

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

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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 cordifolia*, were not effective against *Streptococcus mutans* and *Enterococcus faecalis* respectively. However, *Azadirachta indica*, *Centella asiatica*, *Zingiber officinale* were showing weak 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 remains one of the most prevalent diseases [1]. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are byproducts of 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 of dental 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].

Enterococcus faecalis has been detected in asymptomatic and persistent root canal infections. *E. faecalis* has also been detected in 77% of failed endodontic 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 undergoes chemical reaction with NaOCl forming precipitates para-chloroaniline (PCA) which is carcinogenic [13]. Considering the ineffectiveness, potential side effect and safety concerns of synthetic

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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. The plant materials were authenticated by botanist Dr. AS. Sandhu, NIPER, Mohali,India.

Table 1: List of medicinal plant used in the study

Sr. No.	Botanical name of Plant	Common name	Family	Part Used
1.	<i>Allium sativum</i>	Garlic	Liliaceae	Bulb
2.	<i>Aloe barbadensis</i>	Aloe vera	Liliaceae	Leaves
3.	<i>Azadirachta indica</i>	Neem	Meliaceae	Leave
4.	<i>Centella asiatica</i>	Brahmi	Mackinlayaceae	Leave
5.	<i>Cinnamum zeylanicum</i>	Cinnamon	Lauraceae	Bark
6.	<i>Curcuma longa</i>	Turmeric, haldi	Zingiberaceae	Root
7.	<i>Glycyrrhiza glabra</i>	Mulethi	Leguminosae	Leave
8.	<i>Ocimum sanctum</i>	Tulsi	Lamiaceae	Leave
9.	<i>Piper nigrum</i>	Black Pepper	Piperaceae	Dried Berries
10.	<i>Syzygium aromaticum</i>	Laung, clove	Myrtaceae	Flower buds
11.	<i>Tinospora coridifolia</i>	Giloy	Meninspermeaceae	Stem
12.	<i>Zingiber officinale</i>	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 μ l 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 100 μ l of plant extracts. 2% Chlorhexidine was taken as standard reference. The plates were incubated at 37°C for 24 hours. After incubation, zone of inhibition for extracts were measured in millimeters using veneer calipers.

Results & discussion

The ethanol extracts of twelve medicinal plants were tested against the oral microorganisms viz., *Streptococcus mutans* and *Lactobacillus acidophilus* most common microbes causing 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.	Ethanollic extract of Medicinal Plants	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Tannins	Reducing Sugars	Saponins
1.	<i>Allium sativum</i>	+	+	+	+	+	-	+	-
2.	<i>Aloe barbadensis</i>	+	-	+	+	+	+	+	-
3.	<i>Azadirachta indica</i>	+	+	+	+	+	-	+	+
4.	<i>Centella asiatica</i>	+	+	+	+	+	+	+	-
5.	<i>Cinnamum zeylanicum</i>	+	+	+	+	+	+	+	+
6.	<i>Curcuma longa</i>	+	-	-	+	-	+	+	-
7.	<i>Glycyrrhiza glabra</i>	-	-	-	+	+	-	-	+
8.	<i>Ocimum sanctum</i>	+	+	+	+	+	+	+	+
9.	<i>Piper nigrum</i>	+	+	+	+	+	+	-	+
10.	<i>Syzygium aromaticum</i>	+	+	-	+	+	+	+	+
11.	<i>Tinospora cordifolia</i>	+	+	+	+	+	+	+	+
12.	<i>Zingiber officinale</i>	+	+	+	-	+	+	+	+

The most important of these secondary metabolites include alkaloids, saponins, tannins, steroids and terpenoids, 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 coridifolia*. 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 coridifolia* and *Zingiber officinale*. Plant steroids are known to be important for their cardiogenic 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 and the results are presented in Fig. 1,2,3,4 and 5. 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 *Calendula officinalis* and *Mangifera indica*, were not effective against *Strep. mutans*, *E. faecalis*, *C. tropicalis*, *L. acidophilus* respectively. However, *Lannea coromandelica* (Houtt) Merr and *Rosa centifolia* were showing weak 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

