Asian Pac. J. Health Sci., 2014; 1(3):255-263 ISSN: 2349-0659

Document heading doi: 10.21276/apjhs.2014.1.3.20 Original Article

Phytochemical screening and antimicrobial activity of some medicinal plants against oral flora

Preeti Gauniyal*, Udai Vir Singh Teotia

Research scholar of Shri Venkateshwara University, Gajraula, Amroha (Uttar Pradesh), India
HOD of Life Science, Shri Venkateshwara University, Gajraula, Amroha (Uttar Pradesh), India

ABSTRACT

The present study was carried out to evaluate the phytochemical and antimicrobial activity of some medicinal plants against five microbial strains causing oral infections. The phytochemical analysis carried out revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, reducing sugar and steroids in most of the medicinal plants. The antimicrobial activity of ethanolic extract of medicinal plants were evaluated using well diffusion method against *Streptococcus mutans, Enterococcus faecalis, Lactobacillus acidophilus ,Candida albicans and Candida tropicalis*. Ethanolic extracts of *Aloe barbadensis,Cinnamum zeylanicum* and *Tinospora coridfolia*, were not effective against *Streptococcus mutans*and *Enterococcus faecalis* respectively. However, *Azadirachta indica, Centella asiatica, Zingiber officinale* were showing week and the extract of *Allium sativum, Curcuma longa, Glycyrrhiza glabra, Ocimum sanctum, Piper nigrum,* displaying strong antimicrobial activity against most of the test species. The ethanol extract of *Syzygium aromaticum* showing strong antimicrobial activity against all test species. The results provide justification for the use of the medicinal plants to treat various oral infections.

Keywords: Medicinal plants, well diffusion method, Antimicrobial activity.

INTRODUCTION

Despite great improvements in the global oral health status, dental caries still remainsone of the most prevalent diseases [1]. The early stage of dental caries is characterizedby a destruction of superficial dental structures caused by acids which are byproductsof sugar metabolism by *Streptococcus mutans,* a cariogenic bacterium. Colonization of teeth by cariogenic bacteria is one of the most important risk factor in the development ofdental diseases [2].*S. mutans* and *Candida albicans* are the two microbes often implicated in oral diseases, *C. albicans* is the most common yeast isolated from the oral cavity and a common cause of oral thrush,vaginitis, endocarditis, septicemia and infection of skin, nails and lungs [3,4,5]. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to intracanal medication [6,7].

**Correspondence*
Preeti Gauniyal
Research scholar, Shri Venkateshwara University, Gajraula, Amroha (Uttar Pradesh), India
E Mail: preetidevesh84@gmail.com

Enterococcus faecalis has been detected in asymptomatic and persistent root canal infections. *E.faecalis* has also been detected in 77% of failedendodontic cases and in 50% of cases with chronic apical periodontitis[8].*Lactobacillus acidophilus,Candida albicans, Candida tropicalis* etc are some other microbial species that knowingly cause several oral diseases, such as dental caries, endodontic infections, periodontal diseases and oral Candidiasis[9, 10].

2% Chlorhexidine Gluconate (CHX) has been used as an irrigant and intracanal medicament. CHX is a bis-biguanide that acts by adsorbing onto the cell wall of microorganism resulting in leakage of intracellular components. CHX has a broad-spectrum antimicrobial activity, targeting both Gram-positive and Gram-negative microbes and is biocompatible[11, 12]. But it may have a toxic effect on host tissue if expressed beyond the confines of root canal and impairs healing. It also under goes chemical reaction with NaOCl forming precipitates para-chloroaniline (PCA) which is carcinogenic[13]. Considering the ineffectiveness, potential side effect and safety concerns of synthetic

drugs, the herbal alternatives for oral use might prove as advantageous.

Therefore, there is a continuing need to search for new antimicrobial agents [14]. Over the last decade, plants antimicrobial activity has been studied in different regions of the world[15].

