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DETERMINATION OF ANTIMICROBIAL ACTIVITY OF HERBAL PLANT EXTRACTS AGAINST ORAL AND PATHOGENIC DENTAL ISOLATES AND THEIR PHYTOCHEMICAL ANALYSIS USING GC-MS

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ABSTRACT

Toothpastes and mouth rinse products containing natural herbal plants extracts have always been preferred over synthetic and semi-synthetic counterparts due to their reduced side-effects like irritation, staining etc. Thus, developing oral cleansing products devoid of harmful chemical constituents is a need of the hour. Many herbal plants have beneficial medicinal properties such as antimicrobial, antioxidant, anti-cancerous, anti-proliferative, anti-hemolytic activity, etc. In the present study, plant materials such as. bark of *Acacia catechu* (Khadir, Kattha), Stem of *Glycyrrhiza glabra* L. (Yashtimadhu) and Leaves of *Piper betel* (Paan) were chosen based on their antimicrobial properties. Bioactive compounds were extracted in distilled water and 70% ethanol using Soxhlet extraction system and antimicrobial activity was studied against the oral isolates. The oral isolates were isolated from oral cavity of healthy individuals, decayed tooth and from patients suffering from severe dental caries. The biochemical identification of the oral microflora and dental pathogens was successfully done to the species level using VITEK 2 automated biochemical identification system. The antimicrobial activity was tested using agar well diffusion method. The antimicrobial activity of betel leaf 70% ethanolic extract was found to be better as compared to aqueous extract of betel leaf, 70% ethanolic and aqueous extracts of yashtimadhu and khadir. The antimicrobial activity of betel leaf 70% ethanolic extract against *Streptococcus salivarius, Raoultella ornithinolytica, Stenotrophomonas maltophilia, Pseudomonas aeruginosa* and *Escherichia coli* was almost equivalent to that of standard antibiotic ampicillin and whereas, streptomycin

did not show significant antimicrobial activity at the concentration of 100 µg/ml. Further, commercially available oral cleansing products such as Colgate Plax and Listerine were tested against the oral isolates. Colgate plax showed very less antimicrobial activity whereas Listerine showed no antimicrobial activity against any of the oral isolates. Herbal plants constitute various bioactive components that possess antimicrobial properties. Phytochemical profile of betel leaf ethanolic extract was determined by qualitative analysis as well as using GC-MS. The betel leaf 70% ethanolic extract showed the presence of many bioactive components such as Benzoic acid, 2,3- dimethyl- (35.59%), 4- allyl-1,2- diacetoxybenzene (24.18%), Benzoic acid, 2,5- dimethyl (18.09%), Methyl hydrogen disulfide (10.20%), Phenol, 2- methoxy- 4- (2- propenyl), acetate (7.91%), Phenol, 2- methoxy-3- (2- propenyl)- (1.33%), Phytol (0.26%), n- Hexadecanoic acid (0.11%), etc. and most of them were identified to have antimicrobial property. The outcome of this study has helped in understanding medicinal properties of above herbal extracts against oral isolates. Such preparations can be used for the formulation of herbal tooth paste and herbal mouth rinses.

KEYWORDS: Herbal plants, soxhlet extraction, antimicrobial activity, phytochemical analysis, GC-MS.

INTRODUCTION

The oral cavity consists of various surfaces, each coated with enormous numbers of bacteria. Oral bacteria include *Streptococci*, *Pseudomonas*, *Staphylococci*, *Lactobacilli* and *Corynebacteria* with a great number of anaerobes,[1] *Candida albicans, Porphyromonas gingivalis*, *Actinobacillus*, *Prevotella* and *Fusobacterium* etc.[2] The nutrient environment of mouth makes it a favorable habitat for a variety of micro flora. The colonization of this micro flora results in severe dental problems, formation of dental plaque and tooth decay. Various practices are being used for oral cleansing such as brushing, rinsing, gargling, washing, etc. in order to prevent the colonization. The important global oral health problems are dental caries, periodontal diseases, oral and pharyngeal cancers and oral tissue lesions.[2] The mainstay is to control the plaque and thus the prevention of plaque induced gingivitis.[3] Caries and periodontal disease can be prevented by maintaining good oral hygiene with the use of oral care products such as toothpaste, toothbrush, mouthwash, and oral paste that contain antimicrobial and anti cariogenic properties. However, in the recent years, mouthwashes are being tremendously used as they are relatively easy to use for maintaining oral hygiene.[4] Various synthetic chemical agents have been evaluated over the years with

respect to their antimicrobial effect in oral cavity. Chemical plaque control agents are used as an adjuvant as they possess the capability to prohibit the colonization of bacteria and also inhibit its growth and metabolism; however, these chemical agents are associated with many side effects.[3] The benchmark control in the removal of plaque is chlorohexidine, but it cannot be used for a long duration due to side-effects like altered taste sensation, staining of tongue,[3] vomiting and diarrhea.[2] To overcome the above disadvantages naturally occurring antimicrobial herbs can be used individually or in combination.

Even today in the age of technology people still rely on natural ways to cure quite a number of diseases like dental problems. Thus, patients are going away from modern day medicines, and they prefer using herbal preparations which are effective without causing any side effects. Natural herbs like triphala, tulsipatra, jyestiamadh, neem, clove oil, pudina, ajwain and many more are used as whole single herb or in combination to prevent oral health problems like bleeding gums, halitosis, mouth ulcers and preventing tooth decay and have also been scientifically proven to be safe and effective medicine. The natural active ingredients present in herbal medicines are in combination with other components and hence they are normally considered safer than the non-herbal medicines because. Use of herbs for dental care is very common and herbs like *Terminalia chebula*, Aloe vera, *Azadirachta indica*, *Piper betle*, *Ocimum sanctum* posses antibacterial, ulcer healing, antiplaque and anti halitosis properties.[3] The biologically active compounds are found abundantly in the natural products derived from medicinal plants.[2] Soxhlet extraction method has proven excellent for extraction of such biologically active components.[6] The identification and evaluation of these biologically active components can be done best by using Gas Chromatography-Mass Spectroscopy.[7,8]

The objective of this study includes isolation and identification of oral and dental pathogens, extraction of bioactive components from selected herbal plants using various solvents, identifying bioactive compounds in herbal extracts by performing Qualitative Phytochemical analysis and GC-MS analysis and studying the antimicrobial effects of herbal extracts on the screened oral and dental pathogens.