Sr. No.	Medicinal plant extracts /control groups	<i>Strep. mutan</i>	<i>E. faecalis</i>	<i>L. acidophilus</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
1.	Chlorhexidine (+ve control)	30mm	25mm	25mm	20mm	19mm
2.	Distil water (-ve control)	-	-	-	-	-
3.	<i>Allium sativum</i>	25mm	27mm	18mm	18mm	15mm
4.	<i>Aloe barbadensis</i>	-	15mm	15mm	20mm	15mm
5.	<i>Azadirachta indica</i>	18mm	19mm	23mm	20mm	19mm
6.	<i>Centella asiatica</i>	15mm	10mm	15mm	15mm	14mm
7.	<i>Cinnamum zeylanicum</i>	-	-	20mm	25mm	26mm
8.	<i>Curcuma longa</i>	18mm	21mm	17mm	21mm	23mm
9.	<i>Glycyrrhiza glabra</i>	20mm	25mm	18mm	17mm	18mm
10.	<i>Ocimum sanctum</i>	20mm	22mm	18mm	17mm	16mm
11.	<i>Piper nigrum</i>	-	20mm	18mm	25mm	20mm
12.	<i>Syzygium aromaticum</i>	33mm	25mm	20mm	30mm	25mm
13.	<i>Tinospora coridifolia</i>	-	11mm	14mm	14mm	12mm
14.	<i>Zingiber officinale</i>	18mm	20mm	15mm	15mm	14mm

