

# Antifungal activity and phytochemical screening of *Origanum vulgare* L. growing wild in Kashmir Himalaya.

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## ABSTRACT

Kashmir is well known for possessing rich repository of important medicinal and aromatic plants. The present study describes the antifungal activity and phytochemical profile of *Origanum vulgare* L. The methanolic extract of *Origanum vulgare* showed pronounced activity against all the fungal strains at all tested concentrations (30, 60 and 90 µl) and the diameter of inhibition zone ranged from 5.01 to 13.00 mm in various fungal species and increased with the increase in the concentration of test solution. The highest sensitivity was exhibited against *Candida albicans* and *Saccharomyces cerevisiae* with mean zones of inhibition 13mm and 12 mm respectively at the concentration of 90 µl's. *Penicillium cryogenum* showed the least activity with mean zone of inhibition of 5.01 mm at the concentration of 30 µl. Quantitative estimation of bioactive phytoconstituents showed that the plant contains alkaloids, phenols, flavanoids, tannins, carbohydrates, terpenes and steroids. Our study clearly indicates that *Origanum vulgare* had significant inhibitory effect against all four fungal species included in the study which may find its application in future research for the therapy, food and pharmaceutical industry.

**Key Words:** Antifungal, *Origanum*, Essential oil, Antimicrobial activity.

## INTRODUCTION

Aromatic and medicinal plants are widespread throughout the world. Many of the plant species found can be used medically (Ali-Shtayeh and Abu,1999). Volatile compounds obtained from plants have known antibacterial, antifungal and insecticidal effects (Giordani *et al.*, 2004; Trombeta *et al.*, 2005; Chee and Lee, 2007; Cleff *et al.*,2010). Genus *Origanum* comprises of 42 species and 18 hybrids widely distributed in Eurasia and north Africa (Ietswaart,1980; Duman *et al.*, 1988). *Origanum vulgare* L. is an important multipurpose medicinal perennial plant which belongs to the family Lamiaceae, tribe Mentheae and plays a primary role as a culinary herb in the world trade (Cowann *et al.*,1999). It is locally known as Jungali Tulsi or Himalayan marjoram. It is widely distributed in Mediterranean areas and Northern Africa (Ietswaaart, 1980; Kokkini, 1997). This is the only species of genus *Origanum* which is found in India. It is found in temperate Himalayas from Kashmir to Sikkim at an altitude of 1500 to 3600 m. It is particularly grown in Simla Hills, Gilgit, Nilgris and in the Kashmir valley.

Multiple studies have been reported on the medicinal importance of *Origanum vulgare* L. (Komatis *et al.*,1992; Milos *et al.*,2000 ; Strycharz *et al.*, 2002; Baydar *et al.*,2004; Sahin *et al.*,2004; Viurda -Martos *et al.*,2008). antifungal (Farag *et al.*, 1989; Curtis *et al.*, 1996; Sahin *et al.*, 2004; cleff *et al.*, 2010). The aim of the current study was to analyse the antifungal activity and qualitative phytochemical analysis of *Origanum vulgare* L. growing

in the Kashmir Himalayas so as to evaluate its effectiveness to inhibit the growth of various pathogenic fungi.

## MATERIALS AND METHODS

### Plant Material:

Fresh harvestable aerial parts of *Origanum vulgare* were collected during the month of July from higher reaches of Yarikah (J&K, India) which is at an altitude of 2180mts absl . The collected plant material was properly identified at the Centre of Biodiversity and Plant Taxonomy, University of Kashmir and a specimen Voucher was deposited in Kashmir University Herbaria (KASH) for further reference under voucher specimen No.1822.

### Preparation of methanolic extract:

The aerial parts of the plant were properly cleaned and dried under shade for one week. Dried and powdered plant material weighing 55gms. was extracted with methanol using a soxhlet apparatus at 50-65 °C. The extract was then filtered through Whatmann filter paper No.1. The pellet was discarded and the supernatant was collected and concentrated under reduced pressure at 35-45°C using Buchi rotavapor (R-215). The extract obtained was reweighed and was found to be 1.5 gms. The percentage yield of extract (extract value) was determined as per Banso and Adeyemo,2007.

$E.V = \text{weight of powder} / \text{weight of extract} \times 100$  .

