

NATURAL HERB MOUTH WASH DESIGNING AGAINST CANDIDA ALBICANS CAUSING ORAL CANDIDIASIS

S.SAMELA AND J.VIMALIN HENA

Department of Microbiology,
Hindusthan College of Arts and Science, Nava India, Coimbatore- 641028, Tamilnadu, India.

Received: 22th April 2016; Accepted: 15th May 2016.

Abstract

Plants benefit extensively by harbouring endophytic microbes. They promote plant growth and confer enhanced resistance to various pathogens. Present study experimentally showed that endophytes isolated from rice (*Oryza sativa*), Sugar cane (*Saccharum officinarum*), Banana leaves (*Musa acuminata*), Indian grass (*Sorghastrum nutans*) leaves, Onion (*Allium cepa*) used as the test plant produced two types of interactions; biofilms (bacteria attached to mycelia) and mixed cultures with no such attachments. Acidity, as measured by pH in cultures with biofilms was higher than that of fungi alone, bacteria alone or the mixed cultures. Production of indole acetic acid like substances (IAAS) of biofilms was higher than that of mixed cultures, fungi or bacteria. Bacteria and fungi produced higher quantities of IAAS than mixed cultures. In mixed cultures, the potential of IAAS production of resident microbes was reduced considerably. There was a negative relationship between IAAS and pH of the biofilms, indicating that IAAS was the main contributor to the acidity. However, such a relationship was not observed in mixed cultures. Microbial acid production is important for suppressing plant pathogens. Thus the biofilm formation in endophytic environment seems to be very important for healthy and improved plant growth. As such in vitro production and application of beneficial biofilmed inoculum of endophytes are important for improved plant production in any agro-ecosystem. The conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes may not give the highest microbial effect, which may only be achieved by biofilm formation.

Keywords: Biofilm, Endophytic microbes, Growth hormones, IAA production, Microbial interaction, Other beneficial By-Products.

I. INTRODUCTION

Mouthwash are the liquids that are held in the mouth, swished around or gargled for therapeutic or cosmetic effect, mostly for the removal of plaque and calculus. Plaque is the primary etiologic agent in the development of dental caries, gingivitis and periodontal disease. Mechanical removal of plaque through frequent and efficacious brushing and flossing is the principal means of preventing periodontal diseases and diminishing the risk of caries. Mouth rinsing is easier to perform and may aid in controlling supragingival plaque and gingivitis, but it should always be used in conjunction with mechanical hygiene. Mouthwashes must not be used as a sole measure for oral hygiene.

Specific Ingredients

To design a mouth wash it includes the specific ingredients which are added along with the medicinal herb extract.

Flavouring Agent

Eucalyptol or methanol which give a mouthwash its distinctive taste.

Preservatives

The preservative prolongs the life of the mouthwash and prevents the formation of bacteria and other microbes. e.g. Sodium benzoate.

Water

An essential component of a mouthwash which helps to liquefy all of the ingredients.

Sweetening agents

The sweetening agent enhance the taste of a mouthwash. e.g. sodium saccharine and sucralose.

Fluoride

Fluoride mouthwashes are sometimes used at high risk of dental decay. They strengthen teeth against decay.

Sodium Chloride

Sodium Chloride mouth wash is used after oral surgery to prevent infection. It has a mechanical cleansing and an antiseptic action to act against bacteria and fungal infection. It drains the pus from dental abscesses. Gargling with salt water reduces the sore throat. (Jung et al., 2014)

Detergent - Sodium Lauryl Sulphate

Sodium Lauryl Sulphate prevents the oral hygiene of the mouth wash. It used as a major ingredient in mouthwash. (Rosenberg et al., 2002)

Designing An Anti-Candidial Natural Mouth Wash

The main aim of my research work is to design an anti-candidial herbal mouth wash against oral thrush infection. The mouth wash is prepared by the leaf extracts of the medicinal herbs. The phytochemical and the anti-candidial activity of the medicinal herb leaf extracts were screened and designed an anti - candidial mouth wash.

The present study, To design a natural mouth wash against *Candida albicans* causing oral candidiasis.

