



## EFFICACY OF MISWAK AND CARDAMOM EXTRACT ON DIFFERENT CANDIDAL SPECIES: AN IN VITRO STUDY

<sup>1\*</sup>Dr. Reddy Lavanya, <sup>2</sup>Dr. Mamatha Boringi, <sup>3</sup>Dr. Nallan CSK Chaitanya, <sup>4</sup>Dr. C. Tirumala Ravali, <sup>5</sup>Dr. Lingam Amara Swapna, <sup>6</sup>Dr. Madupu Padma Reddy

<sup>1,4</sup>Assistant Professor, Department of Oral Medicine & Radiology, Government Dental College, Hyderabad.

<sup>2</sup>Reader, Department of Oral Medicine & Radiology, Panineeya Dental College & Hospital, Kamalanagar, Dilsukhnagar, Hyderabad.

<sup>3</sup>Professor, Department of Oral Medicine & Radiology Panineeya Dental College & Hospital, Kamalanagar, Dilsukhnagar, Hyderabad.

<sup>5</sup>Assistant Professor, Department of Surgical and Diagnostic Sciences, DAR al Uloom University, Riyadh.KSA.

<sup>6</sup>Professor, Department of Public Health Dentistry, Panineeya Dental College & Hospital, Kamalanagar, Dilsukhnagar, Hyderabad.

**Corresponding Author: Dr. Reddy Lavanya**

Assistant Professor, Department of Oral Medicine & Radiology, Government Dental College, Hyderabad.

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### ABSTRACT

**Introduction:** Oral health is an integral part of overall health. Poor oral health may often lead to opportunistic infections. Among them most common is candidiasis. Antifungal activities of different plant extracts were shown to be effective against candida. This study was done with an objective, to estimate antifungal efficacy of miswak and cardamom extracts on different candidal species by comparing the growth inhibition zone with both the extracts. **Materials and method:** Commercially available Test organisms, of candidal species, were used for the study to which plant extracts were applied. After incubation, the zones of growth inhibition were measured in mm on agar plates with a loop technique. **Results:** Inhibitory effects of Miswak & Cardamom extracts against different species of candida were examined. Cardamom extract showed greater inhibitory values ranging from 20mm to 10mm as a zone of inhibition when compared with miswak which ranged from 14 to 10mm as zone of inhibition in all species other than candida albicans. **Conclusion:** This in vitro study showed, that homemade products such as miswak and cardamom had greater antimicrobial activity. Future studies should focus on the preparation of ointments along with clinical application in the treatment of oral candidiasis.

**KEYWORDS:** Antifungal, miswak, candidiasis and cardamom.

### INTRODUCTION

Poor oral hygiene may lead to oral diseases which in turn affects the quality of life. When an individual has poor oral health he may be prone to many opportunistic infections. Among them most common is candidiasis.

Candida is a normal commensal present as a part of normal oral flora and accounts for about 30-50% of the population. Oral candidiasis is disease caused by an overgrowth or infection of the oral cavity by a yeast-like fungus, candida (monilia) albicans (most common). Other species involved are C tropicalis, C glabrata, C pseudotropicalis, C guillierimondii, C krusei, C lusitaniae, C parapsilosis, and C stellatoidea. C albicans, C glabrata, C tropicalis C parapsilosis and C krusei which represent more than 80% of isolates from clinical infection and other candida species rare and transient.<sup>[1,2]</sup>

Oral candidiasis can manifest clinically in different types as acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis, and angular cheilitis. Candidiasis is most commonly an asymptomatic disease, but sometimes patient may complain of burning sensation and altered taste. In immunocompromised patient this infection can spread to upper gastrointestinal tract through blood leading to severe infection with significant morbidity and mortality. Systemic candidiasis carries a mortality rate of 71% to 79%.<sup>[1]</sup>

Plant extracts with antibacterial and antifungal actions are commonly preferred medicaments for treating various diseases. Among them Miswak wood (*Salvadora persica* Linn.) has been used for oral hygiene practice since centuries ago, especially by the Arabs.<sup>[3]</sup> The constituents of miswak are fluorides, silica,

tannic acid, resins, alkaloids (salvadorine), volatile oils (simgrins), sulfur, vitamin C, sodium bicarbonate, chlorides, calcium, benzyloisothiocyanate, salicylic acids, sterols, trimethylamine, saponins and flavenoids.<sup>[4,5]</sup>

In literature they have mentioned that most of the antimicrobial/antifungal activity in essential oils and oleoresins derived from spices and culinary herbs is due to phenolic compounds. Among them Cardamom is one such plant species which are used as aromatic, carminative and flavouring agent. In *medicine* it used as a powerful aromatic, antiseptic, stimulant, carminative, stomachic, anti-spasmodic & diuretic.<sup>[6,7]</sup>

Currently in clinical practice relatively few topical and systemic medicaments are available and resistances may develop on repeated use of same drug. Most of the professionals focus on the alternate medicine where we can use herbal extracts which are relatively effective, less toxic with good antibacterial, antifungal activity and anti-inflammatory potential.