MATERIALS AND METHODS

1. Sample collection

Mouth rinse sample: Two subjects having healthy oral environment were asked to rinse their mouth with 20 ml sterile saline for 60 seconds before brushing in the early morning. The saline was then collected in sterile wide mouth screw capped bottle.

Root canal rinse sample: The root canal rinse sample was collected from Safalya Dental Clinic located at Chembur, under the guidance of Dr. Rasik Warbhuvan. The root canal rinse was transferred to sterile liquid dental transport medium (Ref). Two root canal rinse samples of two patients were collected.

Decayed tooth sample: The decayed tooth sample was collected from Safalya Dental Clinic. The decayed tooth was transferred in sterile liquid dental transport medium.

All the collected samples were stored at 4ºC until further processing.

2. Isolation of oral microflora and dental pathogens: The above samples were vortexed thoroughly for 10 minutes. A 1:100 dilution of each sample was streaked on sterile Brain Heart Infusion (BHI) agar. The plates were then incubated at 37ºC for 24 hours. The biochemical identification of the isolates was performed using VITEK 2 automated biochemical identification system.

3. Selection of herbal plants: Healthy and normal parts of plants like Bark of *Acacia catechu* (Khadir, Kattha),[9] Stem of *Glycyrrhiza glabra* L. (Yashtimadhu),[10] Leaves of *Piper betel* (Paan),[11] were selected under the guidance of Dr. Shyam Nabar, Associate Prof. at Sion Ayurveda College. Selected plant materials were washed thoroughly with distilled water. Further they were incubated at 40ºC for drying. The dried plant samples were finely powdered in an electric blender. The powdered samples were filled in clean bottles and kept in desiccators until further use.[11]

4. Preparation of plant extracts: Soxhlet extraction system was employed for preparation of plant extract. For this, 15 grams of powdered sample was added into the thimble and extracted in 150 ml of solvent. Two solvents were used viz. 70% ethanol and distilled water. Temperature of 78ºC was maintained continuously during extraction. The extraction process was continued till a clear solvent solution was observed in the thimble holder. The crude plant extract was removed from the Soxhlet extraction flask and was concentrated to dryness

in rotary vacuum evaporator at 50ºC. The dried extract was weighed, recorded and stored at 4ºC and used for the antimicrobial testing.[11] The dried extracts were dissolved in respective solvents and an equivalent concentration of 5% was maintained throughout for all the samples.

5. Antimicrobial testing: Agar well diffusion method was used for antimicrobial testing. For this, 1ml of culture suspension of each isolate (O.D. adjusted to 0.1 at 530 nm) was added to sterile Muller Hinton (MH) agar butts and transferred to sterile petri-plates. The plates were allowed to solidify.[12] Wells of 8 mm were aseptically punched on the agar using a sterile cork borer. 50 µl of the individual plant extract were introduced into the wells using micropipettes. The plates were then incubated at 37ºC for 24 hours.[13] The results for zone of inhibition were observed and noted. *Escherichia coli* and *Staphylococcus aureus* were used as standard laboratory cultures. Standard antibiotics viz. ampicillin and streptomycin (100 µg/ml each) were used as positive controls. Similarly, commercially available mouthwashes such as Colgate Plax and Listerine were also used as positive controls. The solvents used for extraction i.e. 70% ethanol and distilled water were used as negative controls.

6. **Identification of bioactive components:** Phytochemical analysis of 70% ethanolic extract of betel leaf was carried out to determine the presence of secondary metabolites like phlobatannins, reducing sugar, terpenoids, phenols, flavonoids, cardial glycosides, diterpenes, phytosterol, alkaloids, glycosides, tannins and saponins. The tests were performed as per the standard protocols.[14] The identification of bioactive components was done by using GC-MS[8] with minor modification in which initial oven temperature was set at 300ºC for 5 mins. Helium gas (99.999%) was used as carrier gas at constant flow rate 3.0 ml/min. Total GC running time was 30 minutes.

RESULTS

In all 5 different isolates were obtained from oral samples as mentioned in table 1. *Streptococcus salivarius* is a commensal bacterium of the oral cavity in humans and is responsible for oral health and diseases. Similarly, *Streptococcus sanguinis* is a normal inhabitant of the human oral cavity. It is also one of the most common agents responsible for serious endovascular infection.[15] *Raoultella ornithinolytica* is gram-negative, aerobic, encapsulated bacillus belonging to the Enterobacteriaceae family. It has been isolated from dentin of infected root canals. However, human infections caused by bacteria of the genus *Raoultella* are infrequent.[16] *Stenotrophomonas maltophilia* is a well known human

pathogen and is primarily responsible for nosocomial infections. *Pseudomonas aeruginosa* is commonly isolated from various oral samples. The organism is involved in various types of oral infections.

Table. 1: Microscopic and biochemical identification of the isolates.

Isolates	Gram nature	Morphology	Identified as
MR1	Gram positive	Cocci	*Streptococcus salivarius*
MR2	Gram positive	Cocci	*Streptococcus salivarius*
RC1	Gram positive	Cocci	*Streptococcus sanguinis*
RC2a	Gram negative	Bacilli	*Raoultella ornithinolytica*
RC2b	Gram negative	Bacilli	*Stenotrophomonas maltophilia*
T1	Gram negative	Bacilli	*Pseudomonas aeruginosa*

Key: MR: Mouth Rinse, RC: Root Canal, T: Decayed Tooth.

Antimicrobial effect of herbal extracts on oral micro flora and dental pathogens

1. Antimicrobial activity of 70% ethanolic extracts

Figure 2 represents the antimicrobial activity of 70% ethanolic extracts on isolates. It was observed that Betel leaf extract in 70% ethanol showed maximum zone of inhibition against *Streptococcus salivarius* (MR1), *S. salivarius* (MR2), *S. sanguinis* (RC1), *R. ornithinolytica* (RC2a), *S. maltophilia* (RC2b) and *P. aeruginosa* (T1) i.e. 24.33 mm, 30 mm, 19 mm, 20.33 mm, 27 mm and 22 mm respectively as well as against *E. coli* and *S.aureus* i.e. 19.67 mm and 16.33 mm respectively; Yashtimadhu extract in 70% ethanol showed zone of inhibition against *S. salivarius* (MR1), *S. salivarius* (MR2), *S. sanguinis* (RC1) and *P. aeruginosa* (T1) i.e. 22 mm, 21 mm, 16.67 mm and 11.67 mm respectively; whereas, Khadir extract in 70% ethanol showed zone of inhibition against *S. salivarius* (MR1), *S. salivarius* (MR2) and *S. sanguinis* (RC1) i.e. 17.67 mm, 15.33 mm and 11.33 mm respectively. The negative control 70% ethanol showed no antimicrobial activity against any of the isolates.

