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Evaluation of the anti-mycotic activity of Rosemary Oil Against *Candida albicans*

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ABSTRACT

An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycoses such as athlete's foot, ringworm, candidiasis, serious systemic infections such as Cryptococcal meningitis, and others. In traditional medicine, extracts and essential oil from flowers and leaves are used in the belief they may be useful to treat a variety of fungal disorders. The aim of this study was to analyse the antimycotic properties of rosemary oil and its principal components. The Rosemary oil was screened for antifungal activity by the disc diffusion method. Activated cultures of *Candida albicans* in Sabouraud's broth was adjusted to 0.5 McFarland standards [10^8cfu/ml]. 100 µl of the inoculum was introduced to molten Sabourauds dextrose agar and poured in the sterile Petri plates and allowed to set. Sterile filter paper discs (6.0 mm diameter) impregnated with 25µl, 50µl and 100µl /disc were placed on fungal seeded plates and incubated at 28^{o}C for 48 hrs. Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth inhibition. All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The extract showed good antifungal activity at different concentrations with a maximum zone of inhibition of 38 mm at concentration 100µl. This study provides a sample large enough to determine the antifungal properties of Rosemary oil and suggests further studies for possible therapeutic use.

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INTRODUCTION

Oral health is essential to the general well-being of an individual and relates to the quality of life. Antimicrobial properties of herbs and spices have been recognized and used since ancient times for food preservation and in medicine. Scientific reports on natural antimicrobial agents also date to back more than a century, e.g. Chamberlain reported in 1887 the action of essential oil vapours on anthrax spores. A renewed interest in 'natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance, and a rise in production of minimally processed food joined with 'green' image policies of food industries (Benjilali, 1984).

Numerous studies have documented the anti-fungal and anti-bacterial by the effect of plant essential oils. Examinations of local indigenous herbs and plant material have also been reported from around the world, e.g. India p, Australia, Argentina and Finland. Screening experiments with 13–52 essential oils and major active components against five to 25 micro-organisms have reported that rosemary oil to be some of the best broad spectrum

candidates for inhibition of foodborne pathogens and spoilage organisms (Conner, 1984). However, comparisons of studies that have used different methodologies are difficult, especially regarding minimal inhibitory concentrations (MIC), and the need for uniform and reliable procedures when testing activity has been emphasised (Del Campo, 2000). For thousands of years, medicinal plants have been used as traditional treatments for numerous human diseases in many parts of the world. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, making them an effective source for alternative medicines. Numerous traditional medicinal plants have been evaluated for their potential application in the prevention of mycotic origin. A number of studies have investigated the activity of plant extracts and products against specific oral pathogens (Inouye, 2004).

The other common names for the herb rosemary are Polar Plant, Compass-weed, Compass Plant, and *Rosmarinus coronarium*, Dew of the Sea, Garden Rosemary, Incensier, Mary's Mantle. Rosemary is said to be a native of Mediterranean regions and covers a wide range in Southern Europe, and now it is cultivated throughout the world. The herb of rosemary has several ash coloured branches, and the bark is rather scaly, and it grows up to a height of three feet (Nielsen, 2000). In traditional medicine, extracts and essential oil from flowers and leaves are used in the belief they may be useful to treat a variety of disorders. Rosemary is a powerful anti-oxidant and anti-inflammatory. It contains diterpenes and flavonoids that inhibit free-radical damage and strengthens the capillaries. One of the best-known uses of rosemary oil is that it serves as an extremely effective mouthwash.

The essential oil of the aerial parts of *Rosmarinus officinalis* collected from Konya. Turkey was analyzed by gas chromatography and gas chromatography-mass spectrometry. The oil yield of dried plant (volume/dry weight) obtained by hydrodistillation was 1.9%. Twenty compounds representing 99.93% of the oils were identified. The main constituents of the oils were *p*-cymene (44.02%), Linalool (20.5%), γ-terpinene (16.62%), thymol (1.81%), β-pinene (3.61%), α-pinene (2.83%) and eucalyptol (2.64%). The oil consisted of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons. Also, the inhibition effect of rosemary oil was investigated against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum* (Legan, 1991). The experiment was carried out *in vitro* using disc diffusion to investigate the antifungal action of the oil. The extent of inhibition of fungal growth varied depending on the levels of essential oil used in the experiment.