This study was conducted with an objective to evaluate the antifungal action of extracts of miswak and Cardamom on different species of candida. Based on in vitro inhibitory concentration of the agents on each species.

## MATERIALS AND METHOD

Present study was conducted at Marath Mandal Institute of Dental Sciences, Chandigarh, after procuring samples from centre for microbiological research, they have provided commercially available yeast, different species which includes candida albicans, C tropicalis, C glabrata, and C krusei,

### Plant Extract preparation

A similar modified method which was used by Chevalier, 2003 was used in this study. Initially Miswak sticks were cut into small pieces then ground to powder in a food blender, in the same way Cardamom was also made into powder.

### Procedure for preparing commercially available candida

Markings were made on the ampule near the middle of the cotton wool where they have to break. 0.3 to 0.4ml specified medium was added to make suspension to the carefully removed cotton plug. Streak of few drops were paced on petri plate which then incubated at appropriate temperature to grow the yeast.

### Anti-microbial activity test

Al-Bayati, & Sulaiman, 2008 method (modified agar diffusion method) was used to determine antimicrobial activity. Commercial yeast suspension (one –tenth of the inoculum of tested organisms), wells of 6.0 mm in diameter were punched on each agar plate using a sterile

cork bore. Fixed volume 0.1 ml of each extract was carefully placed in each well. After that these plates were then covered and incubated at 37 °c for 24-48 hr. After incubation the zones of growth inhibition were measured in mm in different species of candida.

## RESULTS

In our study we focussed on the antimicrobial activity of Miswak and cardamom. The zones of inhibition of both the ingredients in different candidal species in different concentrations that is 5, 10, 25, 50 and 75% (w/v) were assessed.

### Test organism Candida albicans

In our study miswak showed a greater zone of inhibition (18mm) when compared with cardamom(16mm) in higher i.e. 75% concentrations. Even in 50% concentrations miswak (14mm) showed greater zone of inhibition then cardamom (13mm). while in 25%, 10% and 5% concentration the organism was found to be resistant. ( Table 1 and Graph 1).

### Test organism Candida glabrata

In this study we observed cardamom showed greater inhibition zones of 21mm in 75% concentration while 19mm at 50% concentrations when compared to miswak which showed 13mm in 75% and 10mm in 50% respectively. Even in 25 % concentration of cardamom showed greater zone of inhibition (12mm) while miswak is found to be resistant at that concentrations. (fig 1 and fig 2) (Table 2 & Graph 2)

### Test organism Candida tropicalis

In our study we observed that cardamom (14mm) showed greater zone of inhibition then miswak (12mm) at 75% concentration. While both extracts showed same inhibition zone in 50% concentration i.e. 10mm. In the remaining concentration the organism was found to be resistant. (fig 3 and 4, Table 2).

### Test organism Candida krusei

In this study it was observed that cardamom showed greater zone of inhibition in both 75% (20mm) and 50% (16mm) concentration when compared with miswak 14mm and 10mm respectively. Even cardamom showed greater zone of inhibition in 25% i.e., 10mm concentration which is equal to miswak zone of inhibition in 50% concentration. (fig 5 and fig 6, Table 2).

This study showed that cardamom extracts having greater inhibitory values ranging from 20mm to 10mm as zone of inhibition when compared with miswak which ranged from 14 to 10mm as zone of inhibition in all species except c.albicans. While in c.albicans miswak showed greater zone of inhibitory values 18mm to 14mm zone of inhibition when compared with cardamom i.e. 16mm to 13mm. (fig 1,2,3,4,5 and 6) ( Table 1 &2).

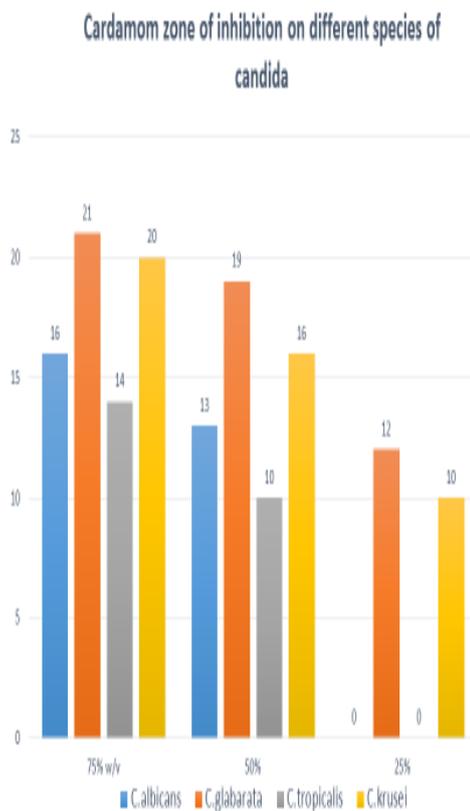
**Table 1: Candida albicans against plant extract at different concentration.**

