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Evaluation of Antimicrobial Efficacy of Mint Extract against oral pathogens and formulation of *Mentha piperita* based Mouthwash

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Abstract

The current study was carried out to check the efficacy of *Mentha piperita* against oralflora.Total 20 samples were collected during the study and further processed for isolation and identification.The predominant organisms were *S. mutans*, *E. coli*, *Bacillus subtilis* , *Staphylococcus aureus, L. bulgaricus, S. pyogenus and S.mitis*. The study was continued with the *S.mutans*.The antimicrobial activity of *Mentha piperita* was checked in different organic solvents such as methanol,ethanol and aqueous.Methanolic extract show excellent activity in controlling oralflora as compare to other solvents. Similary during the study *Mentha piperita* based mouthwash was also formulated.The physical parameters as well as antimicrobial activity of formulated mouthwash was also checked.Mouthwash shows excellent result in terms of zone of inhibition was observed,indicating *Mentha piperita* plants has strong antimicrobial activity in controlling oral microflora.

Keywords: Peppermint,Oral Cavity,Herbal Mouthwash,Antimicrobial activity

INTRODUCTION

Medicinal plants have been discovered for decades and used in traditional medicine practices ever since prehistoric times Medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Among several medicinal plants, Mint (*Mentha piperita*) is a rapid growing perennial herb most widely used in medicinal preparations. It is commonly known as Peppermint, Brandy mint Candy mint, Lamb mint, Balm mint, Vilayati pudina or Paparaminta(Punit and Mello, 2012). The *Mentha piperita* were found a rich source of phytochemical compounds like diterpenes, steroids, tannin, flavonoids, cardial glycosides, alkaloids, phenols, coumarin, and saponin, terpenoids and phenol (Chanchal katariya, *et al.*, 2022).

Dental plaque is the biofilm of microorganisms that grows on surface of teeth prosthetic, implants and dental restorations. Surviving in the oral cavity, *S. mutans* is the primary causal agent and the pathogenic species responsible for dental caries (tooth decay or cavities) specifically in the initiation and development stages (Lisa Simon, 2007). The null hypothesis of the study is that aqueous mint extract does not show any antimicrobial activity against periodontopathic bacteria. The aim of the current study is to assess the antimicrobial activity of aqueous and ethanol mint extract against anaerobic and aerobic bacteria found in subgingival plaque of chronic periodontitis patients (Chanchal katariya,Sankari Malaiappan,2022). Uses of mint mouthwash is to improve oral hygiene, help to control dental plaque, gum diseases, killing germs in oral cavity, freshen breath and covers bad breath, clean septic sockets, relieve pain and inflammation, treatment of Mucositis and Halitosis and also used in Periodontal diseases (Shivani Suresh Uttarwar,2022).

MATERIAL AND METHODS

Selection of Medicinal plant.

Medicinal herbs that are beneficial in treating oral health issues include *Neem, Peppermint, Turmeric, Clove, Spinach,* and *Tulsi*, among many others. We chose the *peppermint* plant since it is one of numerous medicinal plants that grows quickly, spreads swiftly, easy to available and its cultivated everywhere in the world. *Mint* is good for our oral health.

Collection of *Peppermint* Plant.

Peppermint plant was collected from the Akola neighbourhood market. Following leaf collection, they underwent a thorough washing in tap water and a 15-day drying period. After that, the dried leaves were ground using a regular grinder and sieved. The powder was saved for later usage and kept in an airtight container.

Preparation of plant Extract (Ethanol, Methanol and Water) (Gariya *et al.,* 2020.)

5gm of *Mint* powder dissolved in 50ml of solvent like Ethanol, Methanol and Water separately.The suspension was shaken vigorously in shaker for 5 hours then suspension was filtered using Whatman filter paper No. 1. The filtrate was then evaporated on a water bath. After evaporation add 2ml DMSO (Dimethyl sulphoxide) and then stored in airtight sample bottle in a room temperature for their antimicrobial activity.

Phytochemical Analysis

The coarse powder was soaked in 10 ml of distilled water after 1 hours the suspension were filtered using Whatman's no1 filter paper then filtered was used for the phytochemical analysis.

The following method is used for the phytochemical analysis of *Peppermint extract* (Guevara, 2005 and Harborne, 1998).

