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Research article

Antimicrobial activity and phytochemical analysis of ethanolic extracts of twelve medicinal plants against oral micro organisms

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ABSTRACT

The present study was carried out to evaluate the phytochemical and antimicrobial activity of twelve medicinal plants against five microbial strains causing oral infections. The antimicrobial activity of ethanolic extract of plants were evaluated using well diffusion method against *Streptococcus mutans, Enterococcus faecalis, Lactobacillus acidophilus, Candida albicans and Candida tropicalis*. Ethanolic extracts of *Calendulla officinalis* and *Mangifera indica*, were not effective against *Streptococcus mutans, Enterococcus faecalis, Candida tropicalis and Lactobacillus acidophlius* respectively. However, *Lanneacoromandelica (Houtt) Merr*, *Mentha piperita, Citrus sinesis* 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. The phytochemical analysis carried out revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, reducing sugar and steroids in most of the medicinal plants. These results provide justification for the use of the medicinal plants to treat various oral infections.

1. Introduction

Oral diseases impact our quality of life and may lead to systemic and threatening diseases. The relationship between the high incidence of oral diseases and microorganisms is well known. Because of the increased microbial resistance to antibiotics, toxic and harmful effects of few common antimicrobial agents, there is a continuous need for alternative therapies which are affordable, non toxic and effective, such as medicinal plants[1,2]. Alternative medicines are commonly included in therapeutic and diagnostic disciplines outside the conventional health system[3]. In developing countries, access to dental healthcare is restricted and expensive and thus it is limited to emergency dental care procedures[4]**.** Dental caries is one of the most common infection of all oral diseases. It is proved that cariogenic microorganisms, especially *Streptococcus mutans* plays an essential role in the pathogenesis of dental caries. It is involved in the initiation of almost all carious lesions in enamel[5]. The main cause of dental caries is attributing to oral biofilm, also known as dental plaque, a film of microorganisms sticking to the tooth surface[6]. *Streptococcus mutans* has the ability to metabolize dietary sucrose and synthesize glucan by cell surface and extracellular glucosyl transferase. This glucan is an insoluble sticky or slimy gel relatively inert and resistant to bacterial hydrolytic enzymes which causes plaque to adhere tenaciously to tooth surface [7]. *Streptococcus mutans* and other organisms in the plaque produce organic acids such as lactic acid that gradually destroy the enamel to form a cavity[8]. In addition to *Streptococcus mutans, Lactobacillus acidophilus* bacteria probably also play role in acid production in the plaque and are involved in root caries and periodontal diseases[9].

Enterococcus faecalis[10,11] *Candida albicans, Candida tropicalis*[12] etc are some other microbial species that knowingly cause several oral diseases, such as dental caries, endodontic infections, periodontal diseases and oral Candidiasis. The most effective method to eradicate *E. faecalis* and *C.albicans* is the use of sodium hypochlorite and 2% chlorhexidine[13]. Sodium hypochlorite is extremely toxic to periapical tissues if injected beyond apex[14]. Presence of inflammatory exudate and killed microorganisms can inhibit the action of chlorhexidine in root canals[15]. Studies have shown that the combination of NaOCl and chlorhexidine can form para-chloroaniline, a product which is potentially carcinogenic and occludes dentinal tubules[16]**.**

The need for affordable, effective, and nontoxic alternatives has led to the search for compounds from natural sources such as plants[17], which may overcome the high incidence of oral diseases .A few recent studies have focused on the antimicrobial activity against selected oral pathogens from

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natural sources. Chemical agents such as fluoride and chlorhexidine, which have been used to prevent dental caries for several decades, were associated with some side effects such as staining of teeth and fluorosis. Thus, there is no perfect antimicrobial agent to prevent dental caries until now. The use of natural products has been one of the most successful strategies for the discovery of new drugs[18]. Medicinal plants have been used for thousands of years in folk medicine and they are believed to be the new source of antimicrobial agents[19]. There is a need to screen medicinal plants for their promising biological activity. In the present study we studied the antimicrobial activity of ethanolic extracts of twelve medicinal plants against oral micro organisms.