Many studies had shown that the medicinal plants are as good as the conventional ones (in controlling oral bacterial load)[16].The use of plant-derived toothbrushes (chewing-sticks) is a common traditional dental care practice in many parts of the world. Within a given community, chewing-stick plants are often specific, but tend to vary from one culture to the next [17]. As far back as the 1970s, it was suggested that the regular use of the African chewing-stick, acting as an antiseptic, may control the formation and activity of dental plaque and therefore reduce the incidence of gingivitis and possibly dental caries [18].

There is a need to screen medicinal plants for their promising biological activity. In the present study we studied the Phytochemical screening and antimicrobial activity of some medicinal plants against oral flora.

MATERIAL AND METHODS

Collection and Identification of Medicinal plants

The medicinal plants listed in table 1 were collected from the different forest and market of Himachal Pradesh and Uttarakhand.

Table 1: List of medicinal plant used in the study

Sr. No.	Botanical name of Plant	Common name	Family	Part Used
1.	*Allium sativum*	Garlic	Liliaceae	Bulb
2.	*Aloe barbadensis*	Aloe vera	Liliaceae	Leaves
3.	*Azadirachta indica*	Neem	Meliaceae	Leave
4.	*Centella asiatica*	Brahmi	Mackinlayaceae	Leave
5.	*Cinnamum zeylanicum*	Cinnamon	Lauraceae	Bark
6.	*Curcuma longa*	Turmeric, haldi	Zingiberaceae	Root
7.	*Glycyrrhiza glabra*	Mulethi	Leguminosae	Leave
8.	*Ocimum sanctum*	Tulsi	Lamiaceae	Leave
9.	*Piper nigrum*	Black Pepper	Piperaceae	Dried Berries
10.	*Syzygium aromaticum*	Laung, clove	Myrtaceae	Flower buds
11.	*Tinospora coridfolia*	Giloy	Meninspermaceae	Stem
12.	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizome

Preparation of extracts

Air shade dried powdered parts of medicinal plants material (100gm) of table no. 1, were extracted using ethanol (500ml) separately by soaking it for 48hrs at room temperature. The solvents were removed under reduced pressure to obtain crude extracts of ethanol.

Qualitative Analysis of Phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in various extracts of medicinal plants. The above phytoconstituents were tested as per the standard method [19, 20].

Preparation and Standardization of Microbial Inoculum

All the microbial strains used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India-*Streptococcus mutans* (MTCC 890, *Enterococcus faecalis*(MTCC 439), *Lactobacillus acidophilus* (MTCC 10307), *Candida albicans* (MTCC 854) and *Candida tropicalis (*MTCC 184).The microorganisms were subcultured onthe culture media recommended fordifferent microorganisms such as Brainheart infusion agar (*S. mutans and E. faecalis*), Lactobacillus MRS agar (*L. acidophilus*), Sabouraud's Dextrose Agar(*C.albicans* and*C.tropicalis*) and incubatedat 37°C. Turbidity produced was adjusted to match 0.5 McFarland standard (10^{8} cfu/ml) which was further adjusted 10 cfu/ml [21]

Antimicrobial activity

The antimicrobial activity of different plant extracts were evaluated by using the agar well diffusion technique. The 20 ml of sterilized agar's (Brain Heart Infusion Agar, Lactobacillus MRS Agar, Sabouraud's dextrose agar) were poured into sterile petriplate, after solidification, 100 µl of microbial inoculum were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher. The punched agars were filled with 100µl of plant extracts. 2% Chlorhexidine was taken as standard reference. The plates were incubated at 37ºC for 24 hours. After incubation, zone of inhibition for extracts were measured in millimeters using veneer calipers.

Statistical Analysis

The results will be subjected to statistical analysis. All the experiments were performed in triplicates. The values of zone of inhibition expressed in mean ± SD (standard deviation) of three triplicates.