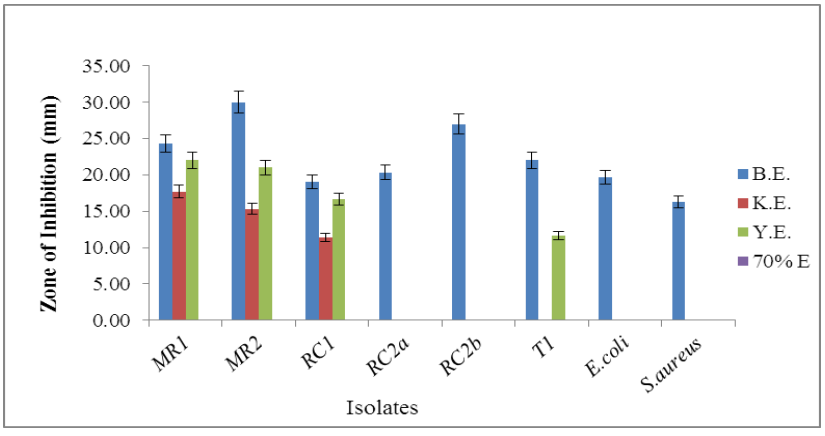


Figure. 1: Antimicrobial activity of 70% ethanolic extracts on isolates.

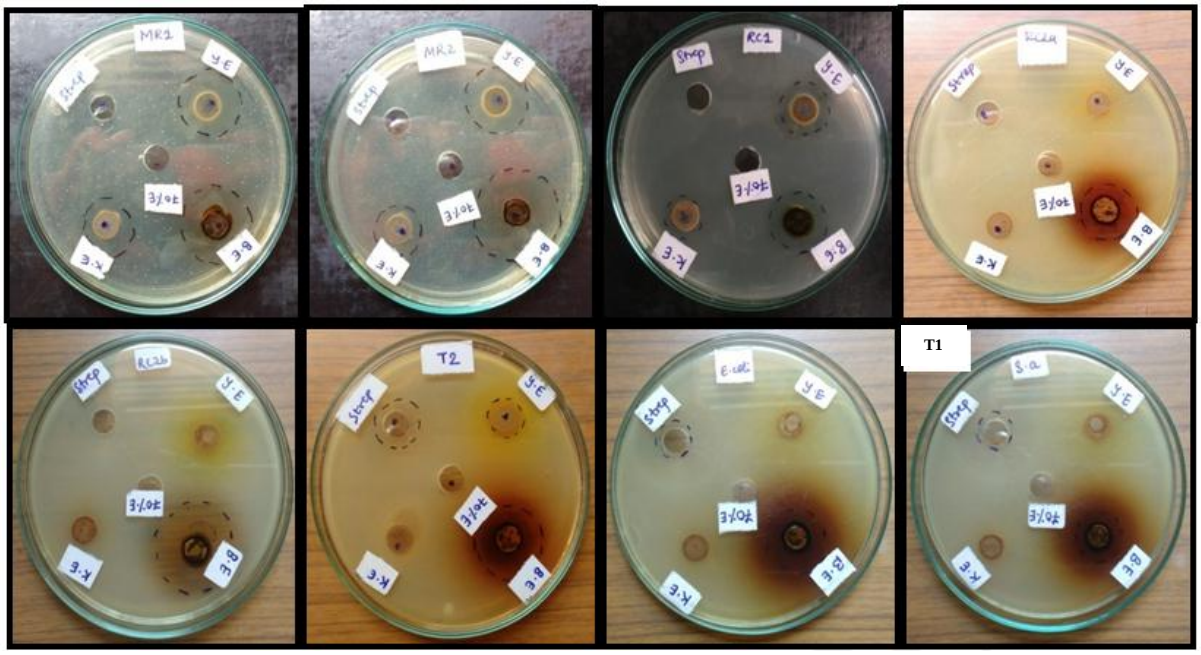


Figure. 2: Antimicrobial activity of 70% ethanolic extracts on isolates.

(Line 1 L-R: MR1, MR2, RC1; Line 2 L-R: RC2a, RC2b, T1, Line 3 L-R: E. coli, S. aureus).

2. Antimicrobial activity of aqueous extracts

Figure 3 represents the antimicrobial activity of aqueous extracts on isolates. It was observed that Betel leaf and Yashtimadhu aqueous extract showed zone of inhibition against only three isolates *S. salivarius* (MR1), *S. salivarius* (MR2), *S. sanguinis* (RC1). The average zone of inhibition for betel leaf extract was observed to be 19 mm, 16.33 mm and 11 mm against *S. salivarius* (MR1), *S. salivarius* (MR2), *S. sanguinis* (RC1) respectively and for Yashtimadhu extract the average zone of inhibition was observed to be 17.67 mm, 14.67 mm and 11.33 mm against *S. salivarius* (MR1), *S. salivarius* (MR2), *S. sanguinis* (RC1) respectively. Khadir aqueous extract showed no activity on any of the isolates. *E. coli* and *S. aureus* were not inhibited by any of the aqueous extracts. The negative control sterile distilled water showed no antimicrobial activity against any of the isolates.

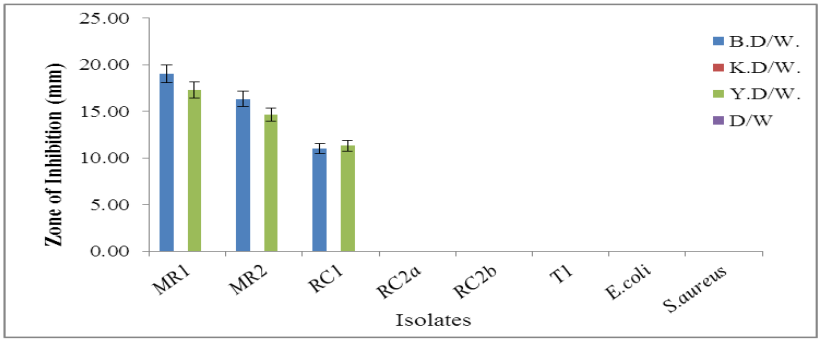


Figure. 3: Antimicrobial activity of aqueous extracts on isolates.

