The effect of *Tamarix ramosissima* and *Cinnamomum zeylanicum* extracts on the growth of *Candida* spp. isolated from the saliva of type II diabetes mellitus patients

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**ABSTRACT**

**Background:** Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. These secondary metabolites could be utilized for benefit of mankind by studying their medicinal properties. The study was performed for isolation and identification of the oral *Candida* species in patients with type II Diabetes mellitus and healthy subjects (control group) and testing the effect of *Tamarix ramosissima* (*T. ramosissima*) and *Cinnamomum zeylanicum* (*Ci. Zeylanicum*) extracts on the isolated species.

**Materials and Methods.** Fasting stimulated saliva were collected by spitting method for each subject. *Candida* species were isolated, purified and identified according to morphological characteristic on chromogenic agar (CHROMagar Candida) and biochemical test on API Candida. Also the study included the effect of *T. ramosissima* and *Ci. zeylanicum* extracts on the isolated species using agar well diffusion method. Aqueous, alcoholic, ethylacetate and aqueous acetone extracts were prepared in different concentrations *T. ramosissima* and aqueous, alcoholic extract from *Ci. zeylanicum*. The activity of the extract was determined by measuring the diameter of inhibition zone.

**Results:** The results showed that *Candida* species was recovered from the oral cavity of 76% of the diabetic group, in contrast to 8.58% of the control group. *Candida albicans* (*C. albicans*) was the most frequently isolated species in both groups it reach to 33 isolates (66%) (in diabetic group follow by *C. krusei* two isolates (4%), *C. glabrata* one isolate (2%), *C. tropicalis* one isolate (2%) and *C. lusitaniae* one isolate (2%) and three isolates (8.58%) of *C. albicans* in the control group. There was highly significant p<0.01 correlation between glycemic control and age, significant p<0.05 correlation between females and diabetes with oral carriage of *Candida* species, also results showed highly significant p<0.01 antifungal effect of *Tamarix ramosissima* aqueous acetone extract on all *Candida* species by using different concentrations there; were diameters of inhibition zones found to increase with increasing the concentration of the extracts. While aqueous, alcoholic and ethylacetate extract of *Tamarix ramosissima* did not show any effect on *Candida* species. Highly significant p<0.01 was shown in aqueous, alcoholic extracts of *Cinnamomum zeylanicum* on all *Candida* species, the diameter of inhibition increase with increasing the concentration.

**Conclusion:** Diabetic patients had a higher oral candidal carriage rate in comparison to non-diabetic. *C. albicans* was the predominant isolate, and a variety of other *Candida* species. Oral candidal colonization was highly significantly associated with fasting blood sugar. *Tamarix ramosissima* leaves (aqueous acetone) extract have antifungal effect against *Candida* while (ethylacetate, aqueous and alcoholic) extract don’t have antifungal effect against *Candida* isolates from the oral cavity of Diabetes mellitus type II. *Cinnamomum zeylanicum* bark (aqueous and alcoholic) extract have antifungal effect against *Candida* isolates from oral cavity of Diabetes mellitus type II.

**Key words:** Antifungal activity, diabetes, *Tamarix* leaves, *Cinnamomum* bark.

**INTRODUCTION**

Subjective oral dryness is a frequent complaint among diabetic patients. This subjective sensation of dry mouth is more frequently reported among these patients than among healthy subjects (4). Recently, there has been a dramatic increase in the use of the plant products and herbs due to increased efficiency of the plant-derived drugs and the growing interest in the natural product and also, because of the side effect of the conventional medicine. One of such plants was *T. ramosissima* is a semi-deciduous, loosely branched shrub or small to medium-sized tree.

It has been reported to be used as a topical application in measles and for skin allergies, as a local application to foul sloughing ulcers and bbebes; and that the powdered, which are rich in tannin, form an efficacious ointment in ulcerating piles and anal fissures (5).

Also *Ci. zeylanicum* is the inner bark of a tropical evergreen tree. It has been reported to be effective in the treatment of type II diabetes, arthritis pain and common colds; also it has a high potency as antioxidant and antimicrobial activity (6) and also, uses in dental and pharmaceutical preparations, toothpastes and mouthwashes (7). It has an active role as anticariogenic agent and treatment of toothache and fight bad breathe (8).
MATERIALS & METHODS

Plant Material:
The bark of *Ci zeylanicum* was brought from local market and leaves of *T. ramosissima* was collected of Baghdad/Iraq in October - November 2010. Freshly collected plant parts were shade-dried at room temperature for 10–15 days. Dried bark and leaf samples were separately crushed and ground into fine powder.