2. MATERIALS AND METHODS

2.1 Plant collection and Processing

The Medicinal plants *Cissus Quadrangularis* (Pirandi), *Cinnamomum Zeylanicum* (Cinnamom), *Glycyrrhiza Glabra* (Mulethi), *Ocimum Sanctum* (Thulsi), *Acorus Calamus* (Vasambu), *Euclea Divinorum* (Adathoda), *Aleo Barbadensis* (Aleo vera), *Azadirachta indica* (Neem), *Piper nigrum* (Black pepper), *Syzygium aromaticum* (Clove) were collected from the agricultural areas of coimbatore.

*Author for correspondence (shareatsudha@gmail.com)

2.2 Solvents used for Extraction

The various solvents that are used in the extraction procedures are:

Water: Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. (Tiwari et al., 2010)

Ethanol: The higher activity of the ethanolic extracts is compared to the presence of higher amounts of polyphenols. They are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. Ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Bimark et al., 2010)

Chloroform: Terpenoid lactones have been obtained by successive extractions of dried barks with activity concentrating in chloroform fraction. (Sharma et al., 2011)

Petroleum Ether: Ether is commonly used selectively for the extraction of coumarins and fatty acids (Wang et al., 2011)

Methanol: It is specially used for the selective extraction of only terpenoids.

2.3 Preparation of extracts

Air shade dried powdered leaf of medicinal herbs like *Cissus Quadrangularis* (Pirandi), *Cinnamum Zeylanicum* (Cinnamom), *Glycyrrhiza Glabra* (Mulethi), *Ocimum Sanctum* (Thulasi), *Acorus Calamus* (Vasambu), *Euclea Divinorum* (Apathoda), *Aleo Barbadensis* (Aleo vera), *Azadirachta indica* (Neem), *Piper nigrum* (Black pepper), *Syzygium aromaticum* (Clove). 5g portions of powdered plant materials were each separately dispersed in Soxhlet extracted with 50ml of water, 70% ethanol, methanol, petroleum ether and chloroform for 48 hours. Each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness invitro and re dissolved in respective solvents and stored at 40C in a refrigerator, until screened for antimicrobial activity.

2.4 Photochemical analysis

The extracts obtained from all the ten medicinal herbs was then subjected to phytochemical analysis.

2.4.1 Test for Alkaloids (Abdul Wadood et al., 2013)

About 0.2g of all the ten plant sample was taken separately in a test tube and 3ml of hexane was added, mixed well, shaken and filtered. 5ml of 2% HCL was added into the tube and heated. The contents were filtered and a few drops of picric acid was added into it. Yellow colour indicates the presence of alkaloids.

2.4.2 Test for Carbohydrates (Prasanth Tiwari et al., 2011)

Benedict's test: The extract of all the ten medicinal herbs was treated with 2 ml Benedict's reagent and heated gently. Orange Red precipitate indicates the presence of carbohydrates.

Test for Glycoides (Sarla Saklani et al., 2012)

Keller-Killani test : 1 ml of glacial acetic acid containing traces of ferric chloride and 1ml of concentrate sulphuric acid were added to all the ten extract carefully. A reddish brown colour formed at the junction of two layers and upper layer turned bluish green indicated the presence of glycosides.

2.4.3 Test for Flavonoids (Abdul Wadood et al., 2013)

In a test tube, 0.5 g of all the ten plant extract was taken and to this 10 ml of distilled water was added separately. To a portion of this, 5 ml of dilute ammonia solution was added followed by 1ml concentrated H₂SO₄. Appearance of yellow colour shows the presence of flavonoids.

2.4.4 Test for Proteins (Yadav et al., 2011)

Millon's Test : Crude extract of all the ten medicinal herbs mixed with 2ml of millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for Saponins : In a test tube, crude extract of all the ten plant was mixed with 5ml of distilled water and was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

2.4.5 Test for reducing sugar (Abdul Wadood et al., 2013)

An amount of 0.50g of all the ten plant sample was added in 5ml of distilled water. In another test tube, 1ml of ethanol and 1ml each of Fehling solution A and Fehling solution B was added. The mixture was heated to boiling and then poured into the aqueous ethanol plant extract. Appearance of colour showed a positive result.