2. Material and method

2.1 Collection and identification of plant material

The medicinal plant materials of the species were collected from market and forest of Uttrakhand and Himachal Pradesh, listed in **table no.1**. Authentication of medicinal plants by department of botany, FRI, Dehradun.

Table 1: Medicinal Plants used in this study

Sr. No.	Botanical name of Plant	Common name	Family	Part Used
1.	*Acacia nilotica*	Kikar, Babul	Fabaceae	Stem
2.	*Calendulla officinalis*	Pot marigold	Asteraceae	Flower
3.	*Citrus limon*	Lemon	Rutaceae	Fruit peel
4.	*Citrus sinesis*	Orange	Rutaceae	Fruit peel
5.	*Emblica officinalis*	Amla	Phyllanthaceae	Fruit
6.	*Juglans regia*	Walnut	Juglandaceae	Bark
7.	*Lannea coromandelica (Houtt.) Merr*	Jhingangummi	Anacardiaceae	Twig
8.	*Mangifera indica*	Mango	Anacardiaceae	Stem
9.	*Mentha piperita*	Peppermint I	Labiatae	Leaves
10.	*Psidium guajava L.*	Guava	Myrtaceae	Twig
11.	*Rosa centifolia*	Red Rose	Rosaceae	Flower
12.	*Withania somnifera*	Ashwagandha	Solanaceae	Root

2.2 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.

2.3 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, steroids, flavonoids and terpenoids in various extracts of medicinal plants. The above phytoconstituents were tested as per the standard methods **[20, 21]**.

2.4 Antimicrobial activity

The antimicrobial activity of different plant extracts were evaluated by using the agar well diffusion test technique. Microbial inoculum were standardized by picking six colonies of each organism into their specific media such as Brain heart infusion broth (*S. mutans and E. feacalis*) (MTCC 890 and MTCC 439), Lactobacillus MRS broth (*L. acidophilus*) (MTCC 10307), Sabouraud's Dextrose broth (*C.albicans* and *C.tropicalis*) (MTCC 854 and MTCC 184) and incubated at 37°C for 18-24hrs.Turbidity produced was adjusted to match 0.5 McFarland standard (10^8 cfu/ml) which was further adjusted 10 cfu/ml. 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 will be taken as standard reference. The plates were incubated at 37ºC for 24 hours. After incubation, zone of inhibition for each extract will be measured in millimeters using veneer calipers.

3. Results & Discussion

The ethanol extracts of twelve medicinal plants were tested against the pathogenic microbes' viz., *Strep. mutans* a most common bacteria of which virulent strains can cause dental plaque and caries; *E. faecalis* associated with various periradicular diseases including primary endodontic infections, persistent infections and asymptomatic chronic periradicular, *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.

Phytochemical constituents such as alkaloids, flavonoids, reducing sugar, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of medicinal plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores[22]. The present study carried out on the medicinal plant samples revealed the presence of medicinally active constituents. The phytochemical constituents and antimicrobial activity of the selected plants investigated are summarized in **Table 2 and 3.**

Table 2: Phytochemical investigation of selected medicinal plants

Sr. No	Ethanolic extract of Medicinal Plants	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Tannins	Reducing Sugars	Saponins
1.	*Acacia nilotica*	+	+	+	+	+	+	+	+
2.	*Calendulla officinalis*	+	+	+	+	+	-	-	-
3.	*Citrus limon*	+	-	+	+	+	+	+	-
4.	*Citrus sinesis*	+	-	+	+	+	+	+	+
5.	*Emblica officinalis*	+	+	-	-	+	+	+	+
6.	*Juglans regia*	+	+	+	-	+	+	-	+
7.	*Lannea coromandelica (Houtt.) Merr*	_	_	+	_	+	+	-	-
8.	*Mangifera indica*	+	-	+	-	+	+	+	-
9.	*Mentha piperita*	+	-	+	+	+	+	+	+
10.	*Psidium guajava L.*	+	+	+	+	-	+	+	+
11.	*Rosa centifolia*	+	+	+	-	+	+	+	+
12.	*Withania somnifera*	+	+	+	+	-	-	+	+