Candida albicans is found in infections of the mouth, vagina, lungs, and skin lesions (Vallverdú-Queralt, 2014). Initial bacterial colonization, plaque evolves to contain a variety of microorganisms, including Candida species, many of which are potential periodontal pathogens. Candida albicans is the most common causative agent of oralcandidias is in human immunodeficiency virus-infected patients (Botelho, 2007). Also, several conditions, such as hyposalivation, diabetes mellitus and prolonged antibiotic and corticoid therapy can predispose to oral Candidiasis (Alviano, 2005). The aim of this study is to evaluate the antimycotic activity of Rosemary oil on Candida albicans.

As there are studies on this aspect, the present study was designed to find scientific evidence on the anti-mycotic activity of rosemary oil.

MATERIALS AND METHODS

Candidaalbicans was isolated from clinical samples and cultured and maintained on subouraud's dextrose agar medium at 30°C. Rosemary oil was obtained commercially and used for the study

Methodology

Different concentrations [25µl, 50µl and 100µl] of the oil were loaded on sterile filter paper discs measuring 6mm in diameter. The discs were dried and kept aseptically.

Screening of antifungal activity [disc diffusion technique]

The Rosemary oil was screened for antifungal activity by the disc diffusion method. Activated cultures of *Candida albicans* in Sabouraud's broth was adjusted to 0.5 McFarland standards [10^8cfu/ml]. 100 µl of the inoculum was introduced to molten Sabourauds dextrose agar and poured in the sterile Petri plates and allowed to set. Sterile filter paper discs (6.0 mm diameter) impregnated with 25µl, 50µl and 100µl /disc were placed on fungal seeded plates and incubated at 28°C for 48 hrs. As a positive control, Fluconazole (10 mcg /disc) and Amphotericin B (100 units /disc) were used. Following an incubation period of 48 hr, plates were removed from the incubator, and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth inhibition. The whole experiment was performed three times to minimize error.

RESULTS

Effect of three different concentrations 25µl, 50µl and 100µl (/disc) of the Rosemary oil was tested against *Candida albicans* using disc diffusion tech-

nique. All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The antifungal activity of the extract against the fungal strains is shown in Table 1. The extract showed good antifungal activity at different concentrations with a maximum zone of inhibition of 38 mm at concentration 100µl.

Table 1: Antimycotic activity of rosemary against C*andida albicans*

Concentration of the extracts	Zone of inhibition [in mm diameter]
25µl	16
50µl	29
100µl	38
Fluconazole (10 mcg /disc)	24
Amphotericin B (100 units /disc)	27

DISCUSSION

The traditional use of plants as medicines provide the basis for indicating which essential oils and plant oils may be useful for specific medical conditions (Korting, 1998). Historically, rosemary oil has been used as topical antiseptics, or have been reported to have anti-mycotic properties (Chami, 2004). Over the past two decades' fungal infections have evolved into the important cause of morbidity and mortality in modern medicine. The prevalence of resistance to antifungal agents has significantly increased. So, it makes it necessary to discover new classes of antifungal compounds to treat fungal infections. The research on natural products derived compounds has accelerated in recent years due to their importance in drug discovery (Jayashree, 2012). Plants are a rich source of bioactive secondary metabolites of a wide variety such as tannins, terpenoids, Camphene, pinene, and flavonoids, which are reported to have in vitro antifungal properties. The rosemary oil is rich in cineole isomers are widely reported to possess high levels of anticandidal activity. A series of molecules with antifungal activity against different strains of fungus have been found plants, which are of great importance (Tharkar, 2010).

Nascimento et al. (2012), reported that assays testing the antifungal activity of essential oils could be inconsistent due to factors such as volatility, water solubility, and viscosity.

