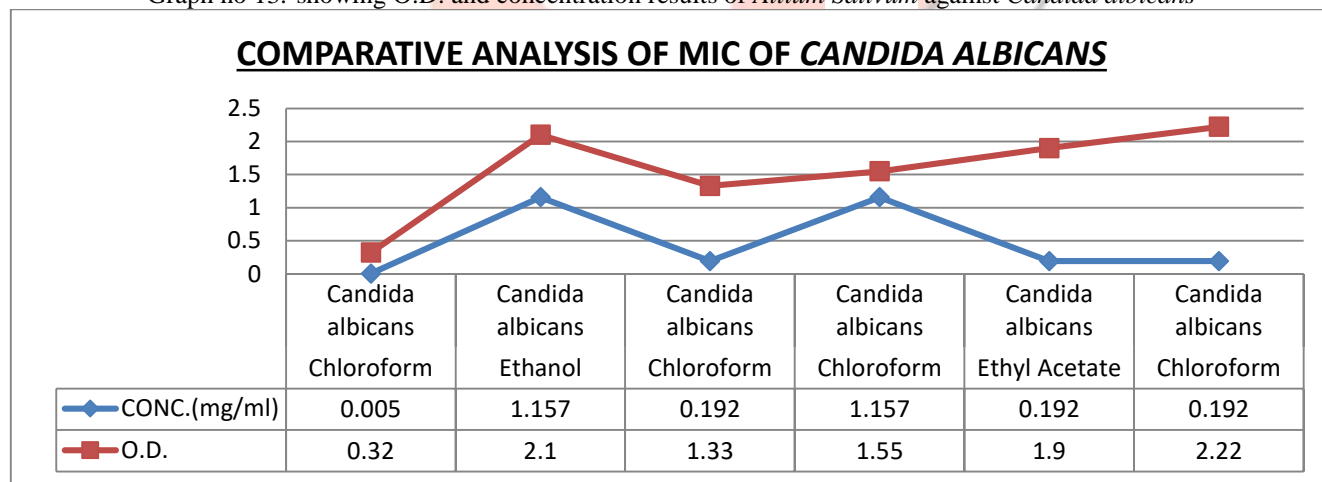
Graph no 11: showing O.D. and concentration results of *Allium Cepa* against *Candida albicans*



Graph no.12: showing O.D. and concentration results of *Zingiber Officinale* against *Candida albicans*



Graph no 13: showing O.D. and concentration results of *Allium Sativum* against *Candida albicans*



Graph no 14: showing O.D. and concentration results against *Candida albicans* of all the selected FST results

#### IV. CONCLUSION

During the complete study of activity of our selected plants against the dental pathogens and fungal species we had observed that, when these microbes were used against different solvents then we had found that *Syzygium aromaticum* showing better result against methanol and against *Streptococcus Mutans sp* it is showing the zone of 0.6 mm. Now using the *Fenugreek* and it shows the best result results against methanol also and it shows best results against *Streptococcus Mutans sp* at the concentration of 200 ul. of zone 1.5mm. After this *Curcuma Longa* were used and it shows good results against methanol and ethyl acetate and when it used against pathogen *Streptococcus Mutans sp*. then it show best results at 200ul. When we use *Allium Cepa* it shows good results against methanol and against pathogens *Streptococcus Mutans sp* it shows satisfactory results at the concentration at 150 ul of showing zone of 1.1 mm. When we are using *Zingiber Officinale* against *Streptococcus Mutans* it shows methanol and ethyl acetate and at concentration of 100ul it shows the best zone of 2.3 nm. During the *Allium Sativum* against *Streptococcus Mutans* it shows best results against methanol and ethyl acetate and at concentration of 100ul and zone of 3.2 mm against *Streptococcus Mutans sp*. all these species are not showing significant results against all the solvents and any fungal strain used so they are not be further studied in this research but they had to studied as ongoing research . As on we had used fungi for fungal

sensitivity test had we had observed the following results against: *Candida albicans* shows ZOI against *Syzygium aromaticum* of 5.6 against methanol, *Fenugreek* against methanol shows 3.6, *Curcuma Longa* shows good ZOI against ethyl acetate of 7.5, *Allium Cepa* shows good results against ethyl acetate of 6.0, *Zingiber Officinale* shows 8.5 against chloroform, *Allium Sativum* shows ZOI of 6.5 against ethanol. When we perform their MIC it show that the best MIC were shown by *Syzygium aromaticum*, *Allium Cepa* and *Curcuma Longa* in the form of O.D. is 1.61, 1.55 and 1.33 respectively. Other extractant are also showing the better MIC as of *Zingiber Officinale*, *Fenugreek* and *Allium Sativum* shows 1.90, 2.10 and 2.22 respectively. From the overall observation we can say that into our research we had found that the solvents as methanol and ethyl acetate are showing good activity then the other solvents and plant extract of fenugreek, *carcuma longa*, *allium sativum* are showing significantly more activity then the other selected plants against *S.mutans*. by observing *Candida albicans* we had found that methanol, chloroform, ethyl acetate good and extract of *Zingiber officinale* and *Curcuma longa* are best

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