2.4.6 Test for Steroids (Yadav et al., 2011)

Crude extract of ten medicinal herbs was mixed with 2ml chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

2.4.7. Test for Terpenoids (Yadav et al., 2011)

1ml of chloroform was added to 2ml of all the ten extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

2.5 Anticandidial activity of ten medicinal herbs

The anticandidial activity was tested against all the ten medicinal herb extract. The inoculation of microorganism was prepared from the candidial culture. About 15-20ml of Sabourauds dextrose agar medium was poured in the sterilized petridish and allowed for solidifying. One drop of *Candida albicans* was inoculated by swabbing with sterile swab over the medium. Wells of 6mm in diameter and about 2cm apart punctured in the culture medium using sterile cork borers. Different concentrations (25, 50, 75, and 100µl) of the medicinal herb extracts were added to the wells and 75µl of ethanol was added to the centre well as control. The plates were incubated at 37°C for 24 hours. Anticandidial activities were evaluated by measuring inhibition zone diameter.

Screening for Natural Mouth Wash-Target selection

Ten plants were extracted and analysed for the mouth wash design in which three plants *Ocimum Sanctum* (Thulasi), *Euclea Divinorum* (Apathoda) and *Glycyrrhiza Glabra* (Mulethi) was selected for the herbal mouth wash based on the higher activity against antibacterial, anticandidial and phytochemical analysis.

The leaf extracts of *Ocimum Sanctum* (Thulasi), *Euclea Divinorum* (Apathoda) and *Glycyrrhiza Glabra* (Mulethi) had high anticandidial activity so they are further used for the mouth wash designing. They were mixed with the specific mouth wash ingredients like Essential oil- Eucalyptus, preservative- sodium benzoate, Fluoride, Detergent- Sodium lauryl sulphate, and sodium chloride were plated on to *Candida albicans* swabbed SDA plate.

2.6 Formulation of Specific Mouth Wash Ingredients

Leaf extracts	: 40 µg
water	: 10 µg
sodium benzoate	: 10 µg
Eucalyptus oil	: 10 µg
Fluoride	: 10 µg
Sodium lauryl sulphate	: 10 µg
Sodium chloride	: 10 µg
Sodium saccharine	: 10 µg

The plates were incubated at 37°C for 24-48 hrs and the zone was measured. It was also tested that the ingredients and the herbal leaf extracts is not causing any defects to the teeth and also inhibits the bacterial growth by plating on to the swabbed Muller Hinton plates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *E.coli*.

3. RESULT & DISCUSSION

3.1 Photochemical analysis

The following are the phytochemical compounds which are analysed by the medicinal plant extracts

2.7 Comparitive study of antibiotic mouthwash and natural herb mouth wash

Natural herb mouth wash was compared with the fungal antibiotic nystatin in which the leaf extracts plays a major role in inhibiting the candida albicans more than the antibiotic nystatin. It also inhibits the growth of bacteria in the mouth and protects the teeth from decay.

3.2 Antimicrobial Activity

Among the Antimicrobial activity *Euclena divinorum* and *Ocimum Sanctum*, and *Glycyrrhiza Glabra* shows the highest value and zone formation

S.No	Medicinal plant extracts-Ethanol	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Reducing Sugar	Saponins
1.	Acorus calamus	+	-	+	+	+	-	-
2.	Aleo Barbadensis	+	-	+	+	+	+	+
3.	Azadirachta Indica	+	+	+	-	+	-	+
4.	Cinnamum Zeylanicum	+	+	-	+	+	+	+
5.	Euclena Divinorum	+	+	+	+	+	+	+
6.	Cissus Quadrangularis	+	+	-	+	+	+	+
7.	Glycyrrhiza Glabra	-	-	-	+	+	+	+
8.	Ocimum Sanctum	+	+	+	+	+	+	+
9.	Piper Nigrum	+	+	+	-	+	-	-
10.	Syzygium Aromaticum	+	+	-	+	+	+	+

Table 1: Ethanol Extract

S.No	Medicinal plant extracts-Ethanol	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Reducing Sugar	Saponins
1.	Acorus calamus	+	-	+	+	+	-	-
2.	Aleo Barbadensis	+	-	+	+	+	+	+
3.	Azadirachta Indica	+	+	+	-	+	-	+
4.	Cinnamum Zeylanicum	+	+	-	+	+	+	+
5.	Euclena Divinorum	+	+	+	+	+	+	+
6.	Cissus Quadrangularis	+	+	-	+	+	+	+
7.	Glycyrrhiza Glabra	-	-	-	+	+	+	+
8.	Ocimum Sanctum	+	+	+	+	+	+	+
9.	Piper Nigrum	+	+	+	-	+	-	-
10.	Syzygium Aromaticum	+	+	-	+	+	+	+

