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Original Article

Phytochemical Screening and Antifungal Activity of Ethanol and Petroleum-Ether Leaf Extracts of *Origanum Majorana*

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ABSTRACT

Despite of the availability of a number of Antimicrobial agents the main matter of concerning the treatment of microbial infections is the limited number of efficacious antimicrobial drugs. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic but not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged periods of administration. The impact is more acute in developing countries due to non availability of desired medicines. There is a real perceived need for the discovery of new compounds that are endowed with antifungal activities, possibly acting through mechanism of actions, which are distinct from those of well known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. The aim of Study to screen the phytochemical constituents and antifungal activity of ethanol and petroleum ether leaf extracts of *origanum majorana* against fungi *Aspergillus niger* and *candida albican*. The ethanol and petroleum ether leaf extracts were analyzed its bioactive constituents which are responsible for antifungal activity by using potato dextrose agar medium. *Origanum majorana* was found to be significantly controlling the test fungi. Data revealed that plants possessing higher glycoside, flavonoids, tannins show antifungal activity. The results indicated that the Ethanol and Petrolium ether extracts showed a varying degree of inhibition of the growth against tested organisms.

Keywords: In-vitro antifungal activity, Bioactive constituents; Medicinal plant; Antifungal activity.

1. INTRODUCTION

A safety and efficiency much superior to that of its isolated and pure active components [1].The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and essential oils which have antimicrobial properties [2, 3]. Increasing microbial resistance of pathogenic microorganisms against antibiotics, natural substances isolated from plants are considered as promising

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sources for prevent harmful infection [4]. Origanum majorana or Majorana hortensis plant is an evergreen herbaceous plant belonging to the family Lamiaceae. It is also known as Sweet marjoram. It is commonly grown in India and distributed widely in temperate regions of the Himalayas from Kashmir to Sikkim at altitudes from 500-1200m. The plant is well known as Marwa in Marathi and Hindi[5]. The genus Origanum houses around 900 different species and many species are extensively used one good home remedy for chest infection, sore throat, rheumatic pain, nervous disorder, cardiovascular diseases, epilepsy, insomnia, skin care, flatulence and stomach disorder and also use for the flavoring of alcoholic beverages, food products and in perfumery owing to their spicy fragrance. In addition to their commercial importance, such plants have been traditionally used as condiments and spices for foods like salads, soups, sausages and meats. Recently antimicrobial, antimutagenic, antihyperglycemic, antilipidemic and antiulcer effect was identified. [6, 7, 8] Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern. The plant possess higher amount of secondary metabolites such as glycoside, flavonoids, tannins identify by phytochemical screening analysis and these phytoconstituents shows antifungal activity. [9, 10]

2. MATERIALS AND METHODS

Collection of plant material

Origanum majorana leaves were collected from Bobbili region, Vijianagaram district, Andhra pradesh, India and used for this study. The lab works are done in Laboratories of Bhaskara institute of Pharmacy, Komatipalli, Bobbili, Andhra Pradesh.

Extraction of plant material

The leaves were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder, this powdered samples (100g/500ml) in Ethanol and petrolium ether for 48 hours at 45ºC. Soxhlet apparatus are used for this extraction. The extract from these solvents are soaked and evaporated under pressure. The leaf extracts were concentrated at 50°C and the residue obtained was stored at 4°C.[11]

Antifungal activity assay by Well Diffusion method

The leaf extracts were also screened for their antifungal activity in comparison with standard antibiotic Clotrimazole (20mg/ml) *in vitro* by well diffusion method [12]. Lawn culture was prepared using the test organism on Potato Dextrose Agar (PDA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. A fixed volume (0.1ml) of the selected extracts of *Origanum majorana* was then introduced into the wells in the increasing concentration. The plates with fungi were incubated at room temperature for 7 days. The activity of the extract was determined by measuring the diameters of zone of inhibition. [13, 14] .The selection of medium depends on the type of organism and nature of compound to be tested. For antifungal sensitivity Potato dextrose agar P^H (5.5-6) was used. The zone of inhibition of mycelial growth was determined by antibiotic zone scale (Hi-media). [12-15]