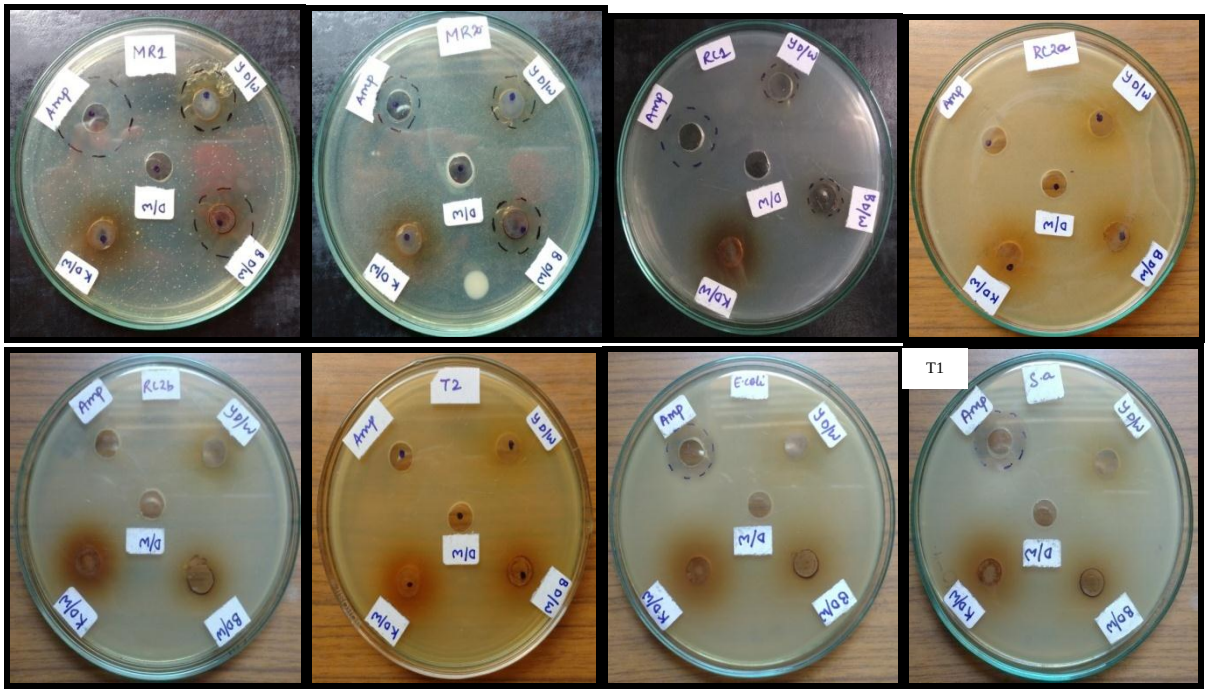
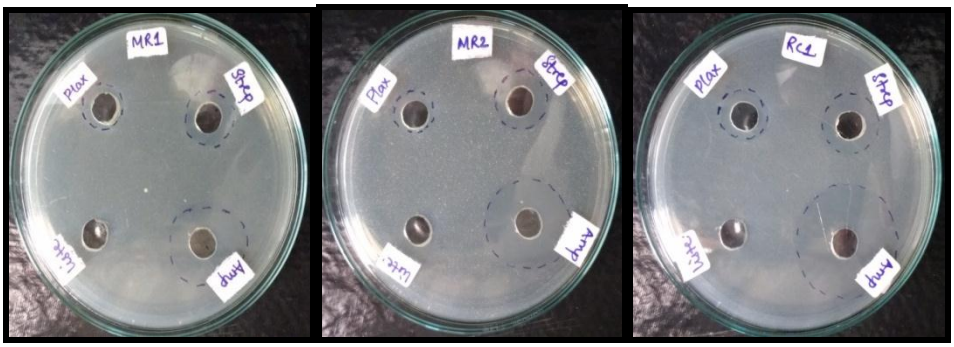


Figure. 4: Antimicrobial activity of aqueous extracts on isolates.

(Line 1 L-R: MR1, MR2, RC1; Line 2 L-R: RC2a, RC2b, T1, Line 3 L-R: E. coli, S. aureus)

3.**Antimicrobial activity of positive controls:** Figure 5 represents the antimicrobial activity of positive controls on isolates. It can be observed that standard antibiotics ampicillin showed equivalent inhibition as 70% ethanolic extract of betel leaf against *S. salivarius, R. ornithinolytica, S. maltophilia, P. aeruginosa* and *E. coli* whereas greater inhibiton was seen against *S. sanguinis* and *S. aureus* whereas streptomycin at the concentration of 100 µg/ml also did not show significant inhibition against these isolates as observed in 70% ethanolic betel leaf extract. Ampicillin proved to be better antibiotic than streptomycin. Further, commercially available oral cleansing products such as Colgate Plax and Listerine were tested against the oral isolates. Colgate plax showed very less antimicrobial activity. No significant antimicrobial activity of Listerine was seen against any oral isolates.