Table 2: Methanol Extract

S.No	Medicinal plant extracts-Ethanol	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Reducing Sugar	Saponins
1.	Acorus calamus	-	-	-	+	+	+	-
2.	Aleo Barbadensis	+	-	+	+	+	+	+
3.	Azadirachta Indica	+	-	+	-	+	-	+
4.	Cinnamum Zeylanicum	+	+	-	+	+	-	+
5.	Euclea Divinorum	+	+	-	+	+	+	+
6.	Cissus Quadrangularis	+	+	-	+	+	+	+
7.	Glycyrrhiza Glabra	-	-	-	+	+	+	+
8.	Ocimum Sanctum	+	+	+	-	+	+	+
9.	Piper Nigrum	+	+	+	+	+	-	-
10.	Syzygium Aromaticum	+	+	-	+	-	+	+

Table 3: Petroleum Ether Extract

S.No	Medicinal plant extracts-Ethanol	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Reducing Sugar	Saponins
1.	Acorus calamus	+	-	-	+	+	-	-
2.	Aleo Barbadensis	+	-	+	+	+	+	+
3.	Azadirachta Indica	+	-	-	-	+	-	+
4.	Cinnamum Zeylanicum	+	-	-	-	+	+	+
5.	Euclea Divinorum	+	+	+	+	+	-	+
6.	Cissus Quadrangularis	+	+	-	+	+	+	+
7.	Glycyrrhiza Glabra	-	-	-	+	+	+	+
8.	Ocimum Sanctum	-	+	+	+	+	-	+
9.	Piper Nigrum	-	+	-	-	+	-	-
10.	Syzygium Aromaticum	-	+	+	+	+	+	+

Table 4: Chloroform Extract

S.No	Medicinal plant extracts-Ethanol	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Reducing Sugar	Saponins
1.	Acorus calamus	+	-	+	+	+	-	-
2.	Aleo Barbadensis	+	-	+	+	+	+	+
3.	Azadirachta Indica	+	+	+	-	+	-	+
4.	Cinnamum Zeylanicum	+	+	+	+	+	+	+
5.	Euclea Divinorum	+	+	+	+	+	+	+
6.	Cissus Quadrangularis	+	+	-	+	+	+	+
7.	Glycyrrhiza Glabra	-	-	-	+	+	+	+
8.	Ocimum Sanctum	+	+	+	+	+	+	+
9.	Piper Nigrum	+	+	+	-	+	-	-
10.	Syzygium Aromaticum	+	+	-	+	+	+	+

Table 5: Water Extract

S.No	Medicinal plant extracts-Ethanol	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Acorus calamus	2.5	1.5	1.8	1.5	2	1
2.	Aleo Barbadensis	1.3	1	1.5	1	1	1
3.	Azadirachta Indica	1.2	1	1.5	1	1	1.5
4.	Cinnamum Zeylanicum	2	1.3	2	2	2	1.5
5.	Euclea Divinorum	2.5	2.3	2.8	2	1.5	1.3
6.	Cissus Quadrangularis	2	1.3	2.5	1	1.5	1.3
7.	Glycyrrhiza Glabra	2.3	1.5	2	1.5	1.7	2
8.	Ocimum Sanctum	1.5	1.5	2.5	1.5	1	1.5
9.	Piper Nigrum	1.3	1	1.2	1.3	1	1
10.	Syzygium Aromaticum	1	1	1.5	1	1	1.5

Table:6 Ethanol Extract - Zone of Inhibition in cm

S.No	Medicinal plant extracts-Ethanol	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Acorus calamus	2.3	1.5	1.8	1.5	2	1
2.	Aleo Barbadensis	1.3	1	1.5	1	1	1
3.	Azadirachta Indica	1.2	1	1.5	1	1	1.5
4.	Cinnamum Zeylanicum	1.6	1.3	2	1.5	1.5	1
5.	Euclea Divinorum	2.5	2.3	2.8	2	1.5	1.3
6.	Cissus Quadrangularis	1	-	2	-	-	-
7.	Glycyrrhiza Glabra	2.3	1.5	2	1.5	1.7	2
8.	Ocimum Sanctum	1.5	1.3	2.5	1.5	1	1.5
9.	Piper Nigrum	1.3	1	1.2	0.8	1	1
10.	Syzygium Aromaticum	1	1	1	1	1	1.5