Test for Carbohydrates: 1 ml of Molisch's reagent was added to 2 ml of the *Mint* 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 *Mint* extract. A greenish black colouration depicts that tannins are present.

Test for Saponins: 2 ml of distilled water was added to 2 ml of *Mint* extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.

Test for Flavonoids: 5 ml of dilute NH, solution was added to 1 ml of *Mint* extract prior to the addition of concentrated sulphuric acid. A yellow colouration depicts that flavonoids are present.

Test for Terpenoids: 2 ml of chloroform and concentrated H2SO4 was added to 0.5 ml of *Mint* 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 *Mint* extract. A green colouration depicts that phenols are present.

Test for Acids: Sodium bicarbonate solution was added to 1 ml of *Mint* extract. Formation of effervescence depicts the presence of acids.

Test for Glycosides: 3 ml of chloroform and 10% NH3 solution was added to 2 ml of *Mint* extract. A pink colouration depicts that the glycosides are present.

Test for Alkaloids: 2ml of concentrated HCl was added to 2 ml of *Mint* extract before a few drops of Mayer's regent were added. A greenish depict that alkaloids are present.

Collection of Samples.

Oral infectious samples were collected by using a sterile oral swab under aseptic condition. Samples of dental plaque, dental caries, and periodontal disease was collected from different Dental clinic such as, MSB dental hospital, Smilez Dental Clinic, Bagadiya Dental Clinic, Shri Krupa Dental Clinical in Akola. From these hospitals, a total of 25 oral swabs were collected**.**

Isolation and Identification

Using the swabbing technique, dental swabs were taken from the hospital and inoculated on Nutrient agar. The inoculation was then let to incubate for 24 hours at 37°C.After isolation the isolates were processed for confirmation by morphological study. On the basis of staining procedure morphology of isolates were observed. The confirmation of isolate was done by Biochemical characterization in which Sugar fermentation test and IMViC test was done. On the basis of Cultural Morphology and Biochemical characterization the isolates were tentatively confirmed (Bergey's manual of Determinative Bacteriology, 1939).On the basis of microscopic examination the isolates were confirmed ie *Streptococcus mutans*. The isolates were streaked on selective agar plates.The selective median for *Streptococcus mutans* is Mitilis Salivarius agar, on selective media the watery colonies are observed.

Antimicrobial Activity.

Exactly 0.2 ml of 24hours old culture of organism was dispensed into 20ml of sterile nutrient broth and was incubated for 3-5hours to standardize the culture to106cfu/ml (Collins *et al.*, 1995).

Agar well diffusion technique

Antimicrobial testing was done using the agar wells diffusion method (Odeyemi and Fagbohun,2005).Broth culture of each isolates was ascetically transferred to the solidified Nutrient agar or Mueller Hilton agar (MHA) and spread evenly on the surface using a sterile cotton swab. Four 6mm wells were bored unto the agar and the wells filled with the plant extract (Ethanol, Methanol and Aqueous) while in the fourth well fill with the distilled water serves as the control. The petri dishes were incubated at 37°C for 24 hours and the inhibition zones were measured (mm).

Formulation of Herbal Mouthwash. [Shweta Patil (2020) and Bodake Ravina (2022)]

Formulation containing ingredients such as *Clove* oil, *Peppermint* extract, Sodium lauryl sulfate, Food colour, *Neem* oil , Honey.

Method of preparation:

- Accurate quantity ingredients where weight.
- Take peppermint extract of 100ml and add 2-3 drop of clove oil and Neem oil.
- Add 2 ml of Honey.
- And then add 1 gm of sodium lauryl sulfate and add colouring agent i.e. food colour.
- Add 100 ml distilled water to make quantity sufficient.
- All ingredients were mixed in beaker.
- Prepared mouthwash was packed into a suitable container, labeled and used for further studies.

Evaluation of Mouthwash at Different Parameters (Bodake *et al.*, 2022)

- **Physical evaluation:** Physical parameter such as color, odour taste and consistency was examined by visual examination.
- **pH:** The pH of prepared herbal mouthwash was measured by using pH strips.
- **Viscosity:** Viscosity is measured with the help of digital viscometer.
- **Microbial Assay:** The antibacterial activities were evaluated by measuring the zones of inhibition (in mm).
- **Stability studies:** Physical parameters like color, odor, consistency and pH was determined at room Temperature and 40°C.
- **Taste:** The taste is strong and remain almost same over the week.
- **Flavor:** The flavor is almost unchanged and has an excellent fragrance of clove and peppermint.