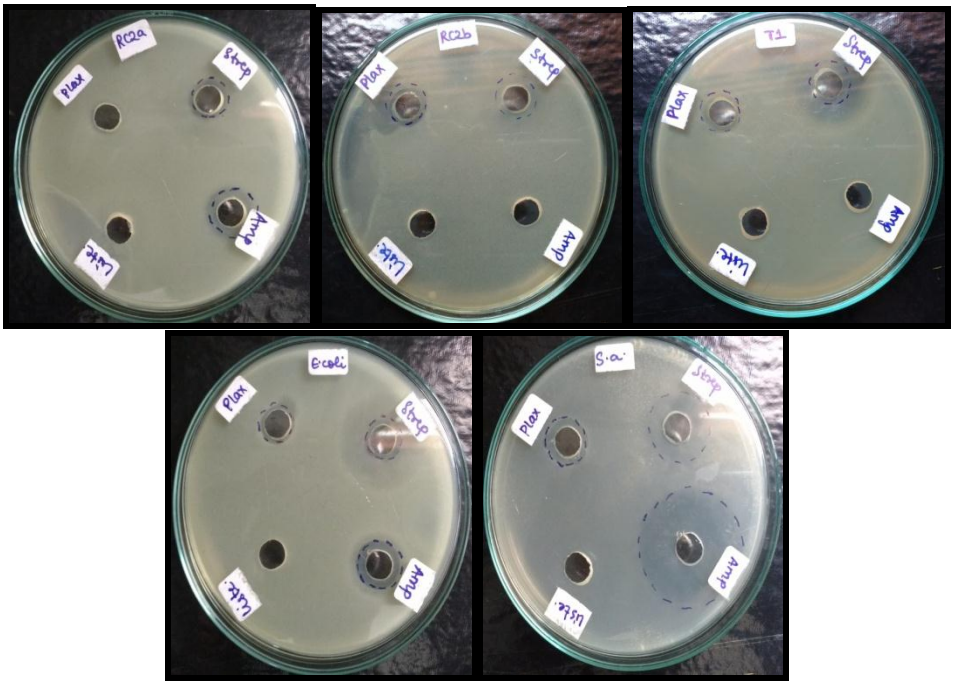


Figure. 6: Antimicrobial activity of positive controls on isolates.

(Line 1 L-R: MR1, MR2, RC1; Line 2 L-R: RC2a, RC2b, T1, Line 3 L-R: E. coli, S. aureus)

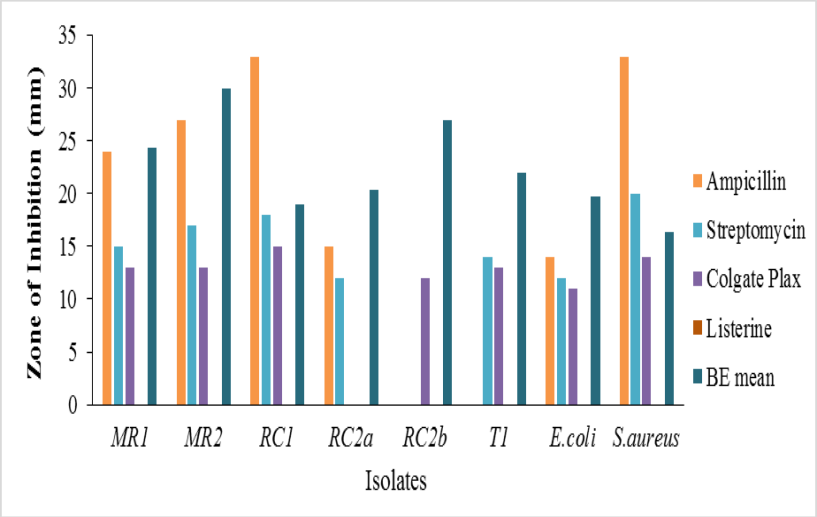


Figure. 5: Antimicrobial activity of 70% ethanol and its comparison to positive controls.

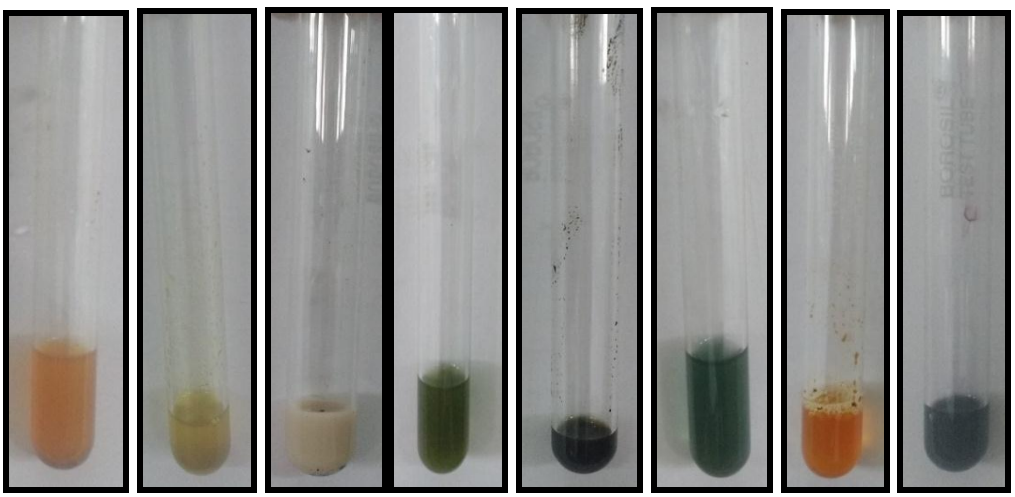
Identification of the bioactive components present in 70% ethanolic extract of betel leaf

1. Qualitative phytochemical analysis

Standard phytochemical analysis was carried out for the 70% ethanolic extract of betel leaf as per the standard protocols given in the reference.[14] The qualitative phytochemical analysis of 70% ethanolic betel leaf extract showed presence of diterpenes, flavonoids, triterpenes, alkaloids, amino acids, tannins and cardial glycosides (Table 2).

Table. 2: Qualitative phytochemical analysis for 70% ethanolic extract of betel leaf.

TEST	OBSERVATION	INFERENCE
Detection of alkaloids		
a) Wagner's Test	Formation of brown color precipitate	Alkaloids present
b) Hager's Test	Formation of yellow colored precipitate	Alkaloids present
Detection of carbohydrates		
a) Molisch's Test	No violet ring was formed at the junction	Carbohydrates absent
b) Benedict's Test	No orange red precipitate	Reducing sugars absent
Detection of phenols		
a) Ferric Chloride Test	No formation of bluish black colour	Phenols absent
Detection of diterpenes		
a) Copper acetate Test	Formation of emerald green colour	Diterpenes present
Detection of proteins and amino acids		
a) Xanthoproteic Test	No formation of yellow colour	Proteins absent
b) Ninhydrin Test	Formation of blue colour	Amino acid present
Detection of flavonoids		
a) Alkaline Reagent Test	No formation of intense yellow colour, which becomes colourless on addition of dilute acid	Flavonoids absent
b) Lead acetate Test	Formation of yellow colour precipitate	Flavonoids present
Detection of tannins		
a) Gelatin Test	Formation of white precipitate	Presence of tannins
Detection of phytosterols		
a) Salkowski's Test	Appearance of golden yellow colour	Presence of triterpenes
Detection of cardiac glycosides		
a) Legal's Test	Formation of pink to blood red colour	Cardiac glycosides present
Detection of glycosides		
a) Modified Borntrager's Test	No formation of rose-pink colour in the ammonical layer	Anthranol glycosides absent
Detection of saponins		
a) Froth Test	No formation of 1 cm layer of foam	Saponins absent
b) Foam Test	Foam was not produced	Saponins absent



