

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*, *Diphtheroid*, *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. aureus*, 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. 200 μ l 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 (50 μ l of a 100 mg /ml) of the methanolic leaves of *Eucalyptus spathulata* into three duplicates wells. A negative control well was too made with 50 μ l 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 37°C 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 \pm standard deviation (mean \pm 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 .

Extracts	The mean of inhibition zone mm (averages±SD)	
	<i>Streptococcus mutans</i>	<i>Candida albicans</i>
<i>Eucalyptus spathulata</i>	12.4=0.80	5.1=0.4
Gentamicin	15.2=1.60	-
Nystatin	-	10.9=0.70

*=significant at0.05

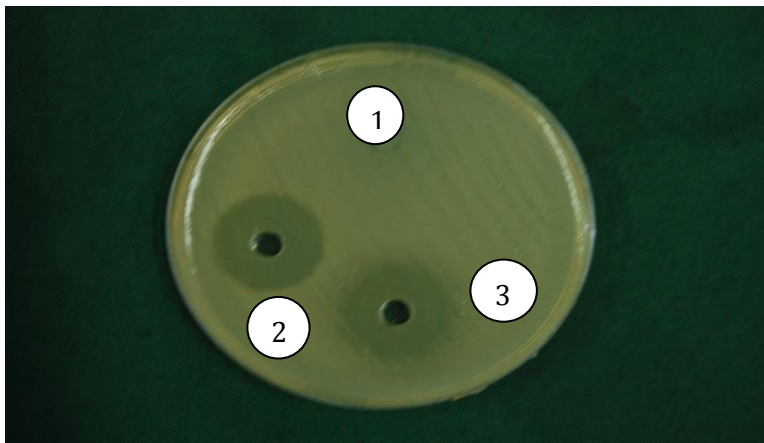


Figure (1): Antibacterial activity of Eucalyptus spathulata leaves extract against Streptococcus mutans. 1=Negative control. 2= Gentamicin. 3= Eucalyptus spathulata.

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*.

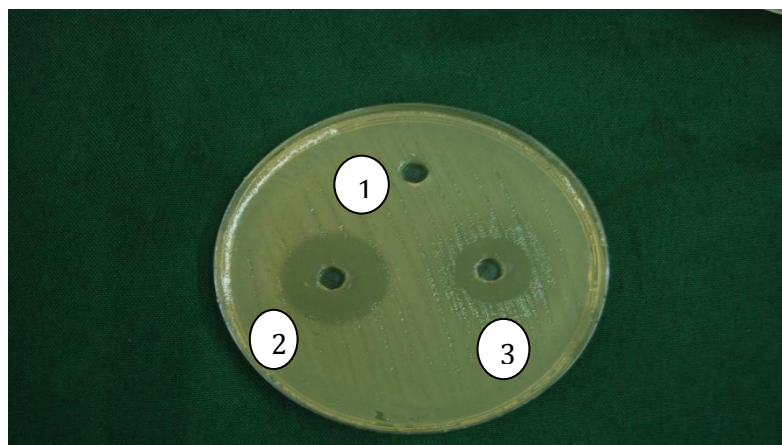


Figure (2): Antifungal activity of *Eucalyptus spathulata* leaves extract against *Candida albicans*. 1=Negative control. 2= Nystatin.3= *Eucalyptus spathulata*.

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

decades, an increasing tendency toward the use of natural substances instead of synthetic ones has been observed [19].

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 *Strept. 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 sources 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

- [1] Sakoulas G., and Moellering R.C. *"Increasing antibiotic resistance among methicillin-resistant Staphylococcus aureus strains"*. Clin. Infect. Dis . 46 Suppl, 2004, pp. 5 360-3675.
- [2] Alkhawajah, A.M. *"Studies on antimicrobial activity of Eucalyptus maculate"*. Am. J. Chin. Med. 25, 1997,175-180.
- [3] *"Bacterial Genomics-Streptococcus mutans"*, National Academyof Sciences, USA, vol. 22, 2002, pp. 14434-14439.
- [4] Jason M., Tanzer, *"Biological active components of some plants oil"*, Journal of Dental Research. vol.74, 1995, pp.1536,
- [5] Scully C., Bagan J.V. *"Chemical composition and antimicrobial activity of Eucalyptus maculate"*, Crit. Rev. Oral Biol. Med. Vol. 15 Issue 4, 2004, pp.221-39.
- [6] *"Oxford Concise Medical Dictionary"*. 5th edition, Oxford University Press. 1997.
- [7] Jensena JL, Barkvoll P. *"Clinical Implications of the Dry Mouth: Oral Mucosal Diseases"*. Annals of the New York Academy of Sci. V. 842, No.1, 1998, pp.156–162.
- [8] Rosin M, Kramer A, Detlef B, Gerrit R, Kocher T. *"The effect of a SCN/H2O2 toothpaste compared to a commercially available triclosan-containing toothpaste on oral hygiene and gingival health –a 6-month home-use study"*. J Clin Periodontol.V. 29,No.12, 2002, 1086–1091.