RESULTS AND DISCUSSION

Phytochemical Analysis

Table 1: Preliminary Phytochemical Analysis of Peppermint plant leave extract.

Sr.No.	Phyto constituents	Observation	Inference
1	Carbohydrates	No purple colouration	Negative
2	Tannins	Greenish black	Positive
3	Saponins	Foam formation	Positive
4	Flavonoids	Yellow colour formation	Positive
5	Alkaloids	No greenish colour formation	Negative
6	Glycosides	No green ring	Negative
7	Terpenoids	Green colour formation	Positive
8	Phenols	White precipitate	Positive
9	Coumarins	Yellow colour formation	Positive
10	Acids	No effervescence	Negative

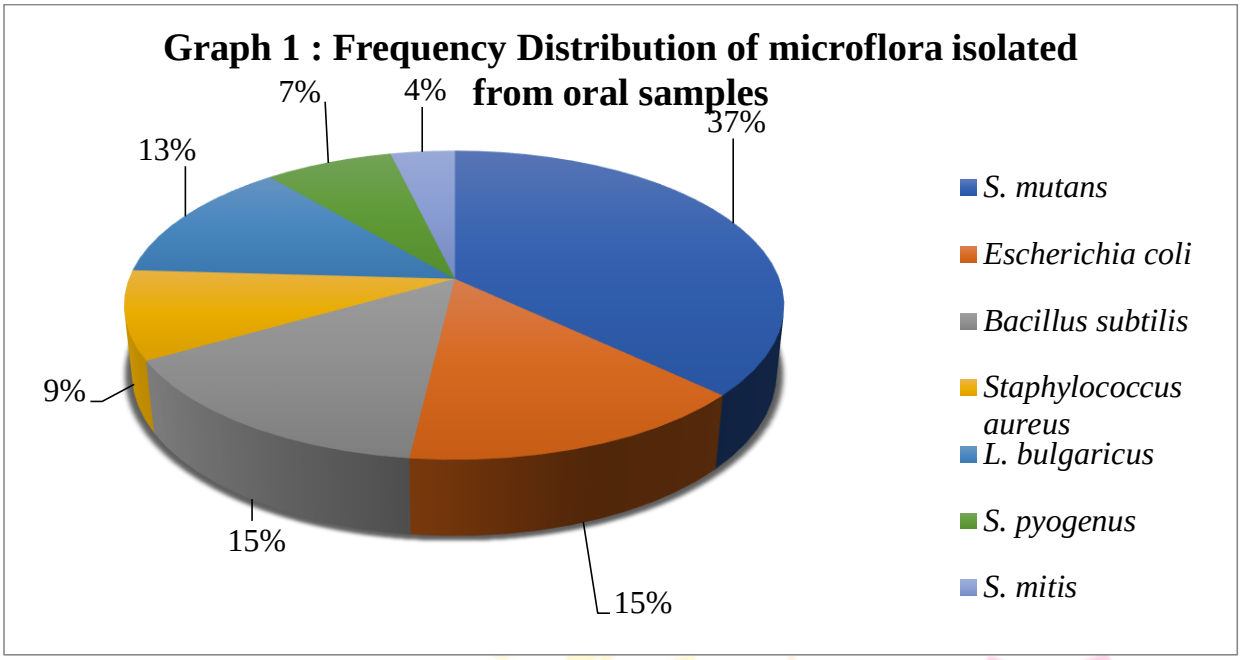
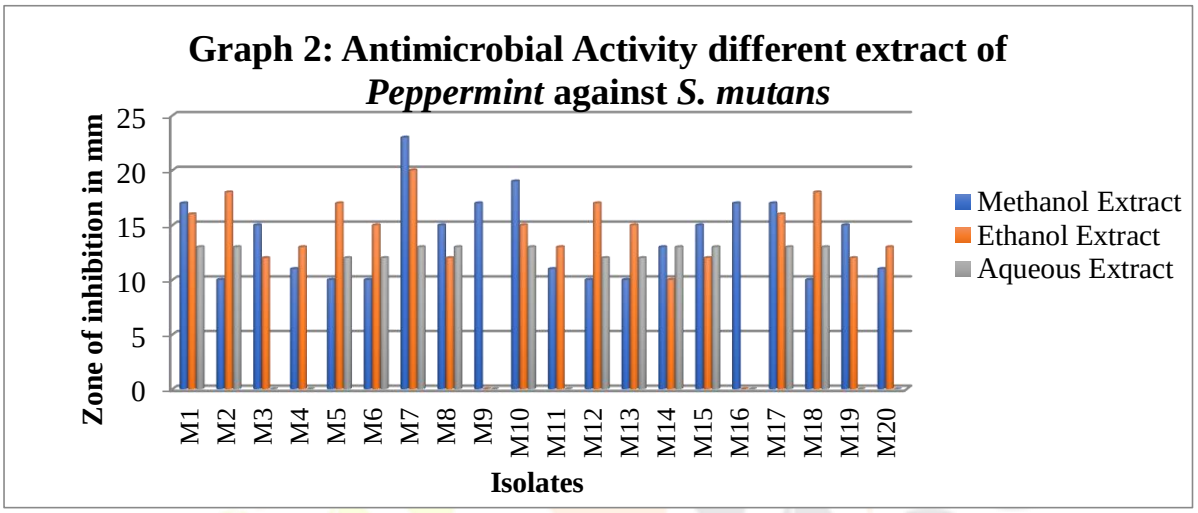