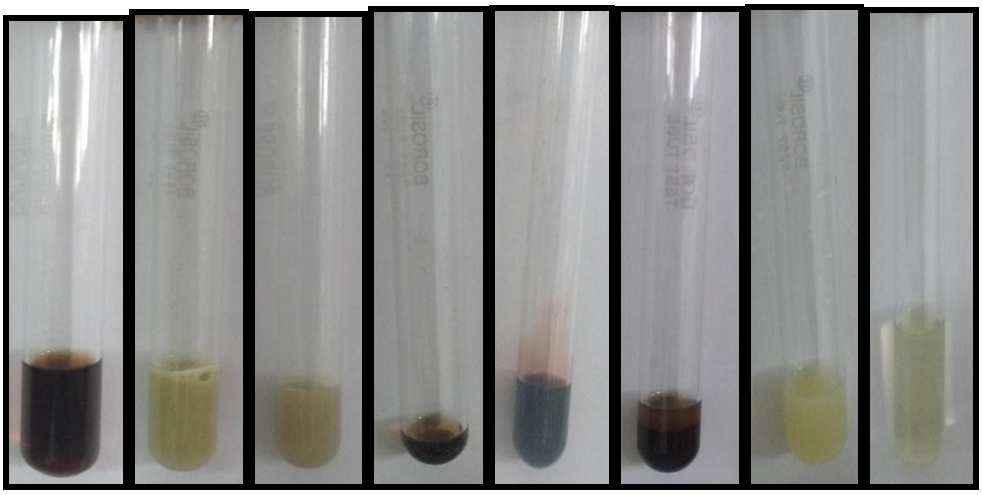


Figure. 7: Qualitative phytochemical analysis of 70% ethanolic betel leaf extract.

(Line 1 L-R: Wagner's test, Hager's test, Molishch's test, Benedict's test, Ferric chloride test, Copper acetate test, Xanthoproteic test, Ninhydrin test; Line 2 L-R: Alkaline reagent test, Lead acetate test, Gelatin test, Salkowski's test, Legal's test, Modified Borntrager's test, Froth test, Foam test).

2. Gas-chromatography and Mass spectroscopy (GC-MS) analysis

The bioactive components from 70% ethanolic extract of betel leaf were successfully analysed using GC-MS (figure 8). 129 components were detected using GC-MS as shown below in Table 10. The components showing higher retention time (RT) i.e. higher peaks in chromatogram were selected and their functions were studied.

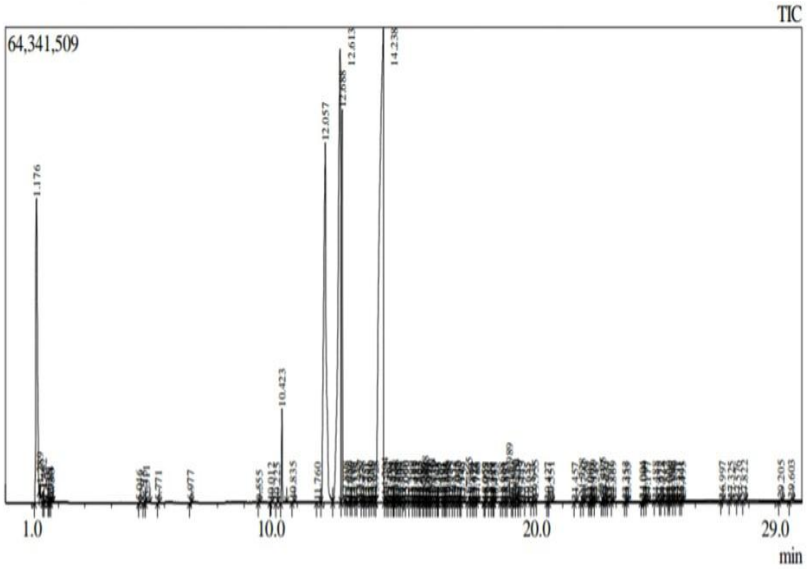


Figure. 8: GC-MS of 70% ethanolic extract of betel leaf.

Table. 10: GC-MS data of important bioactive compounds with their function in 70% ethanolic extraction of betel leaf.

	RT (min)	Peak area (%)	Name	Compound Nature	Function
1.	1.176	10.20	Methyl hydrogen disulfide	-	-
2.	1.289	0.06	Hexane	-	-
3.	1.432	0.10	Acetic acid	-	-
4.	1.475	0.06	Butanal, 3-methyl	Ester	Antimicrobial, Antifungal
5.	5.311	0.08	Benzene acetaldehyde	Aromatic aldehyde	Antimicrobial
6.	10.423	1.33	Phenol, 2- methoxy-3- (2-propenyl)-	-	-
7.	12.057	18.09	Benzoic acid, 2,5-dimethyl	Aromatic carboxcylic acid/ Benzoic acid	Antimicrobial, Food preservation
8.	12.613	24.18	4- allyl-1,2-diacetoxybenzene	-	Antibacterial, Antifungal
9.	12.688	7.91	Phenol, 2- methoxy- 4- (2-propenyl), acetate	Phenolic compound	Antimicrobial
10.	14.238	35.59	Benzoic acid, 2,3-dimethyl-	Aromatic carboxcylic acid/ Benzoic acid	Antimicrobial, Food preservation
11.	14.294	0.11	2- cyclohexene-1-one, 4-(3- hydroxy-1-butenyl)-3,5,5- trimethyl-	-	-
12.	14.688	0.06	Caryophylleneoxide	Terpene	Antimicrobial, Antifungal, Anti- coagulant, Antioxidant,
13.	15.808	0.09	5,5,8a- trimethyl-3,5,6,7,8,8a- hexahydro-2H- chromene	Flavonoid compound	Antimicrobial, Anti-inflammatory, Antioxidant
14.	15.865	0.01	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7- dimethyl-1-(1- methyl)-,(1S- cis)-	Alkenes/Terpenes	Antimicrobial
15.	17.465	0.11	n- Hexadecanoic acid	Palmitic acid	Antimicrobial, hypocholesterolemic, nematicide
16.	18.989	0.26	Phytol	Diterpene	Antimicrobial, Anticancer, anti-inflammatory, Anti-diuretic and Anti-diabetic
17.	19.254	0.11	Erucic acid	Fatty acids	Antibacterial

DISCUSSION

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. The major advantage of using natural products is that they are devoid of artificial chemical constituents and hence they do not possess any side effects. Herbal plants show high antimicrobial activity due to the presence of many bioactive components.