In the present study, these factors were minimized by including Tween 80 as a surfactant, improving the homogeneity of the emulsion, and by keeping the plates in the dark to minimize the degradation of the volatile essential oil (Hood, 2003). We demonstrated that the R. officinalis oil tested in the present study contains high concentrations of active compounds such as α-Pinene and 1, 8-Cineole. Further studies are underway to determine which fractions of the R. officinalis were responsible for the observed activity and whether there is any synergy among active compounds. These results highlight the potential of R. officinalis oil has an antifungal drug candidate. Studies to characterize the composition of the oil, with the aim of determining the concentrations of the active components, are being conducted to further reported the antifungal activity of the essential oil of R. officinalis used (Gomes Neto, 2012).

According to Chandra et al. cells of C. albicans can perform co-aggregation and excrete an extracellular matrix that contributes to cellular adherence to acrylic and polymeric surfaces. Chandra et al. demonstrated that, while in the planktonic state, cells of C. albicans are more susceptible to antifungal action. The authors considered that the secretion of the extracellular matrix and the aggregation state contributed to higher antifungal resistance. It was reported by Chandra et al. that the expression of the fluconazole-resistant gene in C. albicans might be responsible for the increased MIC of the tested chemical compounds against the fluconazole-resistant strains. This finding might be accounted for the increased MIC, and MFC of Rosemary Oil observed among the tested C. albicans isolates (Chandra, 2001). According to Fontenellee (2007), R. officinalis essential oil presented MICs between 0.62 mg/mL and 2.50 mg/mL and MFCs from 1.25 mg/mL to 2.50 mg/mL when was used on C.albicans as determined using the microdilution technique. Likewise, the antifungal activity of R. officinalis essential oil was demonstrated by (Lima *et al.,* 2007) and Packer and Luz who observed fungistatic activity at concentrations above 8% using the agar diffusion technique. According to Scorzoni (2007), the microdilution technique is more sensitive for the determination of MIC and MFC in the assessment the anti-fungal activity of R.officinalis essential oil. According to Yuri Wanderley Cavalcanti (2007), the essential oil of R. officinalis showed anti-adherent activity against C.albicans, as observed by cellular denaturing, fragmentation of structures and a small quantity of adhered components. The interaction of C. albicans with other microorganisms in the constitution of oral biofilms may contribute to the increased susceptibility of this strain to the action of antifungal agents.

In the present study, Rosmarinus officinalis was found to show good antifungal activity at different concentrations with a maximum zone of inhibition of 38 mm at a concentration of 100µg/ml. Despite all the significant advancements in modern

medicine, traditional herbs have given rise to many important drugs. Medicinal plants are being studied for their potential mechanism of action and therapeutic properties. Recent studies suggest a good correlation between medicinal use and the in-vitro anti-mycotic activity of medicinal herbs (NithyaKarpagam, 2017). The results of the present study indicate the antifungal activity of Rosmarinus officinalis essential oil and may be used as a source for the isolation of active compounds that may serve as lead compounds in antifungal drug development.

Further studies on their cytotoxicity or toxicity will be advantageous to know the possible harmful effects of this extract for commonly used by the local communities.

CONCLUSION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Hence the present study has shown the anti-mycotic activity of Rosemary oil on Candida albicans. Anti-mycotic activities could be enhanced if essential oils are purified and used as an additional ingredient in mouthwashes and toothpaste. Thus, the study ascertains the value of plants used in Ayurveda, which could be of considerable interest to the development of new drugs. We identified concentrations of R. officinalis oil that can inhibit the growth of C. albicans. These results suggest a need to further evaluate the antifungal performance of R. officinalis oil in clinical fungal samples.

REFERENCE

Alviano, W. S., Mendonça-Filho, R. R., Alviano, D. S., Bizzo, H. R., Souto-Padrón, T., Rodrigues, M. L., ... & Souza, M. M. G. (2005). Antimicrobial activity of Croton cajucaraBenthlinalool-rich essential oil on artificial biofilms and planktonic microorganisms. Oral microbiology and immunology, 20 (2), 101-105.

Benjilali, B., Tantaoui, E.A., Ayadi, A. and Ihlal, M. (1984) Method to study antimicrobial effects of essential oils: application to the antifungal activity of six Moroccan essences. Journal of Food Protection 47, 748–752.

