

Evaluation of Efficacy of Pudina Extract (*Mentha Arvensis*) on *Candida Albicans* and *Lactobacillus Acidophilus* – An Invitro Study

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ABSTRACT

Introduction: Orthodontic patients have an increase in the microorganisms because of the active sites for the plaque accumulation. Pudina which is a perennial plant has antimicrobial property and can be used as a mouth wash for these patients.

Aim: To evaluate the efficacy of aqueous and methanolic extracts of Pudina (Mint) on *Candida albicans* and *Lactobacillus* species by evaluating their zone of inhibition and determining their minimum inhibitory concentration.

Methodology: Cell suspension with 10⁸ cells were prepared using *Candida albicans* and *Lactobacillus acidophilus*. Using sterile micro tip, wells were made which was about 7- 8mm in diameter. Lawn culture was made on appropriate media.

Then the prepared Aqueous and alcoholic extracts with different concentrations were added on to the wells and observed for zone of inhibition for *Lactobacillus acidophilus* and for *Candida albicans*.

Results: The results showed that there is an increase in the inhibition zone diameter on *Candida albicans* species with the methanolic extract of pudina. There was no inhibitory effect of pudina on *Lactobacillus* species.

Keywords

Pudina, *Mentha Arvensis*, *Lactobacillus Acidophilus*

INTRODUCTION

Orthodontic appliances act as an active site for the attachment of food particles and subsequently microorganisms which later cause caries and periodontal diseases. There are large areas of plaque retention sites in orthodontic patients. There are studies confirming that during fixed orthodontic appliance therapy, the levels of microorganisms are increased despite a pre treatment oral hygiene education and training.^{1,2} Many plants exhibit potent antimicrobial activity against various microorganisms.³ The World Health Organization reported that 80% of the world's population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents. Herbal therapies have limited /no side effects and act against and modulate the factors that are crucial for microbial survival or their activity.⁴ Pudina (*Mentha arvensis*), a perennial aromatic herb belonging to the family Labiatae and genus *Mentha* is an important culinary plant with immense medicinal use. The antibacterial effect of pudina has already been proven against *Streptococcus mutans*.^{5,6} This study is aimed at evaluating the efficacy of extracts of Pudina (Mint) on *Lactobacillus acidophilus* and *Candida albicans* by evaluation of their inhibition zones and their minimum inhibitory concentration levels.

MATERIALS AND METHODS:

Cell suspension with 10^8 cells were prepared using candida albicans (Fig.1) and lactobacillus acidophilus (Fig.2). Using sterile micro tip, wells were made which was about 7- 8mm in diameter. Lawn culture was made on appropriate media.

Then the prepared Aqueous and Methanolic extracts with different concentrations were added on to the wells and observed for zone of inhibition for lactobacillus acidophilus and for Candida albicans.



Fig – 1 : Candida albicans culture Fig – 2: Lactobacillus acidophilus culture

Methods of Pudhina extract preparation

Method – 1: Aqueous extract preparation

Pudina was obtained, air dried and then powdered (Fig.3). Different concentration of pudina extract was prepared (i.e) 5 g in 100ml of water, 10g in 100ml of water, 15 g in 100ml of water, 20g in 100ml of water and 25 g in 100ml of water (5%,10%,15%,20%,25%), it was then individually heated at 40°C for 5-10mins.



Fig – 3: Pudina powder

The preparation was then incubated overnight at 37°C , filtered (fig 4) using sterile whattman filter paper no 1 and then re- filtered using [0.45](#)micrometer filter paper and stored at 4°C in separate containers (fig 5).

