



## ANTIFUNGAL ACTIVITY ANALYSIS OF 3 ESSENTIAL OILS (THYME, AJWAIN AND ORANGE) ON CANDIDA ALBICANS

Vaishnavi Sivakali Subramanian<sup>1</sup>, R.V Geetha<sup>2</sup> and \*Anitha Roy<sup>3</sup>

<sup>1</sup>Dental Surgery, Saveetha Dental College and Hospital, Chennai, India

<sup>2</sup>Department of Microbiology, Saveetha Dental College and Hospital, Chennai, India

<sup>3</sup>Pharmacology, Saveetha Dental College and Hospitals, Chennai, India

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### ABSTRACT

In this paper, existing available literature on numerical solution of singularly perturbed and singular perturbed delay differential equations is reviewed. These equations arise in mathematical modeling of variational problems in control theory, physical and biological phenomena like optically bistable devices, description of human pupil reflex and a variety of models for physiological processes or diseases. The purpose of this work is to investigate the class of singular type problems that are solved till now using different numerical methods. The objective is to motivate the researchers to develop new methods for calculating efficient solution of such problems. There is a lot of work reported in literature to solve these types of equations both numerically and analytically. This paper limits its coverage to the work done by numerous researchers between 2002 and 2017.

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### INTRODUCTION

Candidiasis is the most common yeast infection that range from superficial mucosal infections, such as oral thrush, systemic and potentially life-threatening diseases, such as disseminated candidiasis. In the last two decades, it has been observed a considerable increase in the incidence of deep fungal infections, in both immunocompromised and in healthy subjects (1). The most commonly used classes of antifungal agents to treat *Candida* infections are the azoles, polyenes, and echinocandins; however, the management of *Candida* infections faces many problems, such as toxicity, resistance of *Candida* to commonly used antifungal drugs, relapse of *Candida* infections, and the high cost of antifungal drugs [2, 3]. To elude these problems, investigators are exploiting alternative therapeutic strategies, such as the use of natural products, especially essential oils (EOs) [4–7]. The essential oil from locally grown *T. vulgaris* also contains high concentration of thymol about 20%–54%. Except thymol, the essential oil from this plant also contains some other additional active chemical constituents such as p-cymene, myrcene, borneol and linalool. Therapeutically, thymol is widely used as an antiseptic. The other active chemical ingredient is listerine, commercially used to produce mouthwashes [8]. Thyme oil was also used to medicate

bandages before the discovery of modern pharmaceutical medicine antibiotics [9]. The result for phytochemical screening of hexane, ethyl acetate, chloroform, butanol and methanol extracts were from the leaves of *T. vulgaris* showed the presence of flavonoids, saponins and steroids, but alkaloids, tannins, and triterpenoids not present in the crude extract [10]. *Trachyspermum ammi* yield 2% to 4% brownish essential oil, with thymol as the major constituent (35%–60%) [11]. The nonthymol fraction (thymene) contains p-cymene, terpinene, and  $\beta$  pinenes, dipentene, terpinene and cravacrol [12]. Minute amounts of camphene, myrcene, and -3-carene also have been found in the plant. Alcoholic extracts contain a highly hygroscopic saponin. From the fruits, a yellow, crystalline flavone and a steroid-like substance has been isolated and it also contains 6-O- $\beta$ -glucopyranosyloxythymol, [13] glucoside and yields 25% oleoresin containing 12% volatile oil (thymol, -terpinene, para-cymene, and - and -pinene). [14] The principal oil constituents of *T. ammi* are carvone (46%), limonene (38%), and dillapiole (9%). [15]

The essential oil of orange fruit extracted has mainly dominated by limonene which presented a 85.35%. The rest of compounds were weakly represented with low levels of monoterpenes -pinene, camphor and bornyl acetate of 1.80, 4.81, and 4.21%, respectively. Concerning sesquiterpenes, -humulene (0.16–0.34%) followed by germacrene-D (tr-0.12%) were found to be the most represented compounds [16].

\*Corresponding author: Anitha Roy

Pharmacology, Saveetha Dental College and Hospitals,  
Chennai, India

## MATERIALS AND METHOD

### MATERIALS

Fungal strains such as *Candida albicans* were obtained from microbiological department laboratory, Saveetha Dental College and Hospitals. Samples such as thyme, ajwain and orange essential oil were provided by Pharmacological department, Saveetha dental college and hospitals.