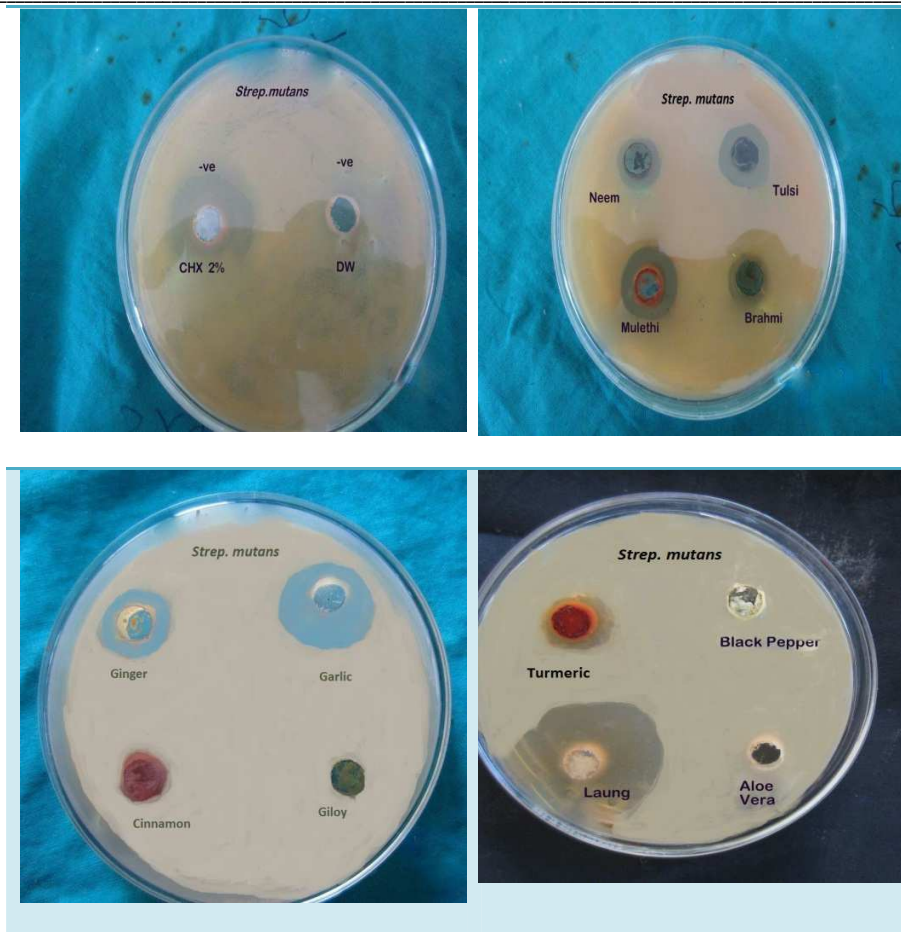


Figure 1: Antibacterial activity of medicinal plants against *Strep.mutans*





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

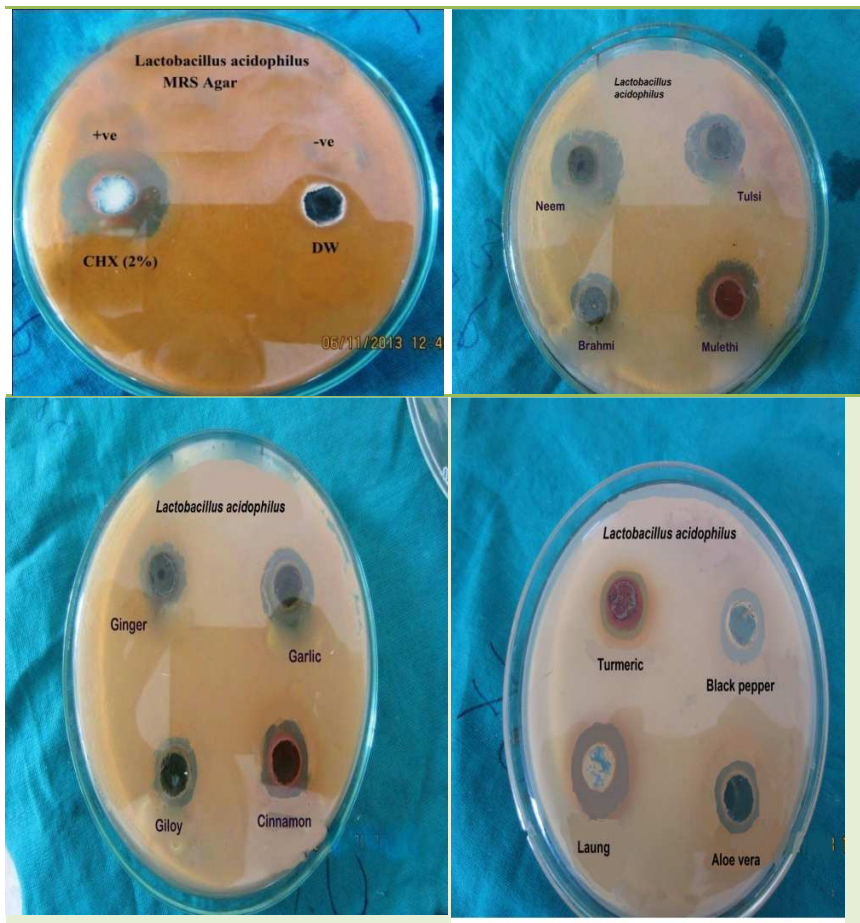


Figure 3: Antibacterial activity of medicinal plants against *Lactobacillus acidophilus*

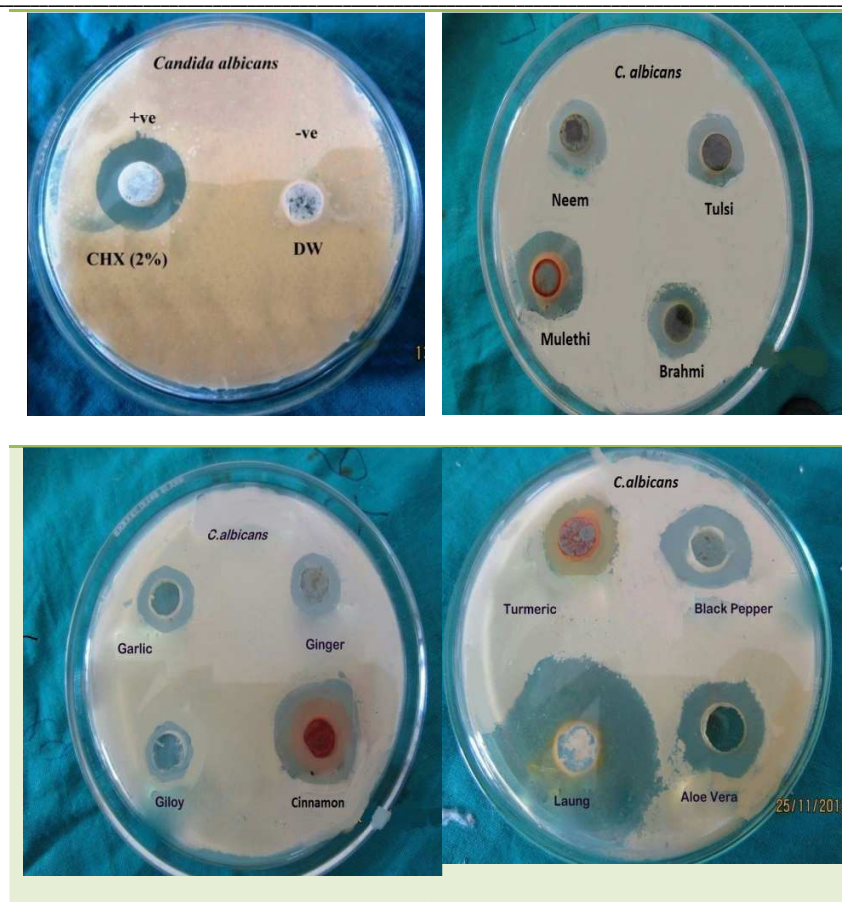


Figure 4: Antifungal activity of medicinal plants against *Candida albicans*





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, *Lansea coromandelica* (Houtt) Merr and *Rosa centifolia* were showing weak 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 cordifolia*. 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.

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