- [9] Collins WJ, Walsh TF. *"Handbook for dental hygienists"*. 1998, pp 272-273.
- [10] Chopra R. N., Nayar S. L. and Chopra R. C. *"Glossary of Indian Medicinal Plants"* (Including the Supplement) Council of Scientific and Industrial Research , New Delhi, 1986, p.11.
- [11] Bown D. *"Encyclopedia of Herbs and their uses"*. Darling Kinderley, London, ISBN O, 1995, pp.7513-020-31.
- [12] Isanga J., and Zhang G.N. *"Biological active components and nutraceuticals in peanuts and related products"*, Review. Food Rev.Intl. 23, 2007, pp. 123-140.
- [13] Miraliakbari H., Shahidi F. *"Oxidative stability of tree nut oils"*, J.Agric. Food. Chem. 56, 2008, pp. 4751-4759.
- [14] Amaral J.S., Casal S., Pereira J., Seabra R., Oliveira B. *"Determination of sterol and fatty acid compositions, oxidative and stability value of (Eucalyptus globulus) cultivars grown in Portugal"*, J. Agric. Food. Chem. 51, 2003, pp. 7698-7702.
- [15] Takahashi T, Kokubo R, Sakaino M. *"Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata"*, Lett Appl.Microbiol. 39 , 2004,pp. 60-64.
- [16] Al-Bayati F.A. and Sulaiman K. D. *"Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity"*. Turk J. Biol., 2008, pp.57-62.
- [17] Sartorelli, P., Marquioreto, A. D., Amaral-Baroli, A., Lima, M. E., and Moreno, P. R. *"Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus"*. Phytother. Res. V.21,No.3, 2007,pp. 231-233.(IVSL).
- [18] Osawa, K., Yasuda, H., Morita, H., Takeya, K., and Itokawa, H. *"From the Leaves of Eucalyptus globules"*, J Nat Prod .V. 59,No.9, 1996,pp. 823-827.

- [19] Pizsolitto A.C, Mancini and B., Fracalanza L. ***“Determination of antibacterial activity of essential oils officialized by the Brazilian pharmacopeia”***, 2nd edition. Chem. Abstr . 86, 1977, pp.12226s.
- [20] Takarada, K., Kimizuka, R., Takahashi, N., Honma, K., Okuda, K., and Kato, T. ***"A Comparison of the antibacterial efficacies of essential oils against oral pathogens”***, Oral Microbiol Immunol . V.19,No.1, 2004,pp 61-64.
- [21] Ahmad, I. and Beg, A. Z. ***"Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens"***, J. Ethnopharmacol . V.74,No.2, 2001, pp.113-123.
- [22] Shahi, S. K., Shukla, A. C., Bajaj, A. K., Banerjee, U., Rimek, D., Midgely, G., and Dikshit, A. ***"Broad spectrum herbal therapy against superficial fungal infections" .*** V.13,No.1, 2000, pp.60-64.
- [23] Fadi Qa'dan, Abdul-Jalil Thewaini, Dalia A. Ali, Rana Afifi, Abdalla Elkhawad and Khalid Z. Matalka. ***"The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms"***. The American Journal of Chinese Medicine, 33, 2005, pp.197–204.
- [24] Salari,M.H.,Amine G.,Shirazi M.H.,Hafezi R.,Mohammadypour M. ***"Antibacterial effects of Eucalyptus globules leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders"***, Clin.Microbiol.Infect.12, 2006, pp.194-196.
- [25] Brantner, A. H., Asres, K., Chakraborty, A., Tokuda, H., Mou, X. Y., Mukainaka, T., Nishino, H., Stoyanova, S., and Hamburger, M. ***Crown gall " a plant tumour with biological activities"***, Phytother Res. V.17,No.4, 2003, pp.385-390.
- [26] Rakotonirainy MS, Lavédrine, ***"Screening for antifungal activity of essential oils and related compounds to control***

the biocontamination in libraries and archives storage areas". International Biodeterioration and Biodegradation. March. V. 55, No.2, 2005, pp. 141-147.

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

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المستخلص:

تعتبر كل من السبحيات الميوتنسية وخميرة المبيضات البيض من عوامل الاصابة الفموية. وان الادوية الاصطناعية لها اعراض جانبية غير مرغوب فيها بالاضافة الى زيادة الاحياء المجهرية التي تصبح مقاومة لتلك الادوية لذلك تسعى الدول المتقدمة للاهتمام بالاعشاب الطبية اكثر. تعتبر النباتات الطبية ومشتقاتها المصدر الطبيعي للمضادات المايكروبية. و السبحيات الميوتنسية لها الدور الاساسي في حدوث بلاك الاسنان والتسوس. لذا تهتم الدراسة الحالية بتقييم الفعالية المايكروبية لخلاصة النبات الطبي التجاري الكالبتوس على هذه البكتريا وتمت دراسة تلك الفعالية من خلال فحص نماذج لعاب لمرضى يعانون من تسوس الاسنان وباستخدام طريقة تنافذ الحفر. وكانت منطقة التثبيط ضد السبحيات الميوتنسية 11,4 ملم لخلاصة اوراق الكالبتوس مقارنة بالمضاد جنتاميسين 15,2 ملم في حين كان معدل منطقة التثبيط ضد المبيضات البيض 5,1 ملم مقارنة بالمضاد نستاتين 10,9 ملم. واطهرت الدراسة ان الخلاصة الميثونولية للاعشاب المستخدمة لها تاثير مثبت على السبحيات الميوتنسية اكثر منه على المبيضات البيض.

كلمات مفتاحية: تسوس الاسنان، الكالبتوس، طريقة التنافذ بالحفر.