Table:7 Methanol Extract - Zone of Inhibition in cm

S.No	Medicinal plant extracts-Ethanol	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Acorus calamus	1.3	1.3	1.5	1.2	1.5	1.5
2.	Aleo Barbadensis	1	1.2	1.5	1	1.3	1.3
3.	Azadirachta Indica	1	1.5	1	1	1	1.3
4.	Cinnamum Zeylanicum	1.5	1.5	1.4	2	1.5	1
5.	Euclea Divinorum	1.5	1.5	2.8	1.8	2	2
6.	Cissus Quadrangularis	-	1.3	2	1.3	-	0.5
7.	Glycyrrhiza Glabra	1.8	1.8	2	1.6	1.8	1.5
8.	Ocimum Sanctum	1.3	1.5	2.4	1.5	1.5	1.5
9.	Piper Nigrum	1	1.3	1.5	1	1	1.2
10.	Syzygium Aromaticum	1.3	1	1.5	1.3	0.8	1

Table:8 Chloroform Extract - Zone of Inhibition in cm

S.No	Medicinal plant extracts-Ethanol	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Acorus calamus	1.3	1	1.5	1.2	1	1.5
2.	Aleo Barbadensis	1	1.2	1.5	1	1.3	1
3.	Azadirachta Indica	1	1.3	1	1	1	1.3
4.	Cinnamum Zeylanicum	1.5	1	1.4	0.8	1.5	1
5.	Euclea Divinorum	1.5	1.5	2.8	1.8	2	2
6.	Cissus Quadrangularis	-	1	1.5	1.3	-	0.5
7.	Glycyrrhiza Glabra	1.8	1.8	2	1.6	1.8	1.5
8.	Ocimum Sanctum	2	1.5	2.4	1.5	1.5	1.5
9.	Piper Nigrum	1	1.3	1.3	1	1	1.2
10.	Syzygium Aromaticum	1	1.5	1.5	1	0.8	1

Table:9 Petroleum Ether Extract - Zone of Inhibition in cm

S.No	Medicinal plant extracts-Ethanol	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Acorus calamus	0.8	1	1	1.2	1	1.5
2.	Aleo Barbadensis	0.5	1	1.5	1	1.3	1
3.	Azadirachta Indica	1	1.3	1	1	1	1.3
4.	Cinnamum Zeylanicum	0.8	1	1.4	0.8	1	1
5.	Euclea Divinorum	1.5	1.5	2	1.8	2	2
6.	Cissus Quadrangularis	-	1	1.5	1.3	-	0.5
7.	Glycyrrhiza Glabra	2.5	1.8	2	2.5	1.8	1.5
8.	Ocimum Sanctum	1	1.5	1.3	1	2.5	2
9.	Piper Nigrum	1	1.3	1	1	1	1.2
10.	Syzygium Aromaticum	1	1	1	1	0.8	1

Table:10 Water Extract - Zone of Inhibition in cm

S.No	Mouth wash Ingredients	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Eucalyptus oil	0.8	1	1	1.2	1	1.5
2.	Sodium Benzoate	0.5	1	1.5	1	1.3	1
3.	Water	0.3	1.3	1	0.8	1	1.3
4.	Sodium saccharine	0.8	1	1.4	0.8	1	1
5.	Fluoride	1.5	1.5	2	1.8	2	2
6.	SLS	1	1	1.5	1.3	0.8	0.5
7.	Sodium chloride	2.5	1.8	2	2.5	1.8	1.5

Table:11 Antimicrobial activity of Mouth Wash Ingredients

S.No	Mouth wash Ingredients	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Eucalyptus oil	1	1.3	1.5	1.2	1	1.5
2.	Sodium Benzoate	0.5	1	1.5	2	1.3	1
3.	Water	1	1.3	1	1	1.3	1.3
4.	Sodium saccharine	0.3	1	0.5	0.8	1	1
5.	Fluoride	1.5	1.5	2	1.8	2	2
6.	SLS	1	1	1.5	1.3	-	0.5
7.	Sodium chloride	2.5	1.8	2	2.5	1.8	1.5