RESULTS AND DISCUSSION

The ethanol extracts of twelve medicinal plants were tested against the oral microorganisms viz., *Streptococcus mutans*and *Lactobacillus acidophilus* most common microbescausing dental plaque and caries; *Enterococcus faecalis* associated with primary endodontic infections, persistent infection and asymptomatic chronic periradicular, *Candida albicans, Candida tropicalis* etc are some other fungal species that knowingly cause several oral diseases, such as endodontic infections, periodontal diseases and oral Candidiasis.

Phytochemical constituents such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins, and several other organic compounds are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects [22]. The present study carried out phytochemical analysis on the medicinal plant extracts revealed the presence of medicinally active constituents. The phytochemical constituents of the selected medicinal plants investigated are summarized in Table-2

Table 2: Phytochemical analysis of selected medicinal plants

Sr. No.	Ethanolic extract of Medicinal Plants	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Tannins	Reducing Sugars	Saponins
1.	*Allium sativum*	+	+	+	+	+	-	+	-
2.	*Aloe barbadensis*	+	-	+	+	+	+	+	-
3.	*Azadirachta indica*	+	+	+	+	+	-	+	+
4.	*Centella asiatica*	+	+	+	+	+	+	+	-
5.	*Cinnamum zeylanicum*	+	+	+	+	+	+	+	+
6.	*Curcuma longa*	+	-	-	+	-	+	+	-
7.	*Glycyrrhiza glabra*	-	-	-	+	+	-	-	+
8.	*Ocimum sanctum*	+	+	+	+	+	+	+	+
9.	*Piper nigrum*	+	+	+	+	+	+	-	+
10.	*Syzygium aromaticum*	+	+	-	+	+	+	+	+
11.	*Tinospora coridfolia*	+	+	+	+	+	+	+	+
12.	*Zingiber officinale*	+	+	+	-	+	+	+	+

The most important of these secondary metabolites include alkaloids, saponins, tannins, steroids andterpenoids, flavonoids etc [23]. Phytochemical screening of twelve medicinal plants studied showed

that most of the extracts have the saponins, tannins, steroids and flavonoids. The maximum numbers of secondary metabolites were found in *Cinnamum zeylanicum, Ocimum sanctum* and *Tinospora coridfolia*. The minimum numbers of secondary metabolites were observed in *Curcuma longa* and *Glycyrrhiza glabra*. The alkaloids have been investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic [24] and anti-inflammatory properties [25]. In the present study all medicinal plants showing presence of alkaloid except *Glycyrrhiza glabra*. The saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anti- cancer, anti-inflammatory, central nervous system activities [25] and weight loss. It is also known to have antifungal properties [26]. The plants having saponins are *Azadirachta indica, Cinnamum zeylanicum, Glycyrrhiza glabra, Ocimum sanctum, Piper nigrum, Syzygium aromaticum, Tinospora coridfolia and Zingiber officinale.* Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti- microbial properties. They are also used in nutrition, herbal medicine and cosmetics [27]. In this study the steroids are present in all medicinal plants except *Zingiber officinale*

Tannins bind to proline rich proteins and interfere with the protein synthesis [28].Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [29].

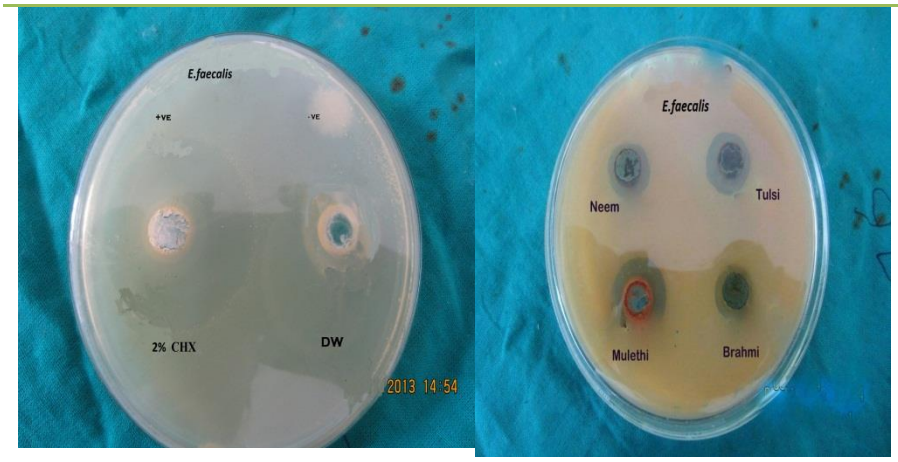
Glycosides were reported to exhibit anti-diabetic characteristics. Cardiac glycosides are known to hamper the Na+ /K+ pump and used in the treatment of congestive heart failure and cardiac arrhythmia [30].

