Invitro: Antimicrobial Activity of Leaves Extracts of Eucalyptus Spathulata Against Streptococcus Mutans and Candida Albicans

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Abstract: Streptococcus mutans and candida albicans, are the most common causes of oral infections and the artificial drugs have unpleasant side effects on the other hand the number of drug resistant microorganisms is increasing so the developing countries are trying to pay more attention to herbal drugs. Plants are natural source of antimicrobial agents and plant- derived medicines have been a part of our traditional health care system. Dental plaque, which caused by Streptococcus mutans plays the primary role in the pathogenesis of the disease.

Present study deals with evaluation of the antimicrobial activity of methanolic extracts of Eucalyptus spathulata traditional medicinal plant. The efficacy of the plant extracts has been assessed by testing on salivary samples of patients suffering
from dental carries. Antimicrobial assay was carried out using agar well diffusion method. Against Strep. mutans the mean of inhibition zone was 12.4mm comparing with 15.2mm for gentamicin and 5.1mm against C. albicans comparing with 10.9mm for nystatin. The study revealed that methanolic extract of Eucalyptus spathulata was effective in inhibiting on Strep. mutans than on C. albicans.

Keywords: Dental careis, Eucalyptus spathulata, Agar well diffusion method

1. Introduction

Nature has been a source of medicinal remedy for thousands of years and since the beginning of man. Artificial drugs have some side effects on the in addition the resistance of microorganisms to these drugs is clear. So recently in the developing countries are trying to pay more attention to herbal drugs [1,2]. It was established that mutans group of Streptococci are the key agents causing dental caries [3]. According to Dr. Keyes and Fitzgerald dental caries was an infectious process of tooth with interplay of plaque, tooth and diet [4]. A wide spectrum of antibacterial medicines is used to treat these infections. But these drugs can sometimes give rise to numerous adverse orofacial manifestations, particularly dry mouth, taste disturbances, oralmucosal ulceration, and/or gingival swelling [5]. There is a continuous need of new antimicrobial components due to rapid emergence of multidrug- resistant pathogens and explosive dreadful infectious diseases. The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora. Mouth bacteria have been linked to plaque, tooth decay and toothache. Plaque [6] (a layer that forms on the surface of a tooth, principally at its neck; composed of bacteria in
an organic matrix) has been linked to gingivitis, periodontal disease, or dental carries [7]. Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpastes and mouthwashes (7+1). There are diverse types of mouth bacteria.

Some are useful, others are hurtful: Neisseria, Staphylococcus, S. pneumoniae, Porphyromonas gingivalis, Diphtheriod, Fusobacteria and Haemophilus [9]. Plants are natural source of effective antibacterial agents. Recent reviews indicate that there is a great potential to find compounds leading to the production of new antibiotics from plant source [10, 11, 12].

The genus Eucalyptus spathulata is known for its rich source of bioactive compounds. [13] It is a source for several unique metabolites which show a variety of biological activities. Studies of medicinal plant extracts have demonstrated broad antimicrobial activity of Eucalyptus [14] against B. Cereus, E.coli, S. areus, fungus including C. albicans isolates [15], and other Gram-positive bacteria. Specific activity against periodontopathic bacteria, such as Porphyromonas gingivalis, Strep.mutans, and S.soribanus [13, 16]. In-Vitro anti-fungal properties have been reported in multiple Eucalyptus species [17].

2. Material and Methods

Preparation of plant extract

Exactly 400g of plant was macerated successively in methanol for 48hrs. The mixture was then filtered under vacuum and the filtrates concentrated using a rotatory evaporator. 10 gram of powdered sample was filled in screw cap bottles with 10 ml of methanol. It was kept at 220 C for fifteen days.

Isolation of microorganisms

The tested bacteria firstly isolated from saliva patients suffering from dental carries and gingivitis on mitis salivarius + bacitracin medium and identification by API-20 Strep. system
(bioMérieux, France) for S. mutans, then subcultured on blood agar and isolated C. albicans on Sabouraud dextrose agar (SDA) (Oxoid, UK) and by yeast identification system API 20C AVX (bioMerieux, France).

**Determination of antibacterial activity**

The bacterial isolates were effectively swabbed on the prepared Mueller-Hinton Agar plates (MHA) (oxoid, UK) and the fungi swabbed on (SDA). The microbial inoculum was standardized at 0.5 McFarland. 200μl of bacteria were aseptically introduced and spread using cotton swabs on surface of Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate. Introduced (50μl of a 100 mg /ml) of the methanolic leaves of Eucalyptus spathulata into three duplicate wells. A negative control well was made too with 50μl of the extracting solvent (methanol). For the positive control Gentamicin (0.5mg/ml: Mast Group UK) and nystatin (0.2mg/ml: HIMEDIA, France), were used for Strep. mutans and C.albicans respectively. The screening was done in triplicates. Sterilized distilled water was used as negative control.

Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37ºC for 24 hours. After incubation all the plates were observed for zones of inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions. Finally the diameter of the zone of inhibition were recorded and expressed in mm [15].

**Statistical analysis:** The results were calculated as mean diameter of zone of inhibition in mm ± standard deviation (mean ± SD). By ANOVA analysis.

**3. Results**

After 24h, the antimicrobial activity was evaluated by measuring the inhibitory zone diameter. This test was repeated 10
times for each plant extract. In the plate of Strep. mutans lack of growth around the methanol wells which were used as negative controls was observed. The inhibition zone around the gentamicin which was used as positive control was observed. The mean of inhibition zone was 15.2, SD. =1.60mm for gentamicin, and 10.9mm, SD. =0.70 for nystatin, 12.4mm for Eucalyptus spathulata. Data presented in Table (1) and figure (1) revealed that methanolic extract of Eucalyptus spathulata was more effective in inhibiting on Strep. mutans.