Botelho, M. A., Nogueira, N. A. P., Bastos, G. M., Fonseca, S. G. C., Lemos, T. L. G., Matos, F. J. A., ... & Brito, G. A. C. (2007). Antimicrobial activity of the essential oil from Lippiasidoides, carvacrol and thymol against oral pathogens. Brazilia

Chami, N., Chami, F., Bennis, S., Trouillas, J., &Remmal, A. (2004). Antifungal treatment with carvacrol and eugenol of oral candidiasis in immunosuppressed rats. Brazilian Journal of Infectious Diseases, 8 (3), 217-226.

Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer JJ, Douglas LJ, Ghannoum MA. Antifungal Resistance of Candidal Biofilms Formed on Denture Acrylic in vitro. J Dent Res 2001;80:903-8.

Conner, D.E. and Beuchat, L.R. (1984) Effects of essential oils from plants on growth of food spoilage yeasts. Journal of Food Science 49, 429–434.

Del Campo, J., Amiot, M.J. and Nguyen, T.C. (2000) Antimicrobial effect of rosemary extracts. Journal of Food Protection 63, 1359–1368.

Fontenelle RO, Morais SM, Brito EH, Kerntopf MR, Brilhante RS, Cordeiro RA, Tomé AR, Queiroz MG, Nascimento NR, Sidrim JJ, Rocha MF. Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippiasidoides Cham. Antimicrob Chemotherher 2007; 59:934-40.

Gomes Neto NJ, Luz IS, Tavares AG, Honório VG, Magnani M, Souza EL. Rosmarinus officinalis L. essential oil and its majority compound 1, 8-cineole at sublethal amounts induce no direct cross-protectiontion in Staphylococcus aureus ATCC 6538. Foodborne Pathog Dis. 2012 Dec;9 (12):1071-6.

Hood JR, Wilkinson JM, Cavanagh HMA. Evaluation of common antibacterial screening methods utilized in essential oil research. J Essent Oil Res. 2003 Nov- Dec;15 (6):428-33.

Inouye, S., Tsuruoka, T., Watanabe, M., Takeo, K., Akao, M., Nishiyama, Y. and Yamaguchi, H. (2002) Inhibitory effect of essential oils on apical growth of Aspergillus fumigatus by vapour contact. Mycoses 43, 17–23.

KortiHans Christiantian, et al. "In vitro susceptibilities and biotypes of Candida albicans isolates from the oral cavities of patients infected with human immunodeficiency virus." Journal of clinical microbiology 26.12 (1988): 2626-2631.

Legan, J.D. and Voysey, P.A. (1991) Yeast spoilage of bakery products and ingredients. Journal of Applied Bacteriology 70, 361–371.

Lima IO, Oliveira RAG, Lima EO, Farias NMP, Souza EL. Atividade anti úngica de óleos essenciaissobree spécies de Cândida. Rev Bras Farmacogn 2006;16:197-201.

N.Jayashree, N.Narayanan, Latasriram., Antibacterial antifungal and anti-mycobacterial studies on some synthetic Dimethoxy flavones, Asain J of pharmaceutical and cresearch, search , 2012; 5 [suppl 1]:101 -103

Nielsen, P.V. and Rios, R. (2000) Inhibition of fungal growth on bread by volatile components

from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. International Journal of Food Microbiology 60, 219–229.

NithyaKarpagam. G, Anitha Roy, Geetha R.V. In vitro Evaluation of Anti-mycotic activity of Carica papaya Extract on Candida albicans. International Journal of Current Research Vol. 9. Issue 06. 53069-53071. June 2017.

Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Mendes-Giannini MJS. Comparative study of disk diffusmicrodilutiondilution methods for evaluation of antifungal activity of natural compounds against medical yeasts Candida spp. and Cryptococcus sp. Rev Cienc Farm BásicaApl 2007;28:25-34.

Thar kar P.R., Tatiya A.U., Shinde P.R ., Surana S.J., Pat il U.K ., Antifungal Activity of Glycyrrhizaglabra Linn. And Emblica Officinalis Gaertn. by Direct Bioautography Method International Journal of PharmTech Research ., 2010; 2 [2] :1547-1549,

Vallverdú-Queralt, A; Regueiro, J; Martínez-Huélamo, M; Rinaldi Alvarenga, J. F.; Leal, L. N.; Lamuela-Raventos, R. M. (2014). "A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay". Food Chemistry. 154: 299–307.