Antifungal efficiency of Miswak & Cardamom Extract on Some Virulence Factors of Candida albicans as Oral Pathogen

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Abstract

In vitro, inhibitory effects of Miswak & Cardamom extracts on some virulence factors belong to C.albicans as oral pathogen was investigated in this study.

Results showed that both plants extract displayed a variable degree of antifungal activity against tested microorganisms but Cardamom extract showed more strongly effect than Miswak extract in which the inhibitory values was 2 mm as inhibitory zone, 15% adherent rate, 10% germination rate at The concentration 100% Cardamom extract compared with 1 mm as inhibitory zone, 35% adherent rate, 29% germination rate when treated with Miswak extract at The same concentration . The above results recommended that Miswak & Cardamom will be a great help for use as oral hygiene tool & fungicidal therapeutically agent for oral infections caused by Candida albicans.

Introduction

In the last decades the problem of sever nosocomial fungal diseases has become more serious especially in developing countries, such as Candida infection especially in individuals whose immune defense mechanisms have been weakened (Doughari & Naya , 2008) . C.albicans can colonize skin and mucosal surfaces of healthy people and thus occurs commensally in the gastrointestinal tract, oral cavity and vagina, often causing superficial infections (Mavar et al., 2005), because of possessing several virulence factors such as: production of different hydrolytic enzymes, the ability to adherence to the host cells & tissues, Morphological dimorphism (kuleta et al., 2009) & Germ tube formation as initial stage in the yeast–hyphal transition (Melero et al.,1998). Treatment recommendations have included local & systemic using of antifungal agents, but these synthetic compounds may produce resistance to the available antifungal agents evaluated difficulty in treatment (Doughari & Naya , 2008).

The antifungal activities of different wild plants have been reported by many workers and proved a good solvent for this situation, such as Salvadora persica a medical plant whose roots, twigs or stems have been used for centuries as oral hygiene tools in many parts of the world, particularly in Saudi Arabia (Abd-El Rahman et al., 2002) , it has different names in different societies, for instance: Miswak, Siwak or Arak (Al Mas & Al Lafi , 1995) . Many studies have demonstrated that extracts of Salvadora persica possess various antiplaque, antiperiopathic, anticaries, anti-inflammatory and antimycotic effects (Abd-El Rahman et al., 2002 ; Al-Bayati, & Sulaiman, 2008).

Where Cardomom is dried fruit of the tall perennial herbaceous plant Elettaria cardamomum, it used as a spice in meat products but in medicine it used as a
powerful aromatic, antiseptic, stimulant, carminative, stomachic, anti-spasmodic & diuretic (Agaoglu et al., 2005). So, the aim for this study was designed to investigate whether the extracts of Cardamom & Miswak has inhibitory activity on some virulence factors of *C.albicans* as oral pathogens such as, germ tube formation and adhesion ability. Because of the necessary for the development of really effective, non toxic, fungicidal therapeutically agents, since those currently available in clinical practice are confined to relatively few chemical classes and resistances are being developed.

**Materials & Methods:**

- **Test Organism**
  A clinical isolate of *Candida albicans* from oral thrush patient were used for this study, obtained from Biology Dep., College of Science for Women, Baghdad University.

  The yeast was maintained on Sabouraud Dextrose Agar, identified classically by using standard Microbiological methods (Evans & Richardson, 1989).

- **Preparation of Inoculum's**
  The inoculums suspensions of *C.albicans* was obtained by taking five colonies from 24h old cultures grown on SDA. The colonies were suspended in 5ml of sterile saline (0.85% Nacl), shaking for 15 s & the density was adjusted to the turbidity of 0.5 McFarland standard (≈1-5x10⁶ Cfu/ml) with sterile saline (Boukraa et al., 2008).

- **Medicinal plant Extraction**
  A modified method was used (Chevalier, 2003).for this purpose, in which: the Miswak sticks were cut into small pieces and ground to powder in a food blender, as well as Cardamom seeds. 10g from each of them were put into sterile screw–capped bottle containing 100ml of sterile hot distilled water. The extract left for 48h at room temperature then centrifuged at 2000 rpm for 10 min, supernatant filtered through filter paper (0.45 µm pore size). The extract was incubated at 37 °C until it became dry and stored in sterile screw capped vials in the refrigerator until needed.

  From this different concentrations 25, 50, 75 and 100% (w/v) of prepared were assayed immediately after dilution.

- **Antimicrobial activity test**
  A modified ages diffusion method (Al-Bayati, & Sulaiman, 2008) was used to determine antimicrobial activity. SDA was inoculated with a microbial cell 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 the each extract was carefully placed in each well. The plates were then covered and incubated at 37 °C for 24-48 hr. After incubation the zones of growth inhibition were measured in mm, studies were carried out for each test strains in duplicate and average measurement calculated.

- **Quantitative assessment of germ tube formation**
  To assay the antifungal activity of plants extract solutions on germ tube formation by the yeast isolates, the modified method was used (Al-Maomory,
of the yeast suspension treated with plants extract solutions in different concentrations (0, 25, 50, 75 and 100%) was added to a test tube containing 2 ml of human serum, mixed & incubated at 35 °C for 2-3hr. Results recorded by taking a drop of the suspension and examined it with a microscope to confirm the presence or absence of germ tubes.