Antimicrobial activities of the medicinal plants investigated are summarized in Table 3 andthe results are presented in Fig. 1,2,3,4and5.Twelve medicinal plants tested for antibacterial and antifungal activity, all plant extracts showed antimicrobial activity by inhibiting one or more oral microorganisms. The zone of inhibition by the test oral microbes against different medicinal plant extracts shows that ethanolic extracts of *Calendulla officinalis and Mangifera indica*, were not effective against *Strep. mutans, E.faecalis, C. tropicalis, L. acidophlius* respectively. However, *Lannea coromandelica (Houtt) Merr* and *Rosa centifolia* were showing week and the extract of *Acacia nilotica, Citrus limon, Emblica officinalis, Juglans regia, Psidium guajava L. and Withania somnifera* displaying strong antimicrobial activity, against all the test species.

Table 3: Antimicrobial activity of medicinal plants against oral microorganisms in millimeters

Sr. No.	Medicinal plant extracts /control groups	*Strep. mutan*	*E. faecalis*	*L. acidophilus*	*C. albicans*	*C.tropicalis*
1.	Chlorhexidine (+ ve control)	30.3 ± 2.0	30 ± 3	25 ± 1	20 ± 2.4	19 ± 1
2.	Distil water (-ve control)					-
3.	*Allium sativum*	25 ± 1.1	27 ± 2	18 ± 1	18 ± 3	15 ± 2
4.	*Aloe barbadensis*		15 ± 1	15 ± 2	20 ± 1	15 ± 1
5.	*Azadirachta indica*	17.6 ± 2.0	17.3 ± 1.5	22.3 ± 2.08	20.3 ± 3.05	19.3 ± 0.5
6.	*Centella asiatica*	15 ± 1	10 ± 2	15 ± 3	15 ± 4	14.6 ± 1.1
7.	*Cinnamum zeylanicum*			19.3 ± 2.0	25 ± 1	26.3 ± 1.5
8.	*Curcuma longa*	18 ± 2	21 ± 2	17 ± 2	21 ± 2	22.3 ± 2.08
9.	*Glycyrrhiza glabra*	20 ± 2	25 ± 3	19.3 ± 1.5	17 ± 2	18 ± 1
10.	*Ocimum sanctum*	20 ± 2	22 ± 2	17.6 ± 2.0	17 ± 2	16 ± 2
11.	*Piper nigrum*		20 ± 4	18 ± 1	25 ± 4	20 ± 2
12.	*Syzygium aromaticum*	32.3 ± 0.5	25 ± 1	21 ± 1	30.6 ± 2.08	25 ± 2
13.	*Tinospora coridfolia*		11 ± 2	14.3 ± 0.5	14 ± 3	12 ± 3
14.	*Zingiber officinale*	18 ± 2	20 ± 1	15.3 ± 1.1	15 ± 2	14 ± 2