It was then dried, labelled and stored at 4<sup>0</sup> C in storage vials for further experimental use .

### Antifungal Activity:

#### Microorganisms tested:

Microbial cultures of four different species of fungi were used for determination of antifungal activity. Four fungal species viz. *Pencillium cryogeneum*, *Candida albicans*, *Aspergillus fumigatus* and *Saccharomyces cereviceae* were standard laboratory isolates obtained from Microbial Type Culture Collection, Chandigarh (India) .All the test cultures were maintained on Potato Dextrose Agar (PDA) media with regular sub-culturing.

#### Assessment of antifungal activity of *Origanum vulgare*:

Methanolic extract of *Origanum vulgare* was assayed for its antifungal activity by the Agar cup bioassay as adopted by Linday *et al.*,1962. The ready-made PDA medium (Himedia,39g) was suspended in distilled water and autoclaved at pressure of 15lb/inc<sup>2</sup> for 20min. Seven days old cultures of test organisms (0.5ml) were inoculated onto the medium. A standard cork borer of 8mm in diameter was used to make uniform wells into which was added 30µl, 60µl and 90µl of methanolic extract of *Origanum vulgare* L. Standard antibiotic Amphotericin (30µg/disc) was used as positive control and DMSO as negative control. The plates were then incubated at 27 ± 1°C for 78h. As the fungi grows it forms a turbid layer except in the region where the concentration of antifungal agent is above the minimum inhibitory zone and a zone of inhibition is seen .The zone of inhibition was measured to the nearest size in mm with the help of standard scale(Norrel *et al.*,1997). The experiments were carried in strict aseptic conditions so as to achieve consistency. The experiments were done in triplicates and results were calculated as mean ±SD.

#### Phytochemical Analysis:

The methanolic extract was used for the preliminary qualitative phytochemical analysis. The important phytochemical examination was carried out for the presence of Alkaloids, Phenols , Flavonoids , Tannins, Terpenes, Saponins and Carbohydrates by using standard qualitative phytochemical methods of Trease *et al.*, 2007 and Harborne 1973. The Terpenes were

identified by using the Salkwaski test .The Alkaloids were detected by using Wagners test, Phenolics by Phenol test, Tanins were determined by Ferric chloride test. Flavonoids were identified by using the Shinoda test. Forth and foam Test was used for the Saponins, similarly Carbohydrates were detected by using Fehling's test.

## RESULTS AND DISCUSSION

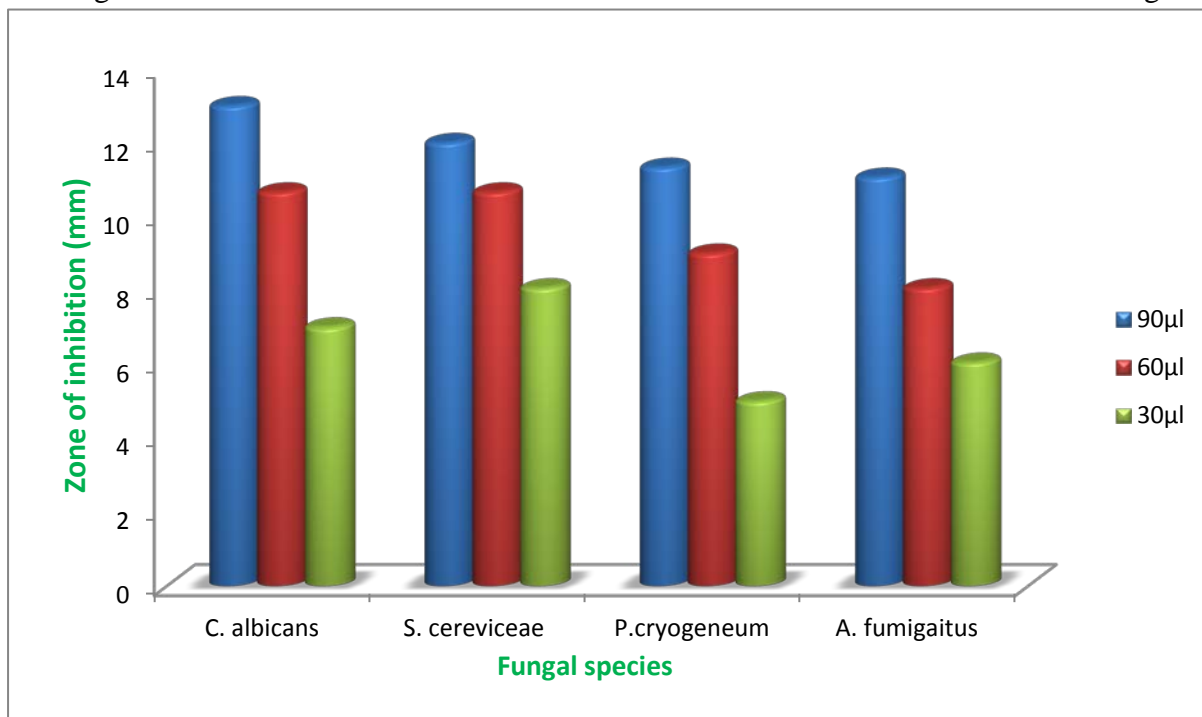
### Antifungal activity:

The methanolic extract of *Origanum vulgare* L. exhibited varying degree of antifungal activity against the tested fungal species(Table- 1).All these fungal species are known to cause serious infections. From clinical point of view Aspergillosis results from the inhalation of spores of *Aspergillus fumigatus*. Once in the lungs the spores of this fungus germinate to form a tangled mass of fungus fibers and blood clots. Fungus spreading increase gradually leading to the destruction of lung tissue, but they do not always spread to other parts of the body (Bansod and Rai, 2008).Similarly, *Candida albicans* leads to candidiasis. Manohar *et al.*, (2001);Tampierie *et al.*,( 2005); and Bozin *et al.*, (2006) showed antifungal effects of essential oil of Origanum on *Candida albicans*. Bennis *et al.*, (2004) showed antifungal activity of *O. vulgare* on *Sacharomyces cereviceae* . Its oil induces deformities in the oil of the yeast cells.*O.vulgare* is especially effective against *Pencillium cryogenum* , which is a threat to patients with compromised immune system.There is an urgent need towards the use of anti-fungal substances, especially with high efficiency and less toxicity compared to currently used drugs (Rapp, 2004; Kauffman, 2006).

All assayed fungi were sensitive to the methanolic extract of *O. vulgare* presenting large growth inhibition zones varying from 5.01 to 13.00 mm and increased with the increase in the concentration of test solution . The results shown in Table- 1 depict that the methanolic extract of leaf and stem exhibited strongest antifungal activity against *Candida albicans* at concentrations of 90  $\mu$ l followed by *Saccharomyces cereviceaea* at same concentration having mean zones of inhibition 13.00mm and 12.00 mm respectively. Relatively modest antifungal activities were observed against *Aspergillus fumigatus* and *Pencillium cryogenum* (11.00 mm and 11.33mm zone of inhibition respectively) at concentration of 90 $\mu$ l. The least antifungal activity was shown against *Pencillium cryogenum* at concentration of 30  $\mu$ l ,having inhibition zone of 5.01mm.

Many screening reports using disc diffusion techniques have established antifungal activity (cleff *et al.*, 2010; Farag *et al.*, 1989; Curtis *et al.*, 1996; Sahin *et al.*, 2004) of *Origanum vulgare* and our results are in confirmity with them. The essentials oil extracts from many plants such as basil, citrus, fennel, lemon grass, oregano, rosemary and thyme have shown their considerable antifungal activity against the wide range of fungal pathogens (Kivanc, 1991). According to the report of Ultee and Smid (2001) oregano and thyme essential oils are apparently amongst the best inhibitors of fungal pathogensAntifungal activity of essential oils and its derivatives has been studied on viable cells count, mycelia growth and mycotoxins producing ability of moulds by Juglal *et al.*, (2002) and concluded that amongst all tested essential oils clove, cinnamon and oregano essential oils are effective against *Aspergillus*. It was found that the phenolic component in the essential oil, such as carvacrol and thymol have a strong antifungal potency which might disrupt the fungal cell membrane. (Farag *et al.*, 1989; Curtis *et al.* , 1996).The current findings listed in Table 1are in accordance with the above ones and it is worth mentioning that *Origanum vulgare* L. growing in Kashmir Himalayas ,for which the biological activity against the said fungal

species has not been reported , to the best of my knowledge, can prove to be a valuable antifungal agent.