Table 3:Antimicrobial Activity different extract of *Peppermint* against *S. mutans*

Sr. No.	Isolates	Zone of inhibition (in mm)		
		Methanol Extract	Ethanol Extract	Aqueous Extract
1	M1	17.0	16.0	13.0
2	M2	10.0	18.0	13.0
3	M3	15.0	12.0	R
4	M4	11.0	13.0	R
5	M5	10.0	17.0	12.0
6	M6	10.0	15.0	12.0
7	M7	23.0	20.0	13.0
8	M8	15.0	12.0	13.0
9	M9	17.0	R	R
10	M10	19.0	15.0	13.0
11	M11	11.0	13.0	R
12	M12	10.0	17.0	12.0
13	M13	10.0	15.0	12.0
14	M14	13.0	10.0	13.0

15	M15	15.0	12.0	13.0
16	M16	17.0	R	R
17	M17	17.0	16.0	13.0
18	M18	10.0	18.0	13.0
19	M19	15.0	12.0	R
20	M20	11.0	13.0	R



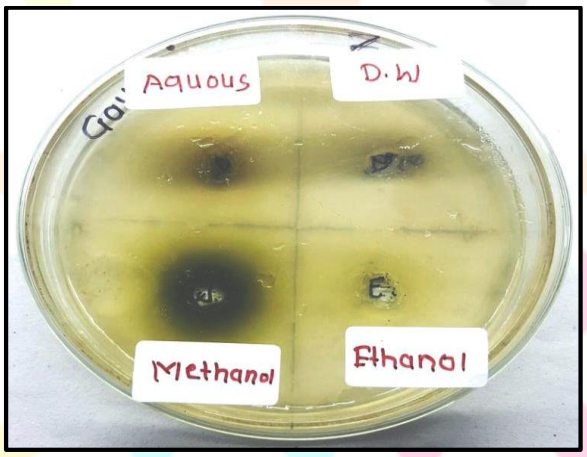


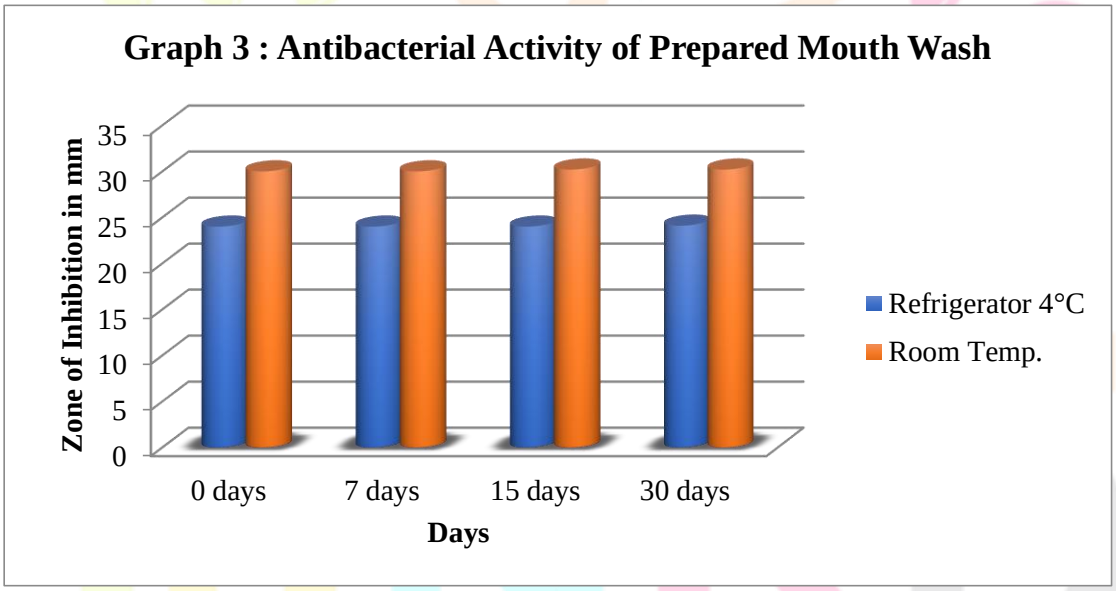
Table 4: Formulation of Herbal Mouthwash

Sr. No.	Ingredients	Botanical Name	Role	Quantity
01	Clove oil	*Syzyqium armaticum*	Anglgesics, Anti-inflammentary	2 ml
02	Peppermint	*Mentha piperita*	Freshner, Antibacterial, Antifungal	20ml

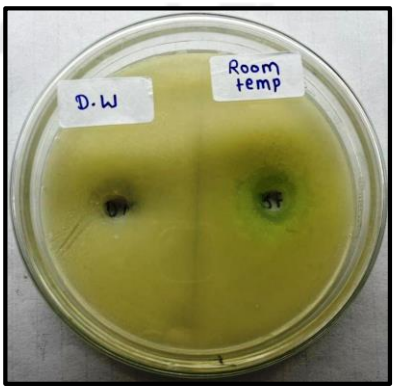
03	Sodium louryl sulphate	Sodium louryl sulphate	Buffering Agent	3 gm
04	Food colour	-	Colouring Agent	1 gm
05	Honey	-	Antibacterial	2 ml
06	Neem oil	*Azadirachta indica*	Preservative	0.5 ml
07	Water	-	Quantity Sufficient	100 ml

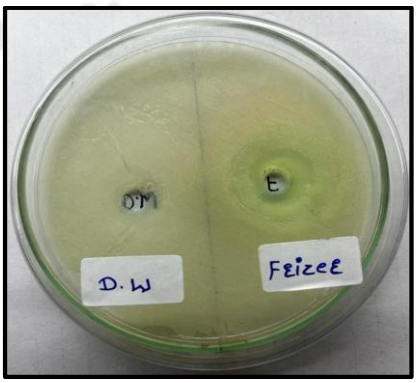
Table No 5 : Antimicrobial Activity of Prepared Mouth Wash

Days	Antimicrobial Activity (Zone of inhibition in mm)	
	Refrigerator 4°C	Room Temp.
0 days	24.0	30.0
7 days	24.0	30.0
15 days	24.0	30.2
30 days	24.1	30.2



Prepared Mouthwash and its Antimicrobial Activity





DISCUSSION :

For the current study Peppermint is preferred because they have many benefits such as it improves digestive health, boost your immuno system, better brain function, support your pregnancy, reduce cold symptoms, keep mouth health, help manage stress, filled with nutrients and many other .Similar study was conducted by R.Eccles (1994). They revealed that the Peppermint incorporates high menthol content, and is usually used as tea and for flavouring dessert, Confectionery, Chewing gum, and toothpaste. The oil also contains menthone and methyl esters, particularly methyl acetate is the oldest and preferred flavor of mint flavoured confectionery. Peppermint could also be used in shampoos and soaps, which give the hair a minty seen and gives a cooling sensation on the skin seasoner is flexible oil: it's analgesic, Anti-inflammatory, anti-viral, Digestive, Anti-septic, an astringent, carminative and anti-spasmodic, Peppermint. Essential oil is used to treat migraine, Bronchitis, sinusitis. Indigestion, nausea, Irritable bowel syndrome, irregular periods and nervous conditions. It is also very useful within the treatment of cold and flu.

