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Effect of Pogostemon Parviflavus Leaves Extracts against Candida Albicans

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Abstract

Introduction: Oral Carcinoma (SCC) is the result of a multistage process from normal to dysplastic lesions. A premalignant or precancerous lesion is defined by the World Health Organization as a morphologically altered tissue in which cancer is more likely to occur. In oral cavity, the oral micro flora may be subsequently replaced by potentially pathogenic microorganisms, such as *Candida* sp., (from 72% to 92%).

Material and Method: Fresh and mature leaves of plants *Pogostemon parviflorus* were collected. Exactly 0.2g of crude DMSO (Dimethyl sulfoxide) extract of *Pogostemon parviflorus leaves* was dissolved in 2ml DMSO (Dimethyl sulfoxide) to get 100mg/ml concentration.

Results: *Pogostemon parviflorus* leaves extracts in DMSO solution gives inhibitory effect against clinically isolated *Candida albicans* spp.

Conclusion: To fight against fungal infections antifungal drugs are used as an important weapon which greatly benefited the health related quality of human life since their introduction but over the past few decades these health benefits are under threats as many commonly used antibiotics have now become less effective due to emergence of drug resistivity.

Introduction

Presently, oral cancer is one of the most prevalent types of disease is a growing health problem around the world and is the second leading cause of the death. Worldwide, there are now more than 10 million cases of cancer per year[1,2]. Oral Carcinoma (SCC) is the result of a multistage process from normal to dysplastic lesions. A premalignant or precancerous lesion is defined by the World Health Organization as a morphologically altered tissue in which cancer is more likely to occur and includes oral leukoplakia, oral erythroplakia, Lichen Planus and possibly oral Sub mucous fibrosis. In oral cavity, the oral micro flora may be subsequently replaced by potentially pathogenic microorganisms, such as *Candida* sp., (from 72% to 92%). *Candida* carriage was reported to be common in oral cancer patients, with *C. albicans* being the predominant species[3,4]. Candidal Oral colonization (up to 93%) and infection (up to 30%) are frequently noted in oral cancer patients[5]. The main reason may be the irradiation-induced histological changes which leads to oral mucositis, together with salivary quantitative and qualitative changes, which facilitate candidal growth. *Candida,* an ubiquitous fungi, are thin-walled, small (4 to 6 microns) reproduce by budding and are one of the most common causes of opportunistic mycoses worldwide. They grow rapidly and mature in 3 days. The colonies of

Candida are cream yellowish in color. The texture of the colony may be pasty, smooth, dry, wrinkled and dull, depending on the species. *Candida* species belongs to the phylum Ascomycota. The genus *Candida* a eukaryotic commensal organism includes about 350 heterogeneous species out of which six are most frequently isolated in human infections. The *Candida* genus includes in a taxonomic group that was originally used to define yeast-like organisms that were not considered to have a sexual reproductive life cycle. Although this fungus reproduces both sexually and asexually by formation of spores.

The prevalence of diseases caused by *Candida* species have been found to increase in recent years, mainly in pregnant, diabetic, elderly or immune-compromised individuals, or those who are receiving antibiotic or corticosteroid treatment as appear to be predisposing factors for *Candida* infection[6].

Clinically important Candida species in humans includes *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsil*osis and *Candida dubliniensis* among which *C. albicans* is the most prevalent pathogenic species which is responsible for the majority of oral and systemic infections[7]. Overgrowth of *C. albicans* (the most common strain of *Candida*) is the culprit behind the majority of infections. These microorganisms have the potential to infect virtually any tissue within the body, however, these are most predominantly found on oral mucosa[8]. In-vitro investigations indicate that *C. albicans* also expresses higher levels of certain putative virulence factors compared with other *Candida* species. Several potential virulence factors have been proposed in the pathogenicity of *Candida albicans* species, with adhesion to host surfaces, secretion of proteinases enzyme and hyphal formation apparently being the most significant. The use of herbs and medicinal plants as the first medicines is a universal phenomenon. During the 1960s, the National Cancer Institute (United States) began to screen plant extracts with antitumor activity[9]. Natural compounds isolated from medicinal plants, as rich sources of novel anticancer drugs, have been of increasing interest since then.

Method and materials

The prospective study was undertaken to probe the frequency and degree of *Candida albicans* in various oral mucosal lesions and its treatment from natural herbs. The main lesions included in this study were Oral submucus fibrosis, Oral leukoplakia, Oral Erythroplakia, and Oral squamous cell carcinoma.