Preparation of extracts:
Powdered plant materials were sequentially extracted with different solvents for 24 h according to the method described by (9) for *T. ramosissima* and (10) for *Ci zeylanicum*. The solvents used for extraction included aqueous, alcohol, ethylacetate and acetone. Then filtered by filter paper (WattmanNo.1), the extract left to dry in glass Petridish at 37ºC in the incubator, the powder was collected in tightly closed glass container and kept in refrigerator until use. Different concentrations of *Ci. zeylanicum* bark alcohol extract were used (20, 40, 80, and 160) mg/ml, three different concentrations of *Ci. zeylanicum* bark aqueous extract were used (500, 750, 1000) mg/ml, different concentrations of *T. ramosissima* leaves aqueous, alcohol and ethylacetate extracts were used (250, 500, 750, 1000) mg/ml and three concentrations of *T. ramosissima* leaves aqueous acetone 1:1 extract were used (250, 500, 750, 1000) mg/ml then sterilized by filtration.

*Ci. zeylanicum* and *T. ramosissima* active materials were tested against a panel of Candida spp. Including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. kruesseland C. lusitaniae* were isolated from type II diabetes mellitus on Sabouraud dextrose agar (SDA) and detection on CHROMagar and APICandida.

Screening for Antimicrobial Activity:
The aqueous acetone 1:1, alcohol, ethylacetate and aqueous Tamarix leaves extract and aqueous, alcohol cinnamon bark extracts were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method (11). In this method, pure isolate of each Candida species was subcultured on CHROMagar at 37ºC for 24h. A plate of each Candida species was taken and a minimum of four colonies were touched and transferred into normal saline (0.85%). Density of suspension was adjusted equal to that of 106 cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. 0.1ml of inoculums of each test Candida was spread onto SDA plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 8mm were made with a sterile borer in the inoculated agar plates. The extracts were reconstituted in distill water and alcohol. A 0.1ml volume of each extract was propelled directly into the wells (in triplicates) of the inoculated SDA plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37ºC for 24h (12). Distill water and alcohol served as the negative control and Candinazole served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm (13). The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

RESULTS

Of the 50 diabetic patients and 35 control subjects screened for the occurrence of Candida species, 76 and 8.5%, respectively, were found positive for Candida Species. In the present study a high number of Candida species was isolated from 38of the 50 diabetic patients. *C. albicans* was the predominant species isolated from patients (66%), the follow species identified were: *C. krusei* 2 of 50 patients (4%), and *C. glabrata*, *C. tropicalis* and *C. lusitaniae* 1 of 50 patient (2%), respectively. In the group of healthy subjects, *C. albicans* was the species colonizing the oral mucosa of 3 of 35 subjects (8.5%) (Table 1).

Chi-square test revealed a highly significant difference (P<0.01) for candidal isolates with age, observed that diabetics older than 70 years showed a greater prevalence for Candida growth in comparison with the healthy controls(Table2) and diabetes value, in which FBS value ≥ 250mg/dl was found to be more associated with oral Candidal isolate specially *C. albicans* (Table 3).Chi-square test revealed a significant difference (P<0.05) for candidal isolates with gender between diabetes and healthy controls (Table 4).Statitical analysis between different concentrations of *T. ramosissima* Aqueous Acetone Extract and Candinazole showed highly significant in relation to *C. krusei* and *C. lusitaniae* while significant in relation to *C. albicans*, *C. glabrata* and *C. Lusitaniae* (figure 1).

The results of aqueous, alcoholic and ethylacetate extracts of *T. ramosissima* leaves showed not antifungal activity against the Candida isolates in (250, 500, 750 and 1000) mg/ml. Statistical analysiswas preformed between different concentrations of *Ci. Zeylanicum*

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aqueous extract and Candidamazole in relation to C. glabrataand C. lusitaniaes showed highly significant while significant in relation to, C. albicans, C. krusei and C. tropicalis. (figure 2). Statistical analysis was preformed between different concentrations of Ci. zeylanicum alcohol extract and Candidamazole in relation to C. albicans, C. glabrata and C. lusitaniae showed highly significant while in relation to C. krusei and C. tropicalis showed significant effect, (figure 3).

DISCUSSION
Candidal infections are a major problem in the world, especially among the immunosuppressed people. Furthermore, increased susceptibility to oral infections with yeasts has long been associated with diabetes mellitus, but the results remain controversial and Contradictory (14).