Phytochemical analysis of *Peppermint* plant of leaves extract show the presences of Tannins (apperance of greenish black colour), Saponins (formation of foam), Flavonoids (yellow colour formation), Terpenoids (green colour formation) Phenol (show white precipitation), Coumarin (yellow colour formation). It shows negative results in case of Carbohydrates (No purple colour formation), Alkaloids (no greenish colour formation), Glycosides (no green ring formation) and Acids (no effervescence) (Table No 1).Similar study was conducted by Pramila *et al.,* (2012). They revealed that the Methanolic mint leaf extract was used for preliminary qualitative screening of phytochemicals as per standard biochemical procedures as previously described by Ga Ayoola *et al.,* (2008). The crude extract was diluted with methanol to the concentration of 1 mg/ml. The qualitative phytochemical analysis of crude methanolic mint leaf was conducted to determine the presence of reducing sugars (glycosides) saponins, tannins, anthraquinone derivatives flavanoids and alkaloids.

The collected samples were further processed for isolation and identification of bacteria on various selective media such as *S. mutans* grows on MSB media, *E. coli* grow on EMB agar, *Bacillus subtilis* grow on MEYP agar, *Staphylococcus aureus* grow on MacConkey agar, *L. bulgaricus* grow on China blue lactose agar, *S. pyogenus* grow on Strep agar *and S.mitis* grow on Mitis Salivarius agar . The frequency obtained isolates were, 20 (37%) isolates were *S. mutans*, 8 (15%) isolate were *Escherichia coli,*8 (15%) isolate were *Bacillus subtilis,*7(13%) isolate were *L. bulgaricus*, 5 (9%) isolate were *Staphylococcus aureus,* 4 (7%) isolate were *S. pyogenus*, 2 (4%) isolate were *S. mitis* (Table 2, Graph 1). As the frequency of occurrence of *S. mutans* in sample was high, the further study was continued with the *S. mutans.*

Antibacterial activity of different extracts of *Pepp*ermint against *S.mutans* isolated from oral cavity sample was done and summarized in Table 3 and Graph 2. It was observed that the Methanolic extract had antibacterial activity against all isolates, with highest zone of inhibition of 23.0 mm and the lowest zone of inhibition given by the Methanolic extract is of 10.0mm. Ethanol extract showed highest zone of inhibition ie 20.0 mm and lowest zone of inhibition is 12.0 mm. Aqueous extract showed very weak antibacterial activity with lowest zone of inhibition of 12.0 mm. According to Chanchal Katariya *et al.,* (2022). The ethanol extract of Mint with zone of inhibition against both aerobic and anaerobic bacteria show 17mm and 16mm respectively The Aqueous extract of Mint show zone of inhibition against aerobic and anaerobic bacteria is 11mm and 9mm respectively.

Formulation of *Mint* mouthwash containing ingredients such as Clove oil, peppermint, Sodium lauryl sulfate, Food colour, Honey, Neem oil and water (Table No 4). On the basis of complete study done on prepared mouthwash. It shows excellent results in terms of colours (green), flavor (fragrance of clove and peppermint), taste (normal), texture (clear liquid) and pH (7-7.5) indicating the mouthwash is excellent in controlling the oral flora. The formulations was found to be free from microbes as they have not produced any microbial growth when they inoculated in the agar medium . Antimicrobial activity of the prepared mouthwash also was checked at Refrigeration ie 4°c the zone of inhibition was 24 mm whereas at room temperature30 mm the zone of inhibition was observed. The *peppermint* mouthwash show highest zone of inhibition at room temperature (Table No 5,Graph 3).

CONCLUSION

The predominant organism from oral cavity was *S. mutans*. The *peppermint* mouthwash is quite effective in controlling the oral microflora. The herbal mouthwash are in high demand because they act on oral pathogens and relief the pain, also shows less side effects. The main purpose of using this mouthwash is that can be used at home as routine to maintain good oral hygiene.

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