Table 3: Antimicrobial activity of medicinal plants against oral microorganisms

Sr. No.	Medicinal plant extracts /control groups	*Strep.mutans*	*E. faecalis*	*L. acidophilus*	*C. albicans*	*C.tropicalis*
1.	Chlorhexidine (+ ve control)	30mm	25mm	25mm	20mm	19mm
2.	Distil water (-ve control)	-	-	-	-	-
3.	*Acacia nilotica*	22mm	25mm	22mm	24mm	20mm
4.	*Calendulla officinalis*	-	-	12mm	18mm	-
5.	*Citrus limon*	18mm	16mm	26mm	20mm	20mm
6.	*Citrus sinesis*	20mm	-	27mm	18mm	20mm
7.	*Emblica officinalis*	25mm	23mm	28mm	19mm	22mm
8.	*Juglans regia*	20mm	19mm	19mm	21mm	20mm
9.	*Lannea coromandelica (Houtt.) Merr*	16mm	13mm	18mm	15mm	-
10.	*Mangifera indica*	-	16mm	-	15mm	12mm
11.	*Mentha piperita*	11mm	-	26mm	23mm	16mm
12.	*Psidium guajava L.*	19mm	20mm	20mm	18mm	20mm
13.	*Rosa centifolia*	15mm	-	11mm	16mm	12mm
14.	*Withania somnifera*	22mm	25mm	20mm	22mm	18mm

Analysis of medicinal plant extracts revealed the presence of alkaloid, flavonoid, glycoside, terpenoids, saponin, steroid, reducing Sugar and tannin in most of the selected medicinal plants which could be responsible for the observed antimicrobial property.

These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis[23]. 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[24].

Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [25]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes**[26]**.

Out of twelve medicinal plants tested for antimicrobial activity, all medicinal plant species showed antibacterial and antifungal activity by inhibiting one or more oral microorganisms. The results of the antimicrobial activity of medicinal plant extracts tested against microorganisms by well diffusion method are shown in Table 3.The results of antibacterial activity were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of microbial growth and it as reported as positive and absence of zone as negative[27].The inhibition zone diameters by the test isolates against different plant extracts shows that ethanolic extracts of *Calendulla officinalis and Mangifera indica,* were not effective against *Streptococcus 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.