Previous studies have demonstrated the antimicrobial activity of certain herbal plants extracts such as peppermint,[17] miswak,[18] amla,[19] etc. In the present study, three herbal plants were used viz. betel leaf, yashtimadhu and khadir to study their antimicrobial activity against 6 oral/dental isolates identified as *S salivarius, S sanguinis, R. ornithinolytica, S. maltophilia, P. aeruginosa* isolated from mouth rinse, root canal rinse and decayed tooth sample. The antimicrobial activity of betel leaf, yashtimadhu and khadir was also studied earlier. *Ghazvini K. et. al., 2012,*studied the antibacterial activity of *Glycyrrhiza glabra* against oral pathogens however, these were laboratory cultures, *Chakraborty D. et al., 2011,*studied the antimicrobial, antioxidative and antihemolytic activity of *Piper betel* leaf extracts, *Geetha R.V. et.al., 2013,*assessed the anti bacterial activity of ethanolic extract of *Heart wood of Acacia catechu willd*, *Aesculus hippocastanum*, *Glycyrrhiza glabra*, *Lakshmi T. et. al., 2011,* studied anti bacterial activity of ethanolic bark extract of *Acacia catechu willd* against enteric pathogens.

In the present study Soxhlet extraction method successfully extracted the bioactive components from the herbal plants using 70% ethanol and distilled water as solvents. This method of extraction was also used by *Datta A. et. al., 2011***.**

70% ethanolic extracts of all three herbal plants showed higher antimicrobial activity as compared to their aqueous extracts. Also, betel leaf extract proved to have higher antimicrobial activity followed by yashtimadhu and the least was observed with khadir. This indicates that *Piper betel* contains broad-spectrum antibacterial compounds which make it a potentially good source of antimicrobial substance. Standard antibiotic ampicillin showed equivalent inhibition as 70% ethanolic extract of betel leaf against *S. salivarius, R. ornithinolytica, S. maltophilia, P. aeruginosa* and *E. coli* whereas greater inhibiton was seen against *S. sanguinis* and *S. aureus* whereas streptomycin at the concentration of 100 µg/ml also did not show significant inhibition against these isolates as observed in 70% ethanolic betel leaf extract. Ampicillin proved to be better antibiotic than streptomycin. Commercially available mouthwashes Colgate Plax and Listerine were also tested for their antimicrobial activity against oral and dental isolates. As compared to betel leaf extract, colgate plax showed very less antimicrobial activity and no antimicrobial activity was observed with Listerine. Thus, we can say that herbal plants extracts are more effective in vivo against the isolates than commercially available mouthwashes.

The qualitative phytochemical analysis of 70% ethanolic betel leaf extract showed presence of diterpenes, flavonoids, triterpenes, alkaloids, amino acids, tannins and cardial glycosides. In a study carried out by *Patil R.S. et al., 2015* the phytochemical screening reveals that, the aqueous, 70% ethanolic and butanolic extracts contains valuable phytochemicals like steroids, diterpenes, tannin, flavonoids etc. and found rich in phytochemicals. in 70% ethanolic betel leaf extract.

The present study also successfully identified the bioactive components present in 70% ethanolic betel leaf extract using GC-MS of which Benzoic acid, 2,3- dimethyl- (35.59%), 4-allyl-1,2- diacetoxybenzene (24.18%), Benzoic acid, 2,5- dimethyl (18.09%), Methyl hydrogen disulfide (10.20%), Phenol, 2- methoxy- 4- (2- propenyl), acetate (7.91%), Phenol, 2- methoxy-3- (2- propenyl)- (1.33%), Phytol (0.26%), n- Hexadecanoic acid (0.11%), 2-cyclohexene-1-one, 4- (3- hydroxy-1-butenyl)- 3,5,5- trimethyl- (0.11%), 5,5,8a- trimethyl-3,5,6,7,8,8a- hexahydro-2H- chromene (0.09%), Benzene acetaldehyde (0.08%), Caryophyllene oxide (0.06%), Butanal, 3-methyl (0.06%) and Naphthalene,1,2,3,5,6,8a-hexahydro-4,7- dimethyl-1-(1- methyl)-,(1S- cis)- (0.01%) were present majorly and were identified by the National Institute of Standard and Technology (NIST). Many of these compounds are known to have antimicrobial, anti-cancerous, anti-inflammatory properties. Similar GC-MS analysis profile has also been reported by *Foo L W. et al., 2015.* Most of the identified compounds obtained in the present study were similar to the compounds obtained by *Foo L W. et al., 2015.*

CONCLUSION

Betel leaf, Yashtimadhu and Khadir were used to study their antimicrobial activity against oral and dental isolates. The bioactive components were extracted in 70% ethanol and distilled water. Based on the current findings it was observed that 70% ethanolic betel leaf extract exhibited good antimicrobial potential than the aqueous betel leaf extract and 70% ethanolic and aqueous extracts of yashtimadhu and khadir. 70% ethanolic betel leaf extract was effective against both gram positive as well as gram negative oral microflora and dental isolates; also 70% ethanolic betel leaf extract showed significant inhibition almost equal to the standard antibiotic ampicillin and much greater than streptomycin at the concentration of 100 µg/ml and the commercially available oral cleansing products viz. Colgate Plax and Listerine. Thus, *Piper betel* leaf should be considered having beneficial potential in dentistry field as oral care products such as toothpaste and mouthwash. The phytochemical analysis

revealed the presence of many bioactive components using qualitative phytochemical analysis and GC-MS. Some of them were found to possess antimicrobial activity. Further studies are needed to be carried out to purify the active compounds present in *Piper betel* and also its antimicrobial activity will be studied to develop herbal mouthwash. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may lead to improvement in the general dental health of the population.