Figure 1: Antibacterial activity of medicinal plants against *Strep.mutans*



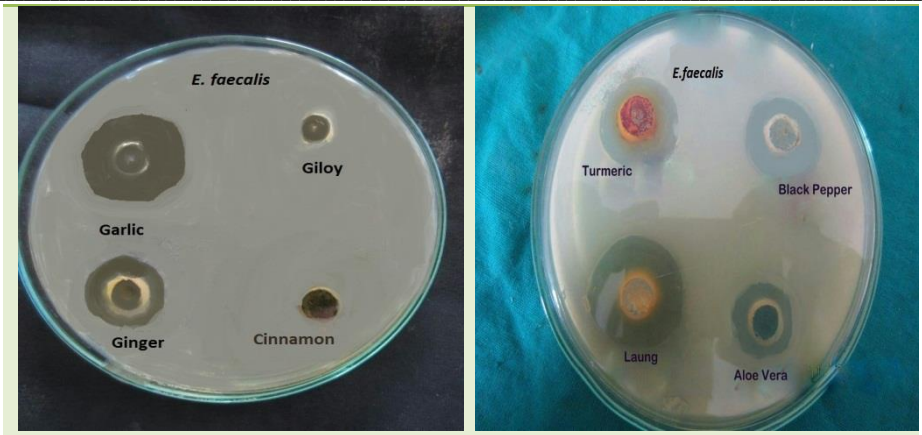


Figure 2: Antibacterial activity of medicinal plants against *E. faecalis*

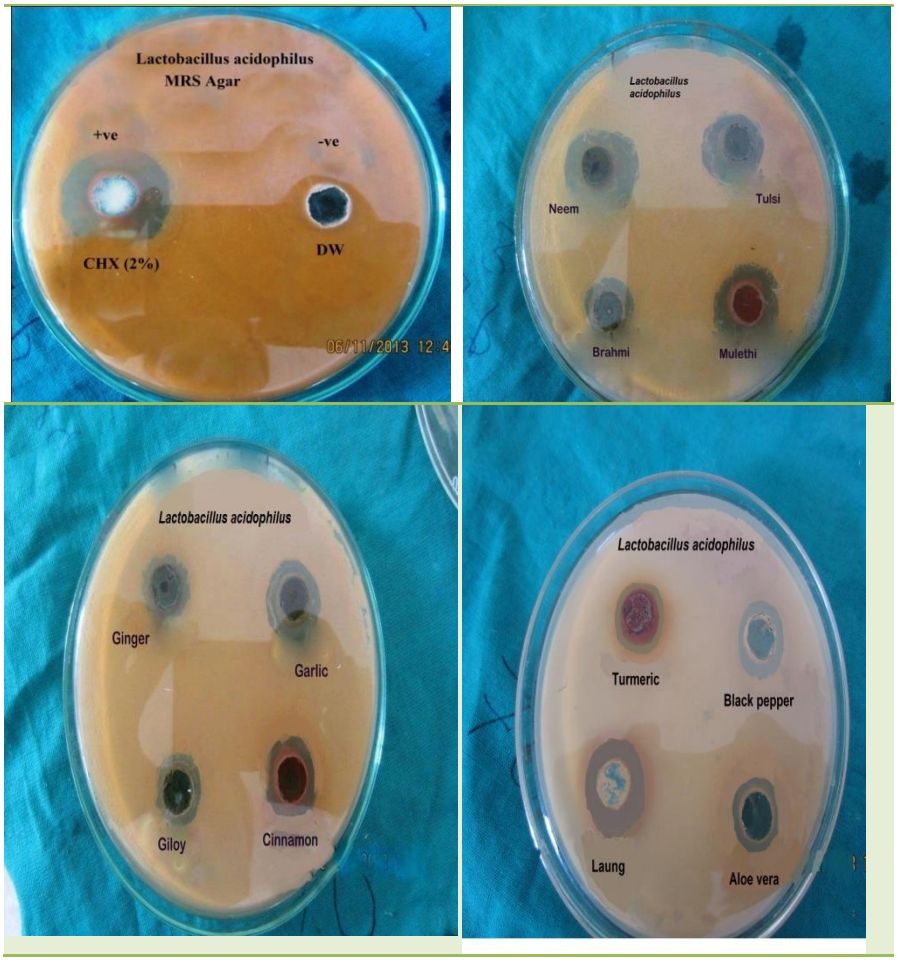


Figure 3: Antibacterial activity of medicinal plants against *Lactobacillus acidophilus*