30 patients for each of the above mentioned four lesions were randomly selected from the Out Patient Department of Oral pathology and Microbiology, KGMU, Lucknow.

The patients were clinically examined by the senior faculty members from the Department of Oral pathology and Microbiology, King George's Medical University, Lucknow and clinical data pertaining to each case was collected.

Study population

The study included a total of 220 samples erythroplakia (30), leukoplakia (30), OSMF (30), OSCC (30) and Control (100) of patients presenting to the Department of Oral Pathology and Microbiology, K.G.M.U, Lucknow. A detailed history of all participants was taken. Individuals were excluded if they had received antibiotic, steroid, or antifungal therapy during the previous three months, if they had a history of underlying systemic disease, or if they were HIV-seropositive or had any other condition that could potentially decrease their immunity. We also excluded oral cancer patients who were undergoing or had undergone radiation therapy or surgical treatment for an oral lesion.

Collection of Sample

Isolates were obtained by using oral swabs and saliva samples. unstimulated saliva, Oropharyngeal secretions (swab from posterior pharyngeal wall) swabs were wiped across mucosal sites were collected with sterile swab in sterilized wide-mouthed universal containers, or sterile cotton to isolate *Candida* species .This specimen was sent to the laboratory for assessment of *Candida* colony-forming units. This was followed by an incision or excision biopsy of the lesion and the biopsy sample was sent for histopathologic examination for the presence or absence of hyphae and to assess the presence or absence of Candidal hyphae in tissue sample.

The patients were informed about the study and the biopsy procedure was explained. After obtaining written consent from the patients, routine blood test comprising of haemoglobin estimation, bleeding time, clotting time, total leukocyte count, differential leukocyte count and blood sugar estimation were carried out. After blood report was received and was found to be satisfactory, the most representative site was selected for the smear to check for the presence or absence of Candidal hyphae. The smears were obtained by thorough scraping of debris with a blunt metal carver and fixed with the spray fixative (95% Ethanol + 3% Glacial acetic acid). Also, incisional biopsy of the lesion was performed under aseptic conditions and local anesthesia. Biopsy specimens were preserved in 10% neutral buffered formalin solution..

The smears taken were stained with Periodic acid Schiff stain according to method described by and the biopsied tissues were processed and embedded in paraffin wax[10]. The paraffin blocks were sectioned with a rotary semi automatic soft tissue microtome into two sections of 5 µm thickness. One section was stained with Hematoxlin and Eosin stain and the other with Periodic acid Schiff stain, according to the procedures described by[11].

Isolation of *Candida* sp

The cotton end of each swab was inserted into 0.5 ml of sterile water in a microcentrifuge tube, the tube was rigorously mixed for 30s with a laboratory tabletop vortex mixer and 0.15 ml of the samples were inoculated on Sabouraud's Dextrose Agar media with antibiotics and incubated at 35^0 C in BOD incubator for one week. SDA plates were observed daily after 24 hr for growth and plates which did not yield any colonial growth after one week were considered as negative. SDA plates with growth were processed. The growth that showed Gram positive budding yeast cells on Gram's staining was further processed by germ tube test, inoculation in SDA broth, for speciation of *Candida*.

The prospective study was done to investigate the herbal plants with antifungal activity towards *Candida albicans* which can further be used in oral candidal infection treatment and in oral mucosal lesions. The pathogen samples were collected from the Department of Oral pathology and Microbiology, K.G.M.U, Lucknow. From patients showing lesions of Oral submucus fibrosis, Oral The patients were clinically examined by the senior faculty members from the Department of Oral pathology and Microbiology, King George' s Medical University, Lucknow and clinical data pertaining to each case was collected.leukoplakia, Oral Erythroplakia, and Oral squamous cell carcinoma.

Pogostemon parviflorus plant was randomly selected to study the antifungal activity against *Candida albicans* isolated from infected oral cavity.

Plant material collection:

Fresh and mature *leaves* of plants *Pogostemon parviflorus* was collected from Department of Botany, University of Lucknow old campus, Lucknow and National Botanical Research Institute, Lucknow during March- May 2015. Samples were collected in sterile plastic bag and brought

to Mycology Laboratory of Botany Department, University of Lucknow, Lucknow, India.

Chemicals

Sabarouds Dextrose Agar (SDA), antifungal discs like Erythromycin (10µg/disc), Minocycline (5 µg/disc), Cotrimoazol (25µg/disc), Fuconazole (10µg/disc) were purchased from Himedia Pvt. Ltd., Mumbai, India. Other chemicals and reagents used for the study were of analytical grade.