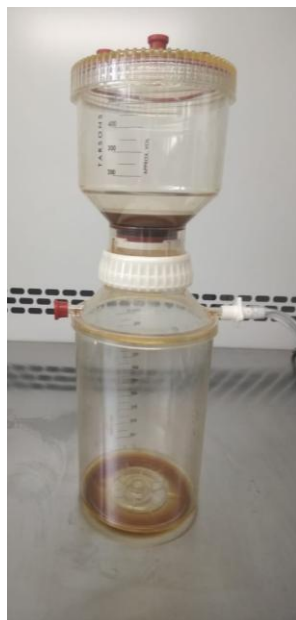


Fig 4 : Filter

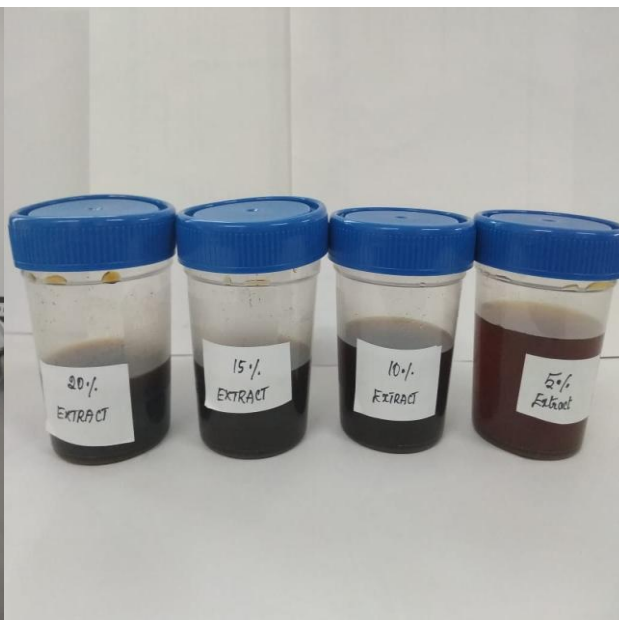


Fig – 5 : Prepared extract with distilled water

Method – 2: Methanolic extract preparation

The pudina leaves were taken, air dried and powdered.

Then 100gms of the pudina powder was soaked in 500ml of 100% methanol and was kept undisturbed for about 2 days (fig 6)



Fig – 6: Pudina powder is soaked in 500ml of 100% methanol.

A clear filtrate of the substrate was obtained by filtration with whatman filter, and then heated at about 60° and reduced to pudina extract residue. From 100gms of pudina, 5 grams of residue (extract) was obtained, so the yield was 5% w/w.

Half of the extract (0.5gm) was dissolved in 10 ml of dimethyl formamide to obtain 5% concentration of extract. One gram of extract was dissolved in 10 ml of dimethyl formamide to obtain 10% concentration of extract and with same procedure 50% concentration was made.⁵ Then the prepared Aqueous and Methanolic extracts with different concentrations were added onto prepared culture wells of candida albicans (Fig.1) and lactobacillus acidophilus (Fig.2). and observed for zone of inhibition for Candida albicans and lactobacillus acidophilus.

RESULTS:

Effect of aqueous extract of Pudhina (Table 1)

Table: 1 Effect of Aqueous extract of Pudina against Candida albicans

Concentration of the extract	Result	Unit
5%	No inhibition zone seen	Diameter
10%	0.3mm	
15%	0.7mm	
20%	0.8mm	

Candida albicans: Partial zone of inhibition was present. (fig .7)

- 5% - No zone of partial inhibition
- 10% - 0.3mm
- 15% - 0.7mm
- 20% - 0.8mm

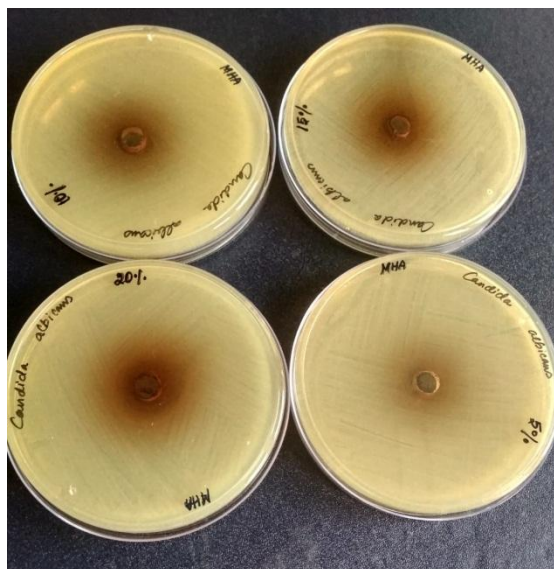


Fig – 7 : Inhibition zone with aqueous extract of pudina on Candida albicans

Effect of methanolic extract of Pudhina (Table 2)

Table: 2 Effect of Methanolic extract of Pudina against Candida albicans

Concentration of the extract	Result	Unit
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5%	No inhibition zone seen	-
10%	16mm,14mm,13mm,14mm	Diameter
50%	17mm,16mm,15mm,17mm	Diameter

a. On *Candida albicans*:

Zones of inhibition (fig.8)

- 5% - no zone seen.
- 10% - 14.5mm
- 50% - 16.25mm

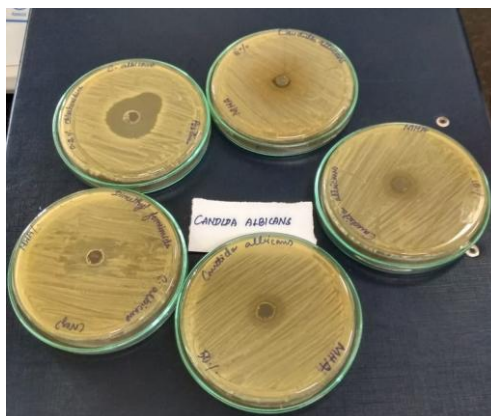


Fig – 8 : Inhibition zone with the methanolic extract of pudina on *Candida albicans*

There was no Zones of inhibition on *Lactobacillus acidophillus* (Fig.9)

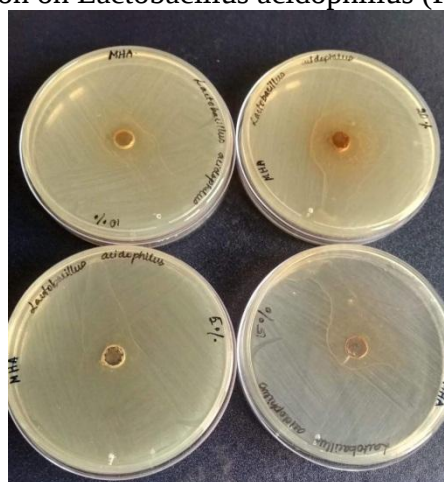


Fig 9: Inhibition zone with the methanolic extract of pudina on *Lactobacillus acidophillus*

DISCUSSION

The medicinal plants have been used for the treatment of medical ailments for a long time. Many plants have been proven to have antibacterial properties. Pudhina's composition (*Mentha arvensis*) is highly complex with many nutrients and biologically active substances, the amount of which may vary with different concentrations of pudina. The therapeutic properties of

pudina is because of the presence of the menthol in it. It is largely used in the treatment of liver and spleen diseases. It is also used to treat asthma and jaundice.⁷

The other main constituents of pudina include esters-menthyl acetate (4.5 to 10%) and ketones (10-20%). The antibacterial effect of pudina is because of these constituents. The menthol in the pudina is more soluble in alcohol than in the water.⁵

Orthodontic patients have lots of plaque retention sites thereby increase in levels of microorganisms during active orthodontic therapy. Many studies have proven that orthodontic treatment have increased the occurrence of carious lesions. There is an increase in the level of *Lactobacillus acidophilus* in patients treated with orthodontic appliance.^{1,6}

There has been studies by Hibino et al (2009) and WisamAlhamadi et al (2017) that proves that there has been an increase in *Candida albicans* levels in orthodontic patients after the initiation of treatment.

This study was done to find the effect of Pudhina against *Candida albicans*. The results showed that the methanolic extract of pudina was effective against the *Candida albicans* with an increased zone of inhibition. (Table:1). Additional assays are required to determine whether Pudhina extract is bactericidal or bacteriostatic. Methanolic extract has more antifungal activity on *Candida albicans* than aqueous extract. Also antifungal activity was dose dependent. As the concentration increased, the inhibition zone was also increased. Flavanoids and Tannis present in the extracts may be responsible for the antimicrobial activity⁸. Hence the pudina mouthwash can be used for the patients being treated with orthodontic appliance, who are more prone to candida infections. Owing to the already proven medicinal properties⁹⁻¹¹, along with the anticandidal property that is proved in our present study, pudinamouthrinse can be used for control of candida infections that occurs during the course of the orthodontic treatment in patients with fixed and removable appliances.

CONCLUSION

Pudinamouthrinse can be used for control of candida infections that occurs during the course of the orthodontic treatment.

Further formulation of pudhinamouthrinse and invivo studies are necessary to prove the efficacy of Pudhinamouthrinse in patients undergoing orthodontic treatment.

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