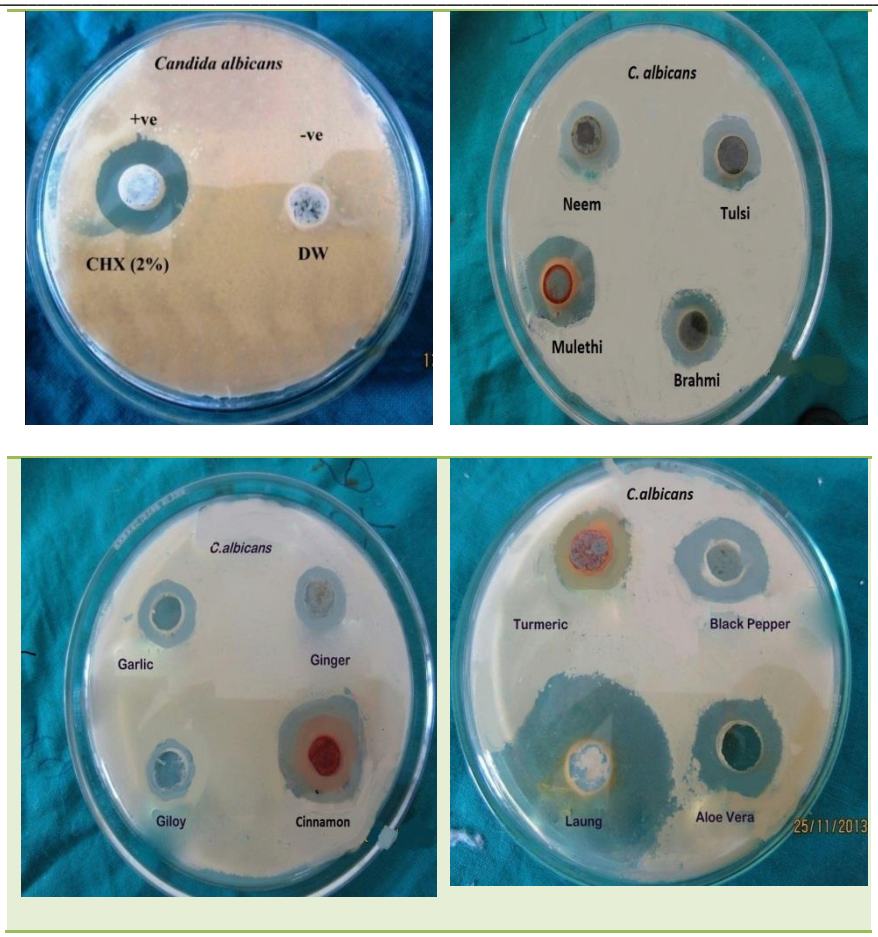
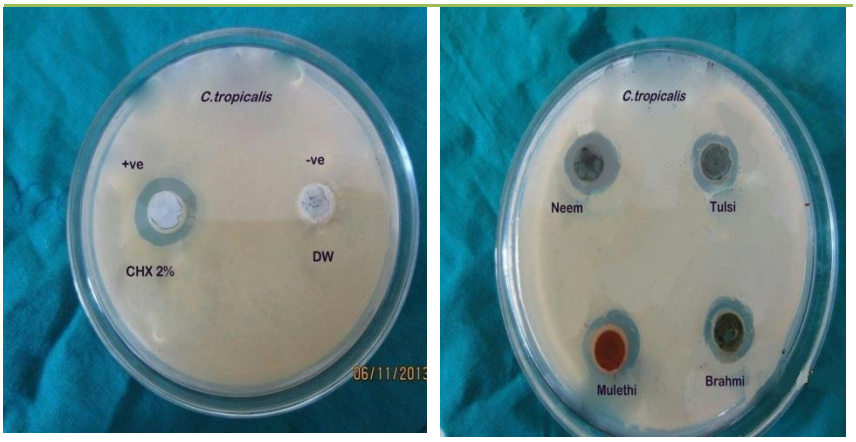