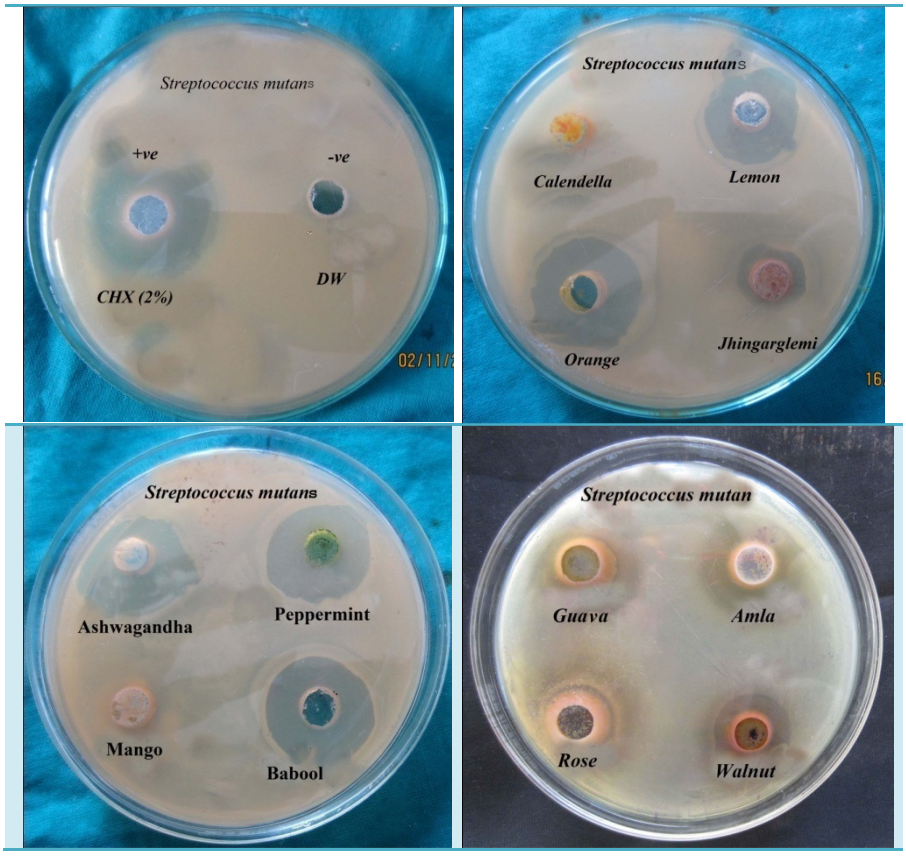
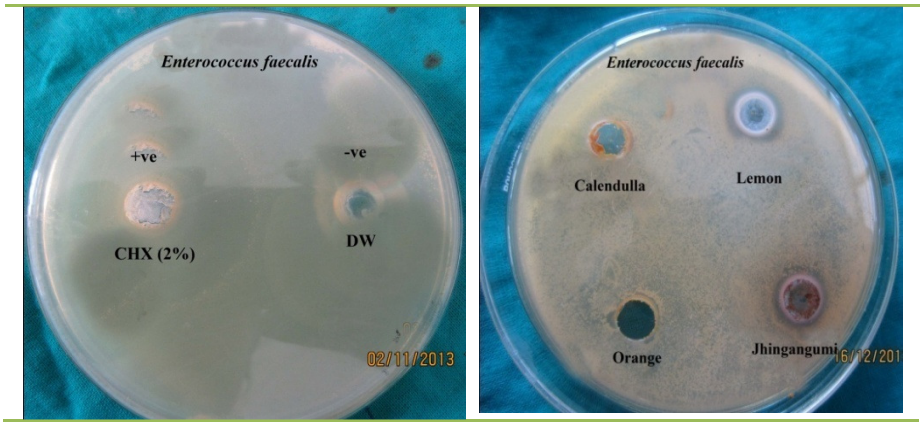


Figure 1: Antibacterial activity of medicinal plants against *Streptococcus mutans*



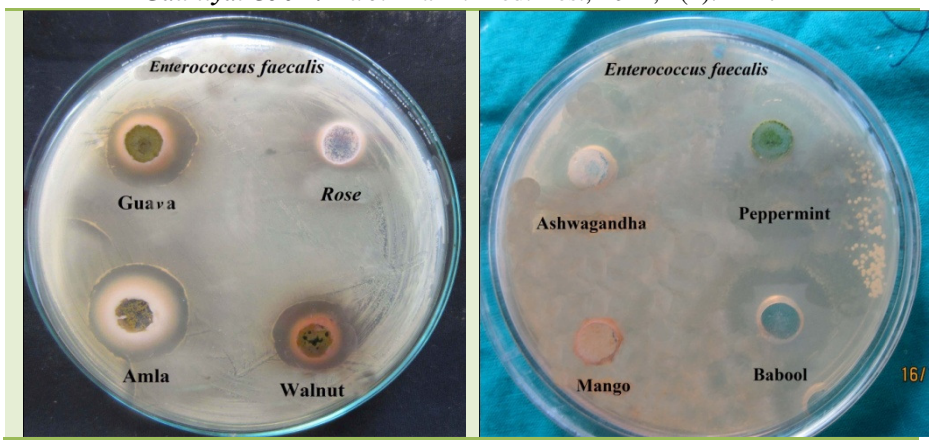


Figure 2: Antibacterial activity of medicinal plants against *Entrococcus faecalis*