### METHOD

#### Preparation of Fungal Spore

Fungi are removed from the substrate surface using fine forceps and were broken and opened in sterilized water in order to provide a spore suspension. The filamentous fungi were grown on Mueller Hinton Agar (MHA) slants at 37°C for 48 hours.

The spores were collected using sterile double distilled water and stored in refrigerator. A glass container is sterilized with ethanol and was sprayed on its surface. A sterilized pipette is used to transfer few drops of sterilized water into the glass slide. Alternatively it is pipetted on to the centre of the agar plate and is carefully shaken to spread the suspension. The prepared spores are checked every 24 hours to establish its germination. Once the spores have germinated, a small piece of spore containing that agar is isolated and examined via compound microscope for its quality.

#### Agar Well Diffusion Method

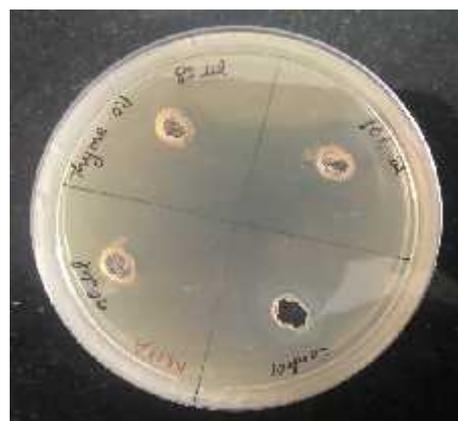
It is a method which shows the movement of the molecules through the matrix that is formed by the gelling of agar. When this method is performed under controlled conditions, the degree of movement of the molecules can be related to the concentration of the molecule. This phenomenon forms the basis of agar diffusion assay that is used to determine the susceptibility of the resistance of the fungal strain to an antifungal agent.

Antifungal activity was carried out using disc diffusion method. Petri plates were then prepared with 20 ml of sterile MHA. The test culture was swabbed on the top of the solidified media and allowed to dry for 10 min. Wells were made on the media using a well borer. Different concentrations of the sample (40, 80 and 100 µL per well) of essential oils diluted in dimethyl sulfoxide (DMSO). Chlorhexidine (0.2% µg/ml) was used as a positive control. These plates were incubated for 48 hrs at 37 °C. Zone of inhibition was recorded in millimeters.

### RESULTS

Thyme and Ajwain oil shows significant antifungal activity against *Candida albicans* at different concentration such as 40,80,100µl/mg. While orange oil did show antifungal activity but comparatively very less. Maximum Zone of inhibition was observed in highest concentration in 3 oils (100µl/mg).

Essential Oil	Concentration µG/ML	Zone Of Inhibition Mm
THYME	40	55
	80	60
	100	65
Chlorhexidine (Control)	0.20%	30



Essential Oil	Concentration µg/ml	Zone Of Inhibition mm
ORANGE	40	22
	80	26
	100	32
Chlorhexidine (Control)	0.20%	23



Essential Oil	Concentration µg/ml	Zone Of Inhibition mm
AJWAIN	40	51
	80	55
	100	60
Chlorhexidine (Control)	0.20%	25



### DISCUSSION

The traditional use of plants as medicine provides the basis for indicating specific medical conditions. It is important to scientifically analyse these plants which have been used in traditional medicines.

The method that is used in extracting oil are Agar well Diffusion method. This study determines the antifungal activity of 3 essential oil against *Candida albicans*, as it is the most common species that affects Candidiasis. The essential oil caused a significant decrease in the activity of the above mentioned fungi and it causes suppression in their growth at concentrations 40µL, 80µL, and 100µL. Chlorhexidine 0.2% was taken as a positive control in well diffusion method.

#### **Petri plates were prepared with 20ml of sterile MHA**

Then the culture was layered down on the top of the solidified media and allowed to dry for 10min. These plates were then incubated for 48hrs at 37 degree Celsius and the zone of inhibition was identified and recorded. The Thyme and ajwain oil shows effective reaction against the fungi at 100µL concentration.

#### **CONCLUSION**

This study was conducted to evaluate the anti fungal activity of 3 essential oils namely thyme, ajwain and orange. Natural products are important source of new drugs which are having importance in modern medicine. Thus Oral candidiasis can be also treated with thyme and ajwain oil giving its maximum effect.

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