Figure 4: **Antifungal activity of medicinal plants against *Candida albicans***



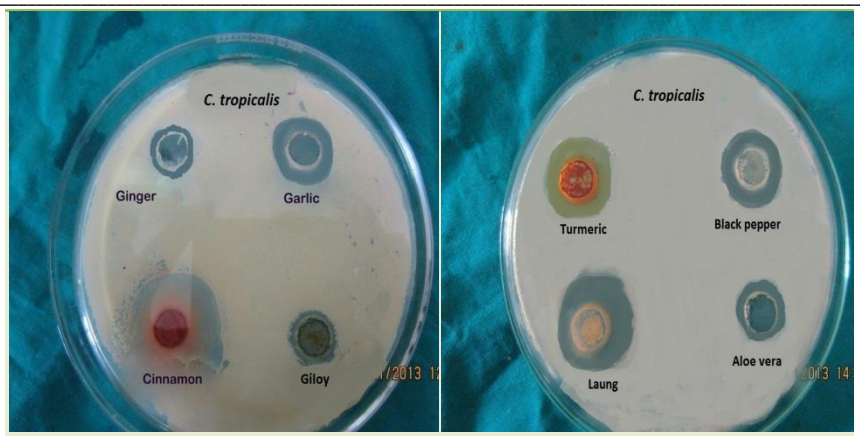


Figure 5: Antifungal activity of medicinal plants against *C.tropicalis*

CONCLUSION

In the present study almost all the medicinal plants showed antimicrobial and phytochemical activity but *Acacia nilotica, Citrus limon, Emblica officinalis, Juglans regia, Psidium guajava* L. *and Withania somnifera* displaying strong antimicrobial activity, against all the test species. However, *Lannea coromandelica (Houtt) Merr* and *Rosa centifolia* were showing week against most of the investigated pathogens.The minimum numbers of secondary metabolites were observed in *Curcuma longa* and *Glycyrrhiza glabra.*The maximum numbers of secondary metabolites were found in *Cinnamum zeylanicum, Ocimum sanctum* and *Tinospora coridfolia.*These plants have potential for development of antimicrobial agents against oral microorganisms, for use in tooth paste, mouth wash etc for preventing and treating oral infections.

Acknowledgment

The authors are greatly thankful to authorities of Himachal institute of dental sciences, Paonta Sahib (H.P), for the provision of laboratory space for the extraction process and equipment to carry out thisresearch and for use of the microbiology laboratory. My sincere gratitude goes to all those that assisted me in one way or another during the course of the reported work.

REFERENCES

1. Van Gemert-Schricks MCM, van Amerongen WE, ten Cate JM, Aartman IHA. The effect of different treatment strategies on the oral health of children: a longitudinal randomized controlled trial. *Clin Oral Invest*. 2008;12: 361-8.
2. Loesche WJ. Role of *Streptococcus mutans* in human dental decay.*Microbiol Rev.* 1986;50: 353-80.
3. Agbelusi GA, Odukoya OA, Otegbeye AF. *In vitro* screening of chewing stick extracts and sap on oral pathogens: immune compromised infections. *Biotechnology*.2007; 6(1): 97-100.
4. Bagg J,1999. Essentials of microbiology for dental students. New York, Oxford University Press, 1-326.
5. Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: Results of an *in vitro* diffusion method study. *J Am Dent Assoc.* 2004;135: 1133-41.
6. Odds FC, 1988. Candida and candidosis: a review and bibliography. 2nd ed. London, Bailiere Tindall, 252-78.
7. Oztan MD, Kiyan M, Gerceker D. Antimicrobial effect, *in vitro*, of gutta-percha points containing root canal medications against yeasts and *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pthol Oral Radio Endod*.2006;102: 410-6.
8. Siqueira JF, Rocas IN. Exploiting molecular methods to explore endodontic infection: Part 2-redefining the endodontic microbiota. *J Endod*. 2005;31:488-98

9. Holloway PJ; Moore, W.J, September, "The role of sugar in the etiology of dental caries". *J Dent.* 1983;11 (3): 189–213.