Microorganisms

Micro-organism namely *Candida albicans* was collected from the Department of Oral Pathology and Microbiology Laboratory, K.G.M.U, Lucknow isolated from the oral cavity of oral cancer patients by sterile cotton swabs.

Plants Leaves Extract preparation

The leaves of plants were washed thoroughly with distilled water and dried at room temperature. Dried leaves were uniformly grounded using a mechanical grinder to yield fine powder. Ten grams of the powder was mixed with 100 ml of each DMSO, methanol and Ethanol in conical flasks and these flasks were kept in shaking incubator at 100 rpm for 24 hours. The mixtures of these plants extracts in different solutions were filtered using Whatman filter paper no 1. Extracts were dried and stored in an airtight container at 4^0C until further use.

Drug / Control	Concentration Of drug(µg/disc) and Negative Controls	Zone of inhibition (mm)	Resistance/ Sensitive
Fluconazole	10µg/disc	09	R
Cotrimoazol	25 µg/disc	18	S
Erythromycin	10 µg/disc	10	R
Minocycline	5 µg/disc	13	S
Negative Control- DMSO	0.2ml	_	R
Negative Control- methanol	0.2ml	_	R

Preparation of extracts in DMSO Solution and isolated fractions for bioactivity test

Exactly 0.2g of crude DMSO (Dimethyl sulfoxide) extract was dissolved in 2ml DMSO (Dimethyl sulfoxide) to get 100mg/ml concentration. This was then serially diluted to obtain 50mg/ml, 25mg/ml, 10 mg/ml and 5mg/ml concentrations. This procedure was repeated for DMSO (Dimethyl sulfoxide) extracts using DMSO (Dimethyl sulfoxide) as a solvent for dilution. Similar dilution procedure was applied for the fractions corresponding to their yield. All of the fractions were prepared in two to three fold dilutions. It should be clear that for those samples in which organic solvents were used for dilution, when antimicrobial test was done filter paper disks after impregnated in the stock solution was left to dry in flat glass (to let the solvent evaporated) and then it was sprayed with sterile distilled water.

Results

Table: 1 Antifungal activity of *Pogostemon parviflorus* leaves extracts against *Candida albicans*

Solution	Volume(ml)	100mg/ml	50mg/ml	25mg/ml	10mg/ml	5mg/ml
DMSO	0.2	19±0.25	15±0.34	12±0.01	11±0.28	09±0.22

Pogostemon parviflorus leaves extracts in DMSO solution gives inhibitory effect against clinically isolated *Candida albicans* spp. The zone of inhibition (mm) at 100mg/ml, 50mg/ml, 25mg/ml, 10mg/ml and 5mg/ml in DMSO was observed to be 19±0.25, 15±0.34, 12±0.01, 11±0.28 and 09±0.22. In DMSO solution the plant leaves extracts shows inhibitory effect at minimum concentration of 50mg/ml.

Table: 2 Antimicrobial inhibition zone (mm) by positive (drugs) and negative control s against *Candida albicans.*

Conclusion

To fight against fungal infections antifungal drugs are used as an important weapon which greatly benefited the health related quality of human life since their introduction but over the past few decades these health benefits are under threats as many commonly used antibiotics have now become less effective due to emergence of drug resistivity. *Candida* sp., also shows to exhibits resistance to antifungals which was firstly reported in 1995 and this has been reported by numerous other researchers also[12,13,14]**.** Presently rise in candidal infections could also be due to a reflection of inherently higher level of antifungal drug resistance in some *Candida* species. The exact mechanism of biofilm resistance to antifungals remains unclear, but it is probably multifactorial. There are no drugs which can effect extremely to treat most oral cancers. Novel natural products offer opportunities for innovation in drug discovery. A considerable number of antitumor agents currently used in the clinic are of natural origin. In fact, natural products play a major role in cancer prevention and treatment by inhibiting the growth of responsible pathogen. For instance, over half of all anticancer prescription drugs approved internationally between the 1940s and 2006 were natural products or their derivatives[15]. Among them, plants have been the chief source of natural compounds used for medicine. The use of herbs and medicinal plant compounds to treat infections is an old age practice through out the world, especially in developing countries, where is there is dependence on traditional medicine for a variety of diseases. All the drugs from the plants are substances with the particular therapeutic actions extracted from the plants.

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