Table:12 Antimicrobial activity of *Euclea Divinorum* - Mouth Wash Ingredients

S.No	Mouth wash Ingredients	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Eucalyptus oil	1	1	0.3	1.2	1	1
2.	Sodium Benzoate	0.4	1	1	1	1.3	1
3.	Water	1	0.4	1	1	0.5	1.3
4.	Sodium saccharine	0.8	1	1.4	0.8	1	1
5.	Fluoride	1.5	1.5	2	1.8	2	2
6.	SLS	1	1	1.5	1.3	1	0.5
7.	Sodium chloride	2	1.8	2	2.5	1.8	1.5

Table:13 Antimicrobial activity of *Glycyrrhiza Glabra* - Mouth Wash Ingredients

S.No	Mouth wash Ingredients	<i>Strep.pyogenes</i>	<i>E.coli</i>	<i>Candiada albicans</i>	<i>Staph.aureus</i>	<i>Pseudomonas</i>	<i>Proteus</i>
1.	Eucalyptus oil	1.5	1	1	1.2	1	1.5
2.	Sodium Benzoate	0.5	1	1.5	1	1.3	1
3.	Water	1	0.4	0.6	1	1	1.3
4.	Sodium saccharine	0.8	1	1.4	0.8	1	1
5.	Fluoride	1.5	1.5	2	1.8	2	2
6.	SLS	1	1	1.5	1.3	0.5	0.5
7.	Sodium chloride	2.5	1.8	2	2.5	1.8	1.5

Table:14 Antimicrobial activity of *Ocimum Sanctum* - Mouth Wash Ingredients

4. DISCUSSION

The leaf extracts of *Ocimum Sanctum* (Thulasi), *Euclea Divinorum*(Adathoda) and *Glycyrrhiza Glabra* (Mulethi) had high anticandidal activity so they are used for the natural herb mouth wash when comparative to the antibiotic mouth wash.

5. REFERANCE

[1] Barnett ML. The role of therapeutic antimicrobial mouthrinses in clinical practice. Control of supragingival plaque and gingivitis. J Am Dent Assoc 2003; 134: 699-701.
[2] Barnett ML. The rationale for the daily use of an antimicrobial [3] mouthrinse. J Am Dent Assoc 2006; 137: 16-21.
[4] Weinberger B. Introduction to the History of Dentistry. St. Louis, Mosby, 1948.
[5] Schroeder HE, Marthaler TM, Mu'hlemann HR. Effects of some potential inhibitors on early calculus formation. Helv Odont Acta 1962; 6: 6-9.
[6] Pitten FA, Kramer A. Efficacy of cetylpyridinium chloride used as oropharyngeal antiseptic. Arzneim Forsch / Drug Res 2001; 51: 588-595.

[7] Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc 2006; 137: 1649-657.
[8] ten Cate JM. Biofilms, a new approach to the microbiology of dental plaque. Odontology 2006;94:1-9. Bowden GH, Hamilton IR. Survival of oral bacteria. Crit Rev Oral Biol Med 1998;9:54-85.
[9] Rodríguez-Morales S, Compadre RL, Castillo R, Breen PJ, Compadre CM. 3D-QSAR, synthesis, and antimicrobial activity of 1-alkylpyridinium compounds as potential agents to improve food safety. Eur J Med Chem 2005;40:840-9.
[10] Perdok JF, Genet M, Rouxhet PG, Busscher HJ. Cetylpyridinium chloride adsorption on the wettability and elemental surface composition of human enamel. Clin Prev Dent 1990;12:25-9.
[11] Sandt C, Barbeau J, Gagnon MA, Lafleur M. Role of the ammonium group in the diffusion of quaternary ammonium compounds in *Streptococcus mutans* biofilms. J Antimicrob Chemother 2007;60:1281-7.
[12] Quisno R, Foter MJ. Cetylpyridinium chloride: I. Germicidal properties. J Bacteriol 1946;52:111-7.
[13] Bereswill S, Vey T, Kist M. Susceptibility in vitro of *Helicobacter pylori* to cetylpyridinium chloride. FEMS Immunol

Med Microbiol 1999;24:189-92.

[14] Giuliana G, Pizzo G, Milici ME, Giangreco R. In vitro activities of antimicrobial agents against Candida species. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87:44-9.