Sample	75	50	25	10	5
<b>C. albicans</b>					
Cardomom	16mm	13mm	R	R	R
Miswak	18mm	14mm	R	R	R

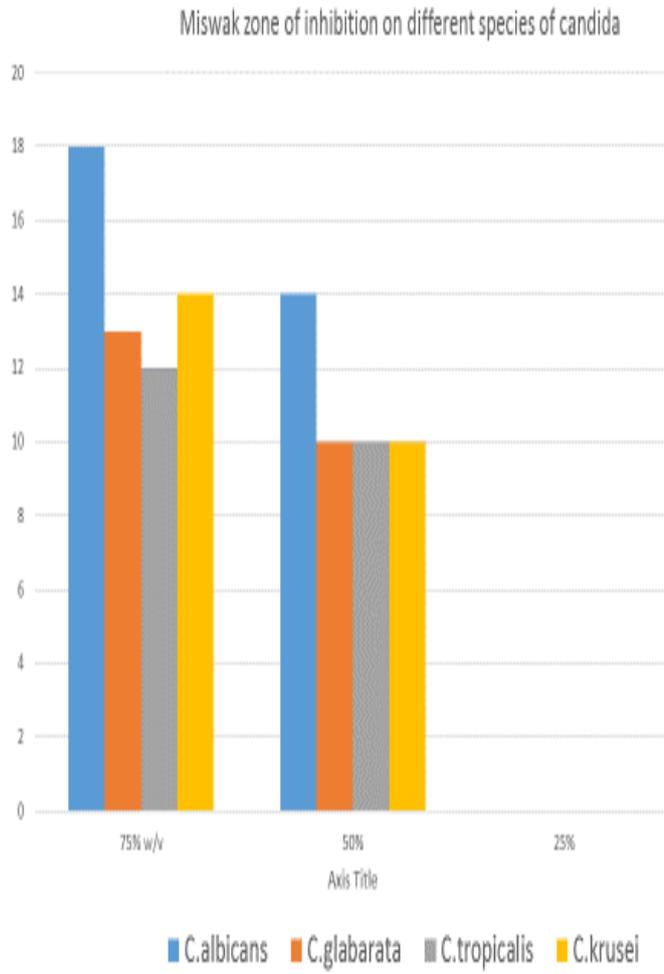
**R: Resistant**

**Table 2: Test organism against plant extract at different concentration.**

Sample	75	50	25	10	5
<b>C. krusai</b>					
Cardomom	20	16	10	R	R
Miswak	14	10	R	R	R
<b>C. tropicalis</b>					
Cardomom	14	10	R	R	R
Miswak	12	10	R	R	R
<b>C. glabrata</b>					
Cardomom	21	19	12	R	R
Miswak	13	10	R	R	R



**Graph 1: Cardamom zone of inhibition in mm on different candida species.**



Graph 2: Miswak zone of inhibition on different species of candida.



Figure 1:



Figure 2:

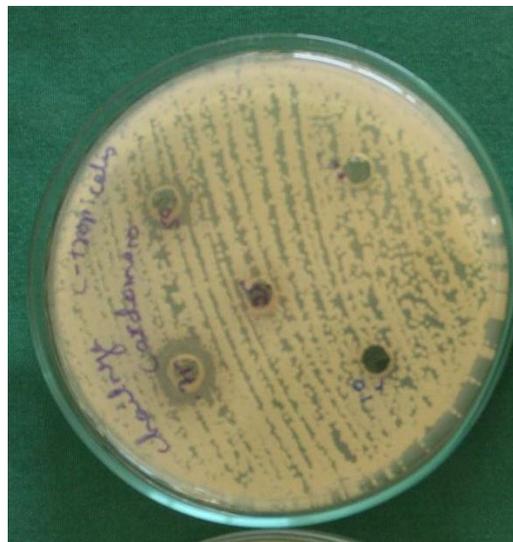


Figure 3:



Figure 4:

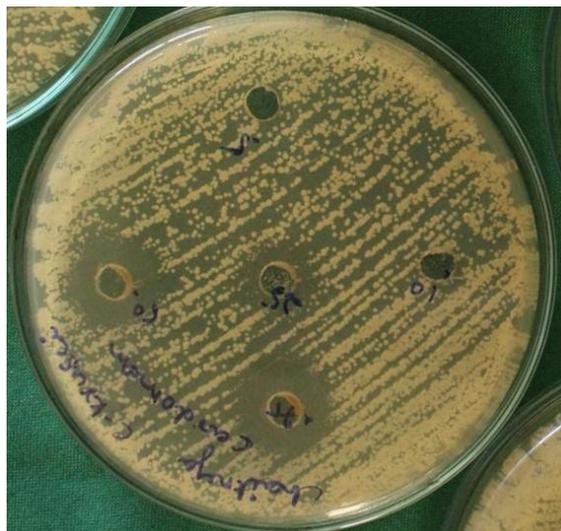


Figure 5:

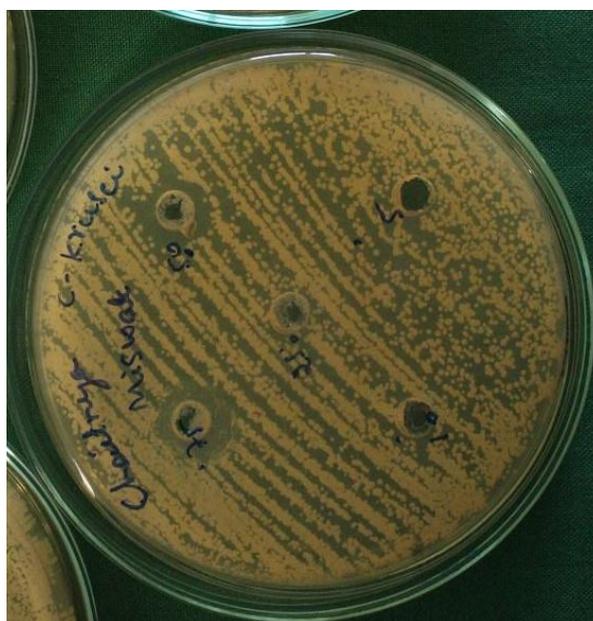


Figure 6:

## DISCUSSION

In this study we noticed that both the plants extract displayed a variable degree of inhibitory effects against this microorganism at 15%, 25% and 75% concentrations.

Cardamom extract showed more strong effect than Miswak extract in all species of candida except candida albicans. While miswak showed greater zones of inhibition in the candida albicans species.

From our results it was observed that both the extracts showing greater zone of inhibition with greater antimicrobial activity against different species of candida in different concentrations and which is in accordance with most of the studies Darout *et al.*, 2000; Al Mas, 2001; Abd-El Rahman *et al.*, 2002; Mohaya *et al.*, 2002;

Agaoglu *et al.*, 2005; Al Mas & Skaury, 2005 ; Al-Bayati, & Sulaiman, 2008; Omar *et al.*, 2009.<sup>[7]</sup>

In our study we observed that miswak showing greater zone of inhibitory values of 18mm to 14mm zone of inhibition when compared with cardamom i.e. 16mm to 13mm. This could be attributed to the Aqueous extracts of miswak in reduction of growth of *Candida albicans*. Such inhibition lasts for up to 36hrs at concentrations of 15% and above.<sup>[8,11]</sup>

Our study findings were in accordance with the study done by Alali *et al* and Noumi *et al* <sup>(8, 9)</sup> in his study he mentioned that the miswak demonstrated the highest inhibitory activity against *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis* strains (with a zone of inhibition range of 10.33-15 mm) using an extract concentration of 300 mg/ml.

Naeini et al<sup>[10]</sup> in his study mentioned that miswak showed the highest zone of growth inhibition for the *Candida albicans* strain, followed by the *Candida dubliniensis* with diameters of the zones of growth inhibition of 10 mm and *Candida glabrata* strains with 7 mm. In contrast, *Candida parapsilosis* and *Candida krusei* were not susceptible which is in accordance with the present study.

In our study Cardamom extract showed greater antimicrobial effect this because presence of high level of phenolic contents and also because of its essential oil while other constituents are believed to contribute little to the antimicrobial effects. It is clear that the essential oil and oleoresins contain phenolic compounds (such as eugenol, carvacrol, methyl eugenol, etc.), and hence antimicrobial activity could be attributed to them.<sup>[6,7,12,13]</sup>

#### Limitations of the study

In this study we observed only zones of inhibition but at which level this extract was acting such as stopping germination tube formation or stopping the adhesion of candida to epithelial cells was not observed or noted.

#### CONCLUSION

In our study we noticed that both the plant extracts were effective against different species of candida. As these extracts are easily available and are less toxic with better antimicrobial effect they can help in the management of oral candidiasis. Further studies in this field may help in using these medicaments topically with good benefit.

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