REFERENCES

1. Daniyan S.Y., Abalaka M.E., Elemba O.M., Aransiola S.A. In vitro Antimicrobial activity and phytochemical screening of Jathropa curcas seed extract. International Research Journal of Pharmacy, 2011; 2(7): 60-64.
2. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evidence-based Complement Altern Med. 2011. doi:10.1093/ecam/nep067.
3. Rajendra Kumar G, Rk G, Devanand G, et al. Antiplaque Efficacy of Aloe Vera… Preliminary antiplaque efficacy of aloe vera mouthwash on 4 day plaque re-growth model: randomized control trial. doi:10.4314/ejhs.v24i2.6.
4. Shafiei Z, Najwa Shuhairi N, Md Fazly Shah Yap N, Harry Sibungkil C-A, Latip J. Antibacterial Activity of Myristica fragrans against Oral Pathogens. Evidence-Based Complement Altern Med. 2012;7. doi:10.1155/2012/825362.
5. M. D. Luque de Castro, L. E. Garcia Ayuso. III /Environmental Applications/ Soxhlet Extraction. 2701-2709, Spain; 2000. Academic Press.
6. Syed Zameer Hussain and Khushnuma Maqbool. GC-MS: Principle, Technique and its application in Food Science. Int. J. Curr. Sci. 2014, 13: E 116-126.
7. Foo LW, Salleh E, Nur S, Mamat H. Extraction and Qualitative Analysis of Piper Betle Leaves for Antimicrobial Activities. SitiNur Hana Mamat Int J Eng Technol Sci Res IJETSR www.ijetsr.com ISSN, 2015; 2: 2394-3386.
8. Jayshree D. Patel, Devang K. Patel, Anshu Shrivastava and Vipin Kumar. Screening of plant extracts used in traditional antidiarrhoeal medicines against pathogenic Escherichia coli. Scientific World, July 2008; 6(6).
9. Anagha K, Manasi D, Priya L, Meera M. Antimicrobial activity of Yashtimadhu (Glycyrrhiza glabra L.) -A Review. Int J Curr Microbiol App Sci., 2014; 3(1): 329-336.
10. Datta A, Ghoshdastidar S, Singh M. Antimicrobial Property of Piper betel Leaf against

Clinical Isolates of Bacteria. Int J Pharma Sci Res (IJPSR), 2011; 2(3): 104-109.

11. Sham Shihabudeen MH, Priscilla HD, Thirumurugan K. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int J Pharma Sci Res., 2010; 1(10): 430-434.
12. Omoregie Gloria Omorowa, Macdonald Idu, Ovuakporie-Uvo Oghale. GC-MS analysis of the aqueous extracts of Buchholzia coriacea engl (capparidaceae), seeds. International Journal of Life science and Pharma Research, 2015; 5(3): ISSN: 2250-0480.
13. Tiwari P. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia | Jan-March 2011; 1(1): 98-106.
14. Sujana P, Sridhar TM, Josthna P, Naidu CV. Antibacterial Activity and Phytochemical Analysis of Mentha piperita L. (Peppermint)—An Important Multipurpose Medicinal Plant. Am J Plant Sci. 2013;4:77-83. doi:10.4236/ajps.2013.41012.
15. Sehmi Paik, Lauren Senty, Sankar Das, Jody C. Noe, Cindy L. Munro, and Todd Kitten, Identification of Virulence Determinants for Endocarditis in Streptococcus sanguinis by Signature-Tagged Mutagenesis, Infection And Immunity, Sept. 2005; 73(9): 6064–6074.
16. Victoria Pulian Morais, Matilde Trigo Daporta, Alberto Fernandez Bao and Marta Garcia Campello, Enteric Fever-Like Syndrome Caused by Raoultella ornithinolytica, Letters to the Editor, Journal of Clinical Microbiology, Mar. 2009; 47(3): 868–869
17. Darout IA, Christy AA, Skaug N, Egeberg PK. Antimicrobial anionic components in miswak extract identification and quantification of some potentially antimicrobial anionic components in miswak extract. Indian J Pharmacol, 2000; 32: 11-14.
18. Saeed S, Tariq P. Antimicrobial activities of Emblica officinalis and Coriandrum sativum against gram positive bacteria and Candida albicans. Pak. J. Bot., 2007, 39(3): 913-917.
19. Agarwal T, Singh R, Shukla AD, Waris I, Gujrati A. Comparative analysis of antibacterial activity of four Piper betel varieties. Advances in Applied Science Research, 2012; 3(2): 698-705.
20. Caburian AB, Osi MO. Characterization and Evaluation of Antimicrobial Activity of the Essential Oil from the Leaves of Piper betle L. E-International Sci Res J. 2010; ISSN(1): 2094-1749.
21. Chakraborty D, Shah B. Antimicrobial, anti-oxidative and anti-hemolytic activity of piper betel leaf extracts. International Journal of Pharmacy and Pharmaceutical Sciences, Vol 3, Suppl 3, 2011, pp. 192-199.
22. Fereshteh Sedighinia, Akbar Safipour Afshar, Saman soleimanpour, Reza zarif, JavadAsili, Kiarash Ghazvini. Antibacterial activity of Glycyrrhiza glabra against oral

pathogens: an in vitro study. Avicenna Journal of Phytomedicine, Summer 2012; 2(3).

23. H. Shyla Jebashree, S. Jayasurya Kingsley, Emmanuel S. Sathish, and D. Devapriya. Antimicrobial Activity of Few Medicinal Plants against Clinically Isolated Human Cariogenic Pathogens—An In Vitro Study. International Scholarly Research Network ISRN Dentistry Volume 2011, Article ID 541421, 6 pages doi:10.5402/2011/541421.
24. Lakshmi. T, Geetha R.V, Anitha Roy. In Vitro Anti Bacterial Activity of Ethanolic Bark Extract of Acacia catechu Willd against Enteric Pathogens. International Journal of Drug Development & Research July-September 2011; 3(3): ISSN 0975-9344.
25. Meghashri, Shubha Gopal. In Vitro Antifungal and Antibacterial Activities of Root Extract of Glycyrrhiza Glabra. Journal of Applied Sciences Research., October 2009 pp.1436-1439.
26. Sita kumari and Nirmala Babu Rao. Phyto chemical analysis of piper betel leaf extract. World journal of pharmacy and pharmaceutical sciences, 4(1): 699-703.
27. Patil RS, Harale PM, Shivangekar KV, Kumbhar PP, Desai RR. Phytochemical potential and in vitro antimicrobial activity of Piper betle Linn. leaf extracts. J Chem Pharm Res. 2015; 7(5): 1095-1101.