**Fig.1: Antifungal assay of methanolic extract of *Origanum vulgare* (L.)**

From the data gathered during this experiment , *Origanum vulgare* shows potential as a natural fungicidal agent. With further experimentation *Origanum vulgare* could serve as a highly effective antifungal agent in the world of medicine.

**Table 1: Antifungal activity exhibited by methanolic extract of *Origanum vulgare* L. against selected fungal species.**

S No	Fungal species used	Zone of inhibition (mm)			
		Methanolic extract of <i>Origanum vulgare</i> )			Standard antibiotic (Amphotericin )
		30µl	60µl	90µl	30µg/disc
1.	<i>Pencillium cryogenum</i>	5.01±0.51	9.00±0.51	11.33±0.51	25.00±1.00
2.	<i>Candida albicans</i>	7.00±2.51	10.66±1.52	13.00±2.51	26.66±1.44
3.	<i>Aspergillus fumigaitus</i>	6.06±1.11	8.06±1.11	11.06±1.11	23.66±1.11
4.	<i>Saccharomyces cereviceae</i>	8.06±1.11	10.66±1.52	12.00±1.00	23.00±3.05

Values are mean zone of inhibition (mm)  $\pm$  S.D of three experiments

**Table 2: Qualitative phytochemical screening of methanolic extract of *Origanum vulgare***

S.No.	Phytoconstituents	Test	Result
01.	Alkaloids	Wagners Test	+
02.	Phenols	Phenol Test	+
03.	Flavonoids	Shinoda Test	+
04.	Tannins	Ferric chloride Test	+
05.	Carbohydrates	Benedict's Test	+
06.	Terpenes	Salkwaski Test	+
07.	Saponins	Frothing Test	-
08.	Steroids	Lieberman- Buchard's Test	+

Key: (+) indicates presence and (-) indicates absence

Generally the methanol extract shows the highest activity against both bacterial and fungal isolates, which is due to the fact that the active ingredients of the plant parts are better extracted with methanol than other solvents. The phytochemical investigation in the present study revealed the presence of secondary metabolites like alkaloids, phenols, flavonoids, tannins, carbohydrates, terpenes and steroids showing the positive test and the saponins was found to be absent by the qualitative test (Table 2) This indicates that the plant extract is a good source of secondary metabolites having a important role in human life. These constituents have a broad range of activities, which may help in protection against chronic diseases known to be biologically active and therefore aid the antimicrobial activities. Therefore, it has been suggested that the essential oils extracts from the medicinal plants might be used as alternative antimicrobial natural substances and also play a great role in the discovery of new drugs.

### CONCLUSION

The current surge of interest for herbal products in preference over the synthetic aroma products has changed the trade scenario much in favour of natural essential oil and aroma chemicals. During the past few years there has been a market increase in the interest shown on many herbal spices, which is used to enhance many kinds of foods. Due to high biological activity, these aromatic plants are widely used these days in food preservation as well as in the cosmetic and pharmaceutical industry. Therefore, for different uses aromatic plants may be grown at different geographic regions for various uses. Our study clearly indicates that the methanolic extract of *Origanum vulgare* from high altitude of Kashmir Himalaya possess significant antifungal activity against diverse fungal strains due to higher concentration of bioactive phytoconstituents like alkaloids, phenolics, flavanoids, terpenes, steroids, carbohydrates and tanins that find an amazing wide application in many industries for scenting and flavorings of all types of consumers finished products. They can be used in perfumery, cosmetics, pharmaceutical, food and flavour industry. Present studies suggested that the agro-climatic conditions of Kashmir are ideal for growing these crops of international standards and can be exploited by giving proper opportunities to the farmers of the region. It



is worth mentioning that *Origanum vulgare L.* growing in Kashmir Himalayas, for which the biological activity against the said fungal species has not been reported, to the best of my knowledge, can prove to be a valuable antifungal agent. Further study is needed to isolate, structurally characterize the pure compounds and evaluate their antimicrobial activity against multidrug resistant microbial strains. Furthermore, since the plant extracts of the genus *Origanum* and its essential oils are used as dietary supplements or for medicinal purposes, it has become crucial to screen them for ensuring authenticity and product quality as toxic adulterants may prove to be life threatening.

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