Table1: Antimicrobial activity of methanol extracts of Eucalyptus.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>The mean of inhibition zone mm (averages±SD)</th>
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<tbody>
<tr>
<td></td>
<td><em>Streptococcus mutans</em></td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td>Eucalyptus spathulata</td>
<td>12.4=0.80</td>
<td>5.1=0.4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>15.2=1.60</td>
<td>-</td>
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<tr>
<td>Nystatin</td>
<td>-</td>
<td>10.9=0.70</td>
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*=significant at 0.05

Figure (1): Antibacterial activity of Eucalyptus spathulata leaves extract against Streptococcus mutans. 1=Negative control. 2= Gentamicin. 3= Eucalyptus spathulata.
In the plate of Candida albicans no zone indicative of the lack of growth around the methanol well which was used as negative controls was observed. The mean of inhibition zone was 10.90mm for nystatin. Table (1) and figure (2) revealed that methanolic extract of Eucalyptus spathulata was less effective in inhibiting on C. albicans than Strept. mutans.

![Figure (2): Antifungal activity of Eucalyptus spathulata leaves extract against Candida albicans. 1=Negative control. 2= Nystatin. 3= Eucalyptus spathulata.](image)

4. Discussion

Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour.

Dental caries is a public oral health problem and an infectious-contagious disease implying an imbalance of normal molecular interactions between the tooth surface/subsurface and the adjacent microbial biofilm [18]. The side effects of synthetic antimicrobial agents are high and the tolerance to these agents is increasing. Therefore, a search for alternative drugs is essential. In recent
In the present investigation, the antimicrobial activity of methanolic leaf extracts of Eucalyptus spathulata was evaluated against Strep. mutans, and C. albicans. The antimicrobial properties of the Eucalyptus spathulata may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids. It is reported that leaves from Eucalyptus spathulata contain monoterpenes and sesquiterpenes, and the bark contains ketones like juglone, regiolone, sterol and flavonoid which were bacteriostatic activity against Strep.mutans [20], by its ability to form hydrogenous bond with protein which lead to stopping protein built up in the cell. The phenol compound of could be considered clinically as antifungal agent [21].The antimicrobial activities of this plant have already been studied with different microorganisms. [22] reported the growth inhibition effect of Eucalyptus spathulata extract against gram positive (Staph.. aureus and Strep..mutans), gram negative (E. coli and P.aeruginosa) and pathogenic yeast C. albicans. [23] Studied the antimicrobial activity of Eucalyptus spathulata leaf extracts, in which they reported the zone of inhibition ranged from 15.8–17.6 mm against P. acnes, 11.3–15.7 mm against Strep. mutans and 12.9–15.5 mm against S.epidermidis by disc diffusion method. Their antimicrobial activities were checked against gram positive (Bacillus cereus, Bacillus subtilis, Staphylococcus aureus) and gram negative bacteria (Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae) and fungi (Candida albicans, Cryptococcus neoformans), revealing activity against the different tested microorganisms [24].The antimicrobial activities of the Eucalyptus spathulata extract due to contain saponins, tannins, steroids and flavonoids [22]. Gentamicin was used as positive control as an antibacterial antibiotic which produced the inhibition zone 15.2mm whereas nystatin was found to make an inhibition zone 10.9mm which was used as positive antifungal. The results are in accordance with those obtained by [25, 26] who reported that the
widespread use of nystatin has led to the appearance of resistant Candida isolates.

The results obtained in this study show that Eucalyptus spathulata could be used as an easily accessible natural source to inhibit the growth of Strep. mutans and C. albicans responsible for dental plaques and oral hygiene problems. Further studies should be developed to identify the molecules responsible for this bioactivity.

References


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دراسة مختبرية: الفعالية المايكروبية لمستخلص أوراق الكالبتوس ضد بكتريا السبحيات الميوتنسية وخميرة المبيضات البيض

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المستخلص:
تعتبر كل من السبحيات الميوتنسية وخميرة المبيضات البيض من عوامل الإصابة الفموية. وان الادوية الاصطناعية لها اعراض جانبية غير مرغوب فيها بالإضافة إلى زيادة الاحياء المجهزية التي تصبح مقاومة لتلك الادوية لذلك تسعى الدول المتقدمة للاهتمام بالاعشاب الطبية أكثر. تعتبر النباتات الطبية ومشتقاتها المصدر الطبيعي للمضادات المايكروبية. والسبحيات الميوتنسية لها الدور الأساسي في حدوث بلاك الأسنان والتسوس.لذا تهم الدراسة الحالية بتقييم الفعالية المايكروبية لخلاصة النبات الطبيعي التجاري الكالبتوس على هذه البكتريا وتمت دراسة تلك الفعالية من خلال فحص نماذج لعاب لمرضى يعانون من تسوس الأسنان وباستخدام طريقة تنافذ الحفر. وكانت منطقة التنافذ ضد السبحيات الميوتنسية 1,1 ملم لخلاصة اوراق الكالبتوس مقارنة بالمضاد جنتاميسين 15,2 ملم في حين كان معدل منطقة التنافذ ضد المبيضات البيض 5,1 ملم مقارنة بالمضاد نستاتين 10,9 ملم. واظهرت الدراسة ان الخلاصة الميثونولية للاعشاب المستخدمة لها تأثير مثبت على السبحيات الميوتنسية أكثر منه على المبيضات البيض.

كلمات مفتاحية: تسوس الأسنان، الكالبتوس، طريقة التنافذ بالحفر