**Adhesion assays**

Yeast adhesion to exfoliated buccal epithelial cells was determined by light microscopy as described previously (Bailey *et al.*, 1995) with slight modification as follows: Human buccal epithelial cells (HBEC) were collected on cotton swabs from healthy female volunteers and transferred to phosphate-buffered saline (PBS) PH 7.2. HBEC was washed three times with PBS and counted by using a hemocytometer, then 1 ml of the yeast suspension treated with plants extract solution in different concentrations (0, 25, 50, 75 and 100)% was added to a test tube containing 1 ml of HBEC at the concentration 1x10^4 cell/ml in PBS were incubated for 1h at 37 °C with shaking, after centrifugation with PBS three times for washing, the sediment were suspended in PBS, one drop from this solution was transferred to microscope slide and stained with crystal violet stain to determine the percent adherence. Adherence was expressed as the percentage of HBEC with adhering Candida cells.

**Results & discussion:**

The inhibitory effects of extracts from Miswak & Cardamom against *C.albicans* as oral Pathogen has been examined in this study as presented in figure (1).

![Graph](image)

Figure (1) Antimicrobial activity of Miswak & Cardamom extracts against *C.albicans* as Inhibition zone values.
According to the findings of this study both plants extract displayed a variable degree of inhibitory effects against this microorganism, but cardamom extract showed more strongly effect then Miswak extract in which the inhibitory values ranged from 0.5-2 mm as inhibitory zone for the different crude extracts compared with the inhibitory values ranged from 0.2-1 mm as inhibitory zone for Miswak extract.

Following our earlier demonstration, the effects of these plants extract on some virulence factors of \textit{C.albicans} such as: germ tube formation and adhesion ability have been done also as summarizes in figures 2 & 3 in which the germination value (%)and adhesion ability(%) of tested yeast isolates show different results depending on which crude extracts was used.

In which our results within the ability of these extracts to inhibit \textit{C.albicans} adhesion to exfoliated buccal epithelial cells figure (2) reveals that \textit{C.albicans} adherent value raged to 85% when treated with cardamom extracts used at a concentration of 25% inhibited to a maximum value 15% when treated with this extract at a concentration of 100%. less inhibitory effects were found about 95% when treated with Miswak extract at a concentration of 25% inhibited to a maximum value up to 35% when treated with this extract at a concentration of 100% compared to controls.

![Graph](image)

**Figure (2) The inhibitory effects of Miswak & Cardamom extracts on adhesion ability of \textit{C.albicans}.

Similar correlations were obtained when we investigated the germination value which tested separately with crude extracts as we seen in figure (3).
Figure (3) The inhibitory effects of Miswak & Cardamom extracts on germ tube formation by *C. albicans*.

Results from the present study showed that cardamom & Miswak extracts had some antimicrobial activity against *c. albicans* as oral pathogens in a variable degree as most studies found (Darout *et al.*, 2000; Al Mas, 2001; Abd-El Rahman *et al.*, 2002; Mohaya *et al.*, 2002; Agaoglu *et al.*, 2005; Al Mas & Skauy, 2005; Al-Bayati, & Sulaiman, 2008; Omar *et al.*, 2009) with no sign to its effect of these extract on the virulence factors belong to this microorganisms.

The selection of Miswak was based on a number of factors: the common using of it, furthermore this sticks are cheap, their taste is agreeable and not unpleasant and reported to have anti-plaque and many other pharmacological properties (Darout *et al.*, 2000; Marongiu *et al.*, 2004; Al Mas & Skauy, 2005) in which the antimicrobial and cleaning effects of Miswak may be attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadourea and salvadorine, saponins, tannins, vitamin C and silica and resin (Darout *et al.*, 2000) in addition to lignan glycosides (kamel *et al.*, 1992), alkaloids, terpenoids, and oleic, linoleic and stearic acids (Abd El Rahman *et al.*, 2003).

On the other hand, the results indicated that cardamom extracts also had antimicrobial activity associations with the age of the content of volatile oil in the seeds which is strongly dependant on the age of the product & storage conditions, The chemical composition of cardamom is: volatile oil, sabinen, myrecene, limonene, terpinolene, terpen, citronellol and geraniol with eugenol and transnerolidol (korikanthimath *et al.*, 1999; Marongiu *et al.*, 2004) therefore, in medicine, it is used as a powerful aromatic, antiseptic, stomachic stimulant, anti-spasmodic and diuretic (Agaoglu *et al.*, 2005).
It could be concluded that Miswak and cardamom are excellent oral hygiene agents, proven to be a great help in developing countries with financial constraints and limited oral health care facilities and their use should be promoted based on scientific knowledge of their benefits and proper use. Because it is widely available in this part of world and is inexpensive.

References:


Candida كفاءة الفعالية المضادة للفطريات لمستخلصات السىاك والهيل على بعض عوامل امراضية

Candida albicans كاحذٖ يًزداث انفى

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الخلاصة

اجريت هذه الدراسة خارج الجسم الحي للتحري عن الأثر التثبيطي لمستخلصات السىاك والهيل ضد بعض عوامل امراضية حمضية C.albicans كاحذٖ ممرضات الفم.

حيث بينت النتائج التي تم الحصول عليها ان كلا المستخلصين اظهر فعال تثبيطيا ولكن بدرجات متفاوتة ضد الكائن قيد الدراسة بحيث أدى مستخلص الهيل كان فعال التثبيطي أكبر على الكائن قيد الدراسة مقارنة بالفعل التثبيطي لمستخلص السىاك من خلال ظهور منطقة تثبيط قطرها 2 مم ومعدل التصاص 15% و نسبة المباث 10% عند الدراسة بمزج المستخلصات يتركز 100% بينما كان قطر منطقة التثبيط 1 مم ومعدل الالتصاص 35% و نسبة المباث 4% عند الدراسة باستخدام مستخلص السىاك عند نفس التركيز. من خلال النتائج
التي تم الحصول عليها نجد أنه بالإمكان استخدام المستخلصات النباتية لنباتي الهيل والسواك كعسلات مطهرة للنفط الوقاية والعلاج من الإصابات الفموية بهذه الخصائص المرضية.