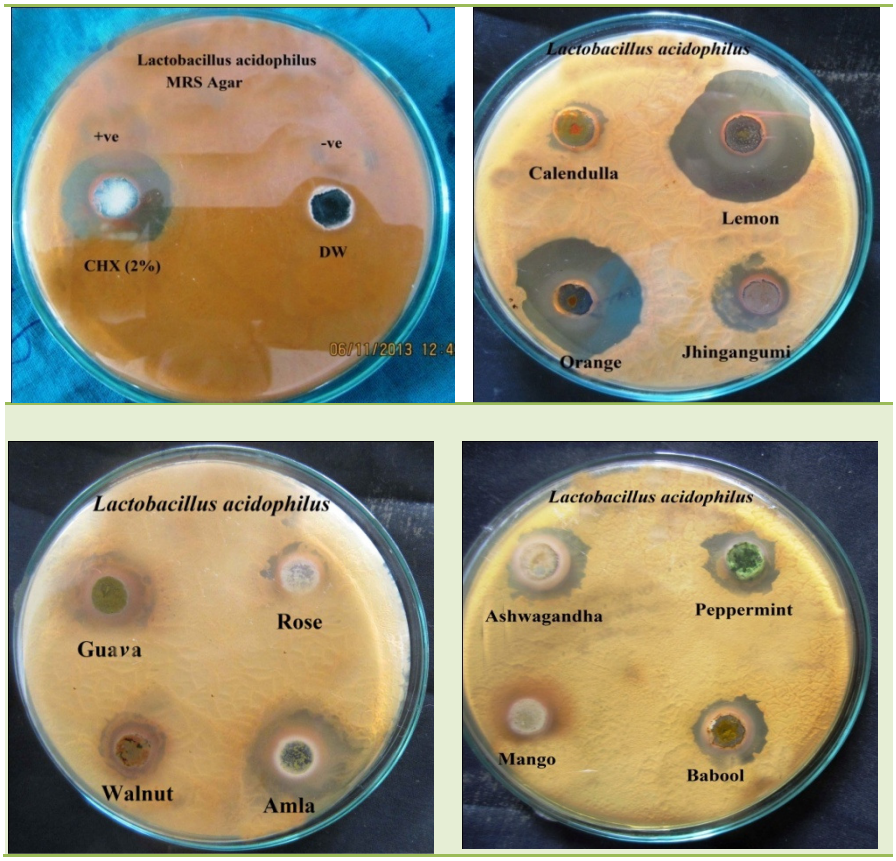


Figure 3: Antibacterial activity of medicinal plants against *Lactobacillus acidophilus*