In the present study 38 of the diabetic patients (76%) were found to carry Candida spp. in their oral cavity. This finding showed a statistical highly significance for the patients compared with the control group. The predisposition of the diabetics to infections by pathogenic fungal species has been explained in terms of enhancement of yeast growth by elevated tissue fluid glucose levels. Moreover, the presence of a high concentration of salivary glucose combined with low salivary secretion may enhance growth of yeasts and their adherence in epithelial oral cells (15). Furthermore, as the age groups were combined the results revealed a great significance in candidal growth in diabetics older than 70 years. There could be several etiologies for such an association. Among older adults, medical problems, increased prescription and over-the-counter medication intake, poor oral hygiene, dietary selections and increased dental problems are some of the potential factors that could influence candidal growth in the oral cavity.9 Also The result of the study revealed that females had higher Candidal Carriage in comparison with males which was statistically significant P<0.05 in diabetics This may be related to different volumes of salivary glands, as it was found male submandibular glands were 50% larger than female glands (16) due to males had higher salivary output when in comparison with females. Also may be attribute to the hormone levels alteration (e.g. estrogen and progestron) (17).

The results of the study showed higher Candidal carriage with increase FBS values. The result means that increase blood glucose level (hyperglycemia), that might increase osmotic ingredients within the salivary glands, thereby limiting secretion (18), combined decrease salivary flow rate, associated suppression of antimicrobial proteins and peptides in saliva which, may promote overgrowth of Candida (19).

T. ramosissima leaves aqueous acetone extracts showed antifungal activity at a concentration of (250, 500, 750 and 1000) mg/ml inhibit the growth of all isolates. The action of T. ramosissima reflect the activity of the extracts may be due to the presence of polyphenolic compounds such as flavonoids and tannins (20). The polyphenolic compounds of the extract bind with active site of the cell enzyme by its hydroxyl group which have the ability to form hydrogen bonds with these sites then inhibit the important metabolic activities exerted by these enzyme such as the growth, reproduction and protein synthesis (21).

The diameters of inhibition zones were found to increase with increasing the concentration of the aqueous acetone extract. The amount of the dissolved active constituents of the extract will be more abundant as the concentrations increase causing increasing antifungal activity of the extract.

The results of alcoholic, ethylacetate and aqueous extracts of T. ramosissima leaves showed no antifungal activity against the test strains may be due to presence of active components in insufficient quantities in the crude extracts to show the activity with the dose levels employed.

Cinnamomum zeylanicum bark extracts (aqueous and alcoholic) were found to inhibit the growth of all Candida isolates. The action of Ci. zeylanicum reflect the activity of the cinnamon extracts which may be due to the presence of cinnamondehyde, an aromatic aldehyde. Cinnamon bark is rich cinnamondehyde (50.5%) which is highly electronegative and interferes in biological processes involving electron transfer and react with nitrogen containing components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms (22). Phytochemical screening of the C. zeylanicum has revealed that extract form bark posses at least three to four of the classes of secondary metabolites. Phenols, flavonoids, trepenoids, alkaloids and saponin (23) therefore, the presence of these phytochemicals could be some extent justify the observed antifungal activity through the inhibition of cytochrome p450 demethylase, RNA and DNA synthesis (24). Also the cinnamon bark contains tannins consisting of polymeric 5,7,3,4–tetrohydroxy flavan-3,4–diol units (25) have a great affinity to precipitation of proteins forming proteins complex which will affect the cytoplasmic membrane function integrity.
The differences in the sensitivity of Candida isolates to the different concentrations of the extracts of *Ci. zeylanicum* and Candimazole could be due to hereditary contents of the isolates which may alter the susceptibility of the organisms by modifying the targets to be attacked by the active constituents like proteins and lipids of the microbial membrane or inhibiting the constituents of the barks’ extract or modifying the structure of these constituents by some enzymes rendering them to less effective compounds.

**REFERENCES**


**Table 1: Distribution of oral Candidal isolate in diabetic and control groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number</th>
<th>Candida isolates (C. albicans, C. krusei, C. glabrata, C. tropicalis, C. lusitaniae)</th>
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<tr>
<td>Diabetic</td>
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<td>Total</td>
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The effect of *Tamarix*
Table 2: Age distribution with oral Candidal carriage

<table>
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<th>Age in Years</th>
<th>Diabetic (N=50)</th>
<th>Non-diabetic (N=35)</th>
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<tr>
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<td>45-50</td>
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<td>Total</td>
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Table 3: Relationship between oral Candida isolation and diabetic value

<table>
<thead>
<tr>
<th>FBS values</th>
<th>C. albicans</th>
<th>C. glabrata</th>
<th>C. kruzei</th>
<th>C. tropicalis</th>
<th>C. lusitaniae</th>
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<tr>
<td>mg/dl</td>
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Table 4: Gender distribution with oral Candida carriage

<table>
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<th>Non-Diabetic</th>
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<td>Total</td>
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<td>76</td>
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Figure 1: Antifungal effect of *T. ramosissima* aqueous acetone extract and Candimazole on Candida isolates
Figure 2: Antifungal effect of *Ci. zeylanicum* aqueous extract and Candidimazole on Candida isolate

Figure 3: Antifungal effect of *C.zeylanicum* alcoholic extract and Candimazole on Candida isolates