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Study of Antifungal Activity of Essential Oil of *Thymus serpyllum*

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This work is dedicated in memory of Professor Dr. Mushtaq Ahmad (Late).

ABSTRACT

The determination of antifungal activity of *Thymus serpyllum* L. was the major focus of this research work. The oil of *Thymus serpyllum* was checked for antifungal activity against three test microorganisms i.e., *Aspergillus niger, Rhizopus oligosporous, Trichoderma viride* and it was carried out by taking 50, 100, 150 and 200 mcg of essential oil. In order to compare the results with standard, three antifungal drugs i.e., Natamycin, Nystatin and Miconazole were employed. The significant antifungal activity of *Thymus serpyllum* oil was observed for *Aspergillus niger*.

Keywords: *Thymus serpyllum,* Essential oil, Antifungal activity, Minimum inhibitory concentration, *Aspergillus niger, Rhizopus oligosporous, Trichoderma viride.*

1. INTRODUCTION

Several researchers reported that a large number of medicinal herbs, their extracts and essential oils have attributed potential applications towards cosmetics, phyto-preparations, food technology, fragrance and folk medicines[1-3]. Moreover, an endless source of medicines was achieved from natural resources in the past. Since new chemical entities (NCEs) always remained an essential need of our society due to increased resistance against the older compounds in microorganisms and plants provided a basis for new chemical entities regarding health care purposes. The relatively higher therapeutic window and less serious side effects of herbal drugs have increased the use of such drugs along with the economical factor. The scientific basis of their therapeutic actions is an area of extensive studies for various researchers[4]. The drug resistant strains of microorganisms presented a new challenge to the scientific community and infection issues are still prevailing even after the development of antibiotics. The extensive uses of herbal drugs have been reported for centuries in literature[5]. The great potential and hope is associated with essential oils. The biocidal effects of various essential oils have been found in literature against different types of bacteria, fungi, viruses, protozoa, insects and plants[6-7]. *Thymus serpyllum* is used as a gastronomic herb, as well as for perfume and for routine medicinal purposes well known to Kashmiri as Jawand. The varieties, hybrids and ecotypes of *Thymus* (almost three hundred in number) are collectively described by the word "*Thyme*" and most of them are native perennial herbs distributed in the regions of Asia and Europe. Certain remarkable characteristics are attributed to this plant. Many traditions related to the use of these plants for their tonic character are known. The strong antiseptic, antispasmodic, carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative and tonic properties have been reported for this plant[8-12]. The seeds of *Thymus serpyllum* are used as vermifuge[13]. The treatments of bronchitis, catarrh, laryngitis, flatulent indigestion, painful menstruation, colic and hangoversare carried out even on international scale by such plants[14]. In case of minor injuries, mastitis, mouth, throat and gum infections etc, it is applied externally.

The plant was used for embalming purposes by Egyptians. It was also used by ancient Greeks for taking bath to get more vigour. The sprigs of *Thymus* were burnt to cleanse the indoor air and considered good for protection against plague. The oil of thyme was used during the First World War as an antiseptic. The use of thyme for preserving liquids, protecting paper from mould and conserving anatomy and botany specimens is still in practice[15].

The antifungal activity of essential oils of *Thymus serpyllum* was investigated for the microorganisms such as *Aspergillus niger, Rhizopus oligosporous, Trichoderma viride* and the data was compared with the standard drugs like Natamycin, Nystatin and Miconazole.

2. EXPERIMENTAL

2.1 *Materials and methods*

i. The investigations were carried out on the seeds of *Thymus serpyllum*
ii. Test fungi (*Aspergillus niger, Rhizopus oligosporous, Trichoderma viride*)
iii. Natamycin, Nystatin and Miconazole were used as standard antifungal drugs.

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2.2 *Extraction of essential oil of Thymus serpyllum*[16]

The seeds of *Thymus serpyllum* were weighed and taken into the flask, connected with Dean stark apparatus. It was heated on a heating mental after the addition of water. The steam, thus produced, was fed on the material from top. In this way the oil containing mixture of steam was obtained from the area where matter was controlled by means of a grill. The condensate was composed of two layers, the above layer contained oil and the lower layer was comprised of water. The water layer was removed from lower opening. In this case, steam also diffuses along with the oil and for this reason the process is known as hydro-diffusion distillation. In order to remove moisture completely, the layer containing oil was further treated with anhydrous sodium sulphate. The sample bottle was filled with the oil for further investigations.