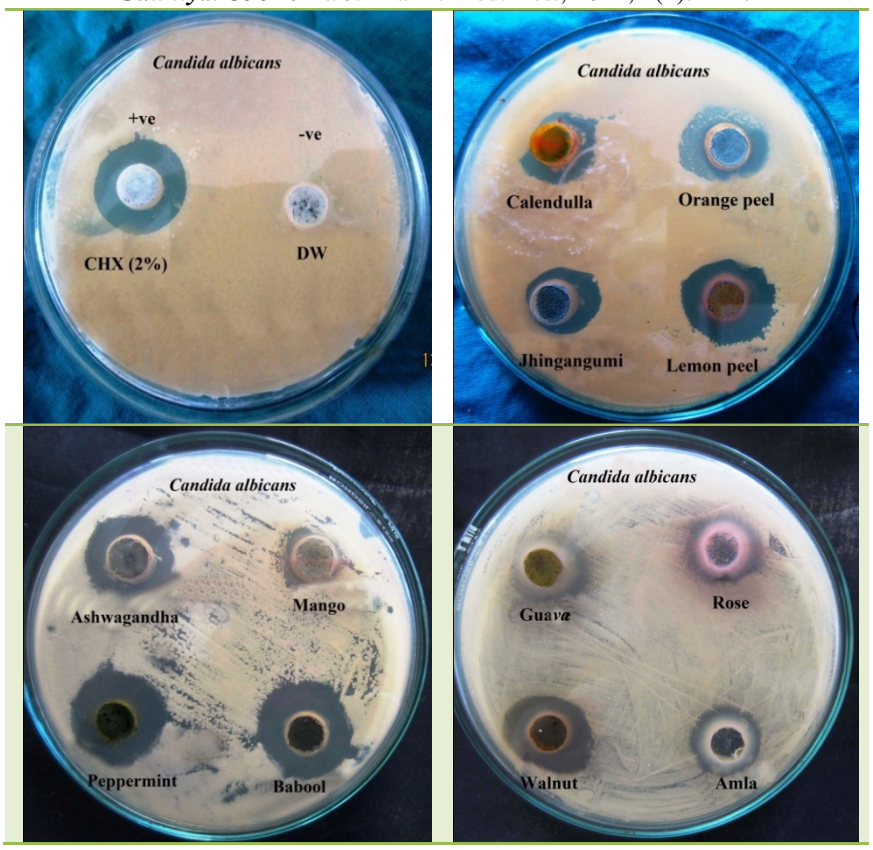


Figure 4: Antifungal activity of medicinal plants against *Candida albicans*

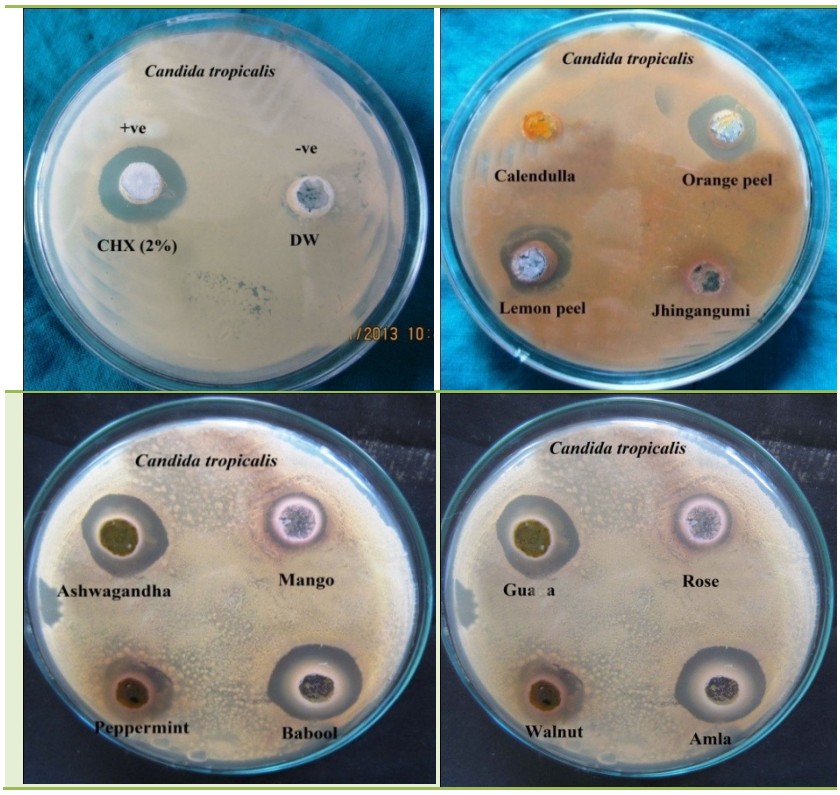


Figure 5: Antifungal activity of medicinal plants against *Candida tropicalis*

4. Conclusion

It can be concluded from the result ethanolic extract of twelve medicinal plants that, extract of *Acacia nilotica, Citrus limon, Emblica officinalis, Juglans regia, Psidium guajava L and Withania somnifera* used in the present study possess significant microbial activity against tested oral microbes. These medicinal plants have potential for development of antimicrobial agents against oral microorganisms, for use in tooth paste, mouth wash, chewing gum etc for preventing and treating oral infections. However, *Lannea coromandelica* (Houtt) Merr and *Rosa centifolia* were showing week activity against some microbes. *Calendulla officinalis and Mangifera indica were* showing week activity against many microbes.

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