10. Akpan, A. Morgan. Oral candidiasis-Review. Postgrad. *Med. J*., 2002;78:455–459.

11. Delany GM, Patterson SS, Miller CH, Newton CW,The effect of Chlorhexidine Gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. *Oral Surg Oral Med Oral Path Oral Radiol Endod*. 1982;53:518–23.

12. Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established andpotential root canal irrigants. *J Endod*. 1995;21:513–5.

13. Basrani Bettina R. Manek Sheela, RanaN.S, Edward Fillery, Aldo Manzur. Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate. *J Endod*.,2007;33:966 –969

14. Cai L, Wu CD. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J Nat Pro.*, 1996;59 :987–90

15. Janovska D, Kubikova K, Kokoska L. Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. *Czech J Food Sci*, 2003;21: 107-110.

16. Van der Weijden, G.A, C.J. Timmer, M.F. Timmerman, E. Reijerse, M.S. Mantel and U. Velder. The effect of herbal extracts in an experimental mouth rinse on established plague and gingivitis, *J.Clin. Periodontol*., 1998;25: 399-403

17. Wu C. D, Darout I. A, Skaug N. Chewing sticks-Timeless natural toothbrush for oral cleansing. *J. Periodontal Res*. 2001;36, 275–284.

18. Akpata E. S, Akinrimisi E. O. Antibacterial activity of extracts from some African chewing sticks. *Oral Surg. Oral Med. Oral Patho*. 1977;44, 717–722.

19. Kokate CK, Khandelwal KR, Pawar AP, Gokhale SB,1995. Practical Pharmacognosy 3rd ed.,NiraliPrakashan Pune: p.137.

20. Trease and Evans, 1989. Text Book of Pharmacognosy 12th ed., ELBS Publications. pp.49, 126, 132-137, 205, 248.

21. Odebiyi, A. and A.E. Sofowora. Antimicrobial alkaloids from a Nigeria chewing stick *Fagara zanthozyboides*. *Plantamedica*, 1979;40: 204-207.

22. Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal medicine of south east regions of Iran, *J Biol Sci*;2004;4: 405-412.

23. Anpin Raja, R.D., S. Jeeva, J.W. Prakash, M. Johnson and V. Irudayaraj. Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac. J. Trop. Med.*, 2011;4: 375-378.

24. Akindele, A.J. and O.O. Adeyemi. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpuscoccineus*. Fitoterapia, 2007;78: 25-28.

25. Malairajan, P., G. Geetha, S. Narasimhan and K. JessiKalaVeni. Analgesic activity of some Indian medicinal plants. *J. Ethnopharma*, 2006;19: 425-428.

26. Argal, A. and A.K. Pathak. CNS activity of *Calotropisgigantea*roots. *J. Ethnopharma*, 2006;106: 142-145.

27. Callow RK. Steroids, 1936. ProcRoyal Soc. London Series A. 157:194.

28. Shimada T. Salivary proteins as a defence against dietary tannins, *J. Chem. Ecol*, 2006; 32 (6): 1149-1163.

29. Marjorie C. Plant Products as Antimicrobial Agents. *Clinical Microbiology Rev*., 1999;12:564-582

30. Ogbonnnia, S.O., N.V. Enwuru, E.U. Onyemenem and G.A. Oyedele. Phytochemical evaluation and antibacterial profile of Treculia africana Decne bark extract on gastrointestinal bacterial Pathogens. *African Journal of Biotechnology*,2008; 7(10): 1385-1389.

Source of Support: NIL
Conflict of Interest: None