2.3 *Antifungal assays*

2.3.1 *Agar tube dilution assay*[17-21]

The most significant requirement for curing various plant and human diseases due to fungi, is to discover new compounds with toxic effects for such fungi. Potato late blight, hop downy mildew, tobacco blue mould, ergot of rye, grape down mildew corn blight cereal rusts, and Dutch elm disease are among the common fungal diseases associated with plants. However, athlete foot, *aspergillosis*, *actinomycosis*, *histoplasmosis* and *coccidioidomycosis* are among the human diseases due to fungi. Anyhow, there are certain beneficial fungi which save human being from harmful insects.

2.3.1.1 *General procedure*

The agar tube dilution assay was performed by following steps:

i. The stock solution was prepared by dissolving sample in sterile DMSO.
ii. Sabouraud 4 % glucose agar was mixed with agar in distilled water to prepare saboured dextrose agar.
iii. It was then dissolved with the help of a magnetic stirrer and a known amount was introduced to the screw capped test tubes.
iv. The media containing test tubes were autoclaved for 15 min at 121 ^{o}C.
v. The samples of appropriate concentration were pipetted out from the stock solution after cooling the tubes to 50 ^{o}C and added to the non solidified sabouraud agar media.
vi. Then tubes were kept in slaunting position at room temperature to solidify them.
vii. A piece of inoculum i.e., 4 mm in size, was removed from a seven day old culture of fungi to inoculate each tube.

All the tubes having culture were inoculated for 7-10 days at optimum temperature 28-30 °C. An open pan of water was placed in the incubator to control humidity level (40 % to 50 %). During incubation cultures were observed two per week. Minimum inhibitory concentration (MIC) of the test sample was determined by using the tubes with no visible growth of microorganisms after the lapse of 7-10 days and it was expressed in $\mu g\ mL^{-1}$

Table-1: Antifungal activity of *Thymus serpyllum* oil compared with Natamycin, Nystatin and Miconazole.

Organisms	Zone of inhibition (mm)				Control DMSO (100 µL)	Zone of inhibition (mm)		
	Thymus serpyllum oil (mcg)					Standard antibiotics (100 mcg)		
	50	100	150	200		Natamycin	Nystatin	Miconazole
A. niger	12.60	38.50	45.00	52.00	+	19.50	28.50	21.25
T. viride	13.50	18.00	24.00	31.00	+	27.50	33.50	19.75
R. oligosporous	13.63	17.00	25.00	33.50	+	25.00	28.50	25.50

2.3.1.2 *Procedure adopted for measurement of zone of inhibition*

The procurement of microorganisms such as *Aspergillus niger*, *Rhizopus oligosporous*, *Trichoderma viride* was carried out from testing laboratory and microorganisms were then maintained on potato dextrose agar slants. Dimethyl sulphoxide (DMSO) was used to prepare stock solution with concentration of 1 mg/ml. The solutions of 50, 100, 150 and 200 mcg were made from the stock solution by simple dilution methods. The standards employed for this work include Natamycin, Nystatin and Miconazole. The comparison studies were conducted by using standard antibiotics with a concentration of 100 mcg prepared in DMSO. Agar dilution method was employed for determining the antifungal activity of essential oils on the basis of effects of essential oils on growth of microorganisms. The culture was mixed with potato dextrose agar to prepare 15 mL media containing potato dextrose agar plates. Such plates were inoculated with 5mL inoculums of the respective organism.

Fig-1: Antifungal activity of *Aspergillus niger.*

Fig-2: Antifungal activity of *Rhizopus oligosporous.*

Fig-3: Antifungal activity of *Trichoderma viride***.**

The cork borers were used to make 6.0 mm wells after the settlement of agar. There replicates were made for each standard antibiotics and oil dilution. In this experiment incubation was conducted for 24 hours at 37 ^{o}C. The diameter of the clear zone around the well was recorded after the end of incubation period.

3. RESULTS AND DISCUSSION

The essential oils of *Thymus serpyllum* L. were checked against three strains. The table-1 and fig 1-3 shows the zones of inhibition for three standard antibiotics Natamycin, Nystatin and Micronazole and different concentrations of essential oil for each susceptible microorganism. The essential oils were found to have greater zone of inhibition than the three standards against *Aspergillus niger* upon using 100 mcg of each antibiotics and the essential oils were used whereas lesser antifungal activity was observed for essential oils upon using 50 mcg of each. Among different concentrations, the essential oil with concentration of 100 mcg against each susceptible organism was found much greater. However, the comparison of data with standards at 100 mcg showed that the activity against specific microorganism might be higher in case of Nystatin which possesses a lower activity as compared to other standards at the same concentration. It was observed that essential oils possess antifungal activity at lower concentrations (50 mcg) but higher concentration of the essential oils was needed to compare with standard antibiotics.

4. CONCLUSION

The present study proves that the *Thymus serpyllum* possesses considerable antifungal activity against *Aspergillus niger*.

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