Activity index = Zone of inhibition of sample/Zone of inhibition of reference

Microorganisms used

Two human pathogenic microorganisms, Fungi *Aspergillus niger* and *candida albican* were used in the study for the evaluation of the antifungal activity.[16] 2 Strains of Fungi were the laboratory isolate. All the strains were collected from the Department of Microbiology, Bhaskara Institute of Pharmacy,Komatipalli, Bobbili, Andhra Pradesh, India.

Table 1: Qualitative Analysis -Leaf Extracts

S.NO	Phytochemicals	Ethanol Extract	Petroleum Ether Extract
1	Alkaloids	+	+
2	Flavonoids	+ + +	+ + +
3	Saponins	+	+
4	Tannins	+ +	+ +
5	Phlobatanins	-	-
6	Glycosides	+ + +	+ + +
7	Sterols	+	+ +
8	Resins	+ +	+ +
9	Phenols	+	+ +
10	Anthraquinones	-	-
11	Terpinoids	-	-
12	Cardiac glycosides	+ +	+ +

3. RESULTS AND DISCUSSION

Phytochemical analysis of bioactive compound in different solvent extracts of *origanum majorana*

The leaf extracts in different solvents were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of glycosides, flavonoids, tannins are in most prominent amount while cardiac glycoside, alkaloid and phenol are in least amount and terpenoid, pholbatannin and anthraquinone are absent in organic solvent. Resin is present. [18, 19, 20]

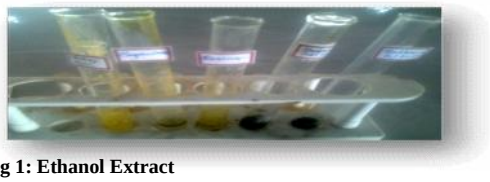

Fig 1: Ethanol Extract

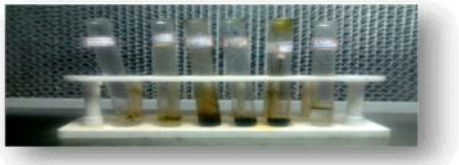

Fig 2: Petroleum ether extract

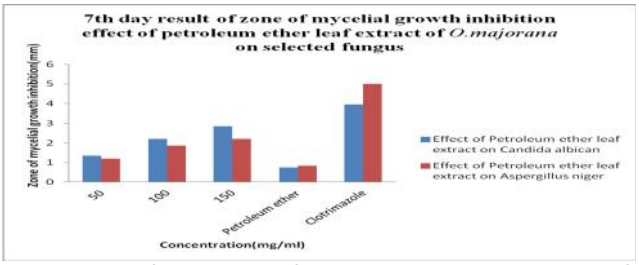

Fig 3: The antifungal activity of Petroleum ether extract (diameter of the inhibition zone, mm) of *Orignum majorana* on two different human pathogenic fungi.

Table 2: 7th Day Result Of Petroleum Ether Leaf Extract Of *O.Majorana* On *Candida Albican* And *Aspergillus Niger*

Concentration(mg/ml)	Zone of mycelial growth inhibition(mm)	
50	1.35	1.2
100	2.2	1.85
150	2.85	2.2
Petroleum ether	0.74	0.82
Clotrimazole	3.9	5

Petroleum ether leaf extract of *Origanum majorana (50,100,150* mg/ml)

Table 3: 7th Day Result of Ethanol Leaf Extract of *O.Majorana* On *Candida Albican* And *Aspergillus Niger*

Concentration(mg/ml)	Zone of mycelial growth inhibition(mm)	
50	1.88	2
100	2.45	2.85
150	3.56	3.78
Ethanol	0.9	0.92
Clotrimazole	3.96	5

Ethanol leaf extract of *Origanum majarona (50,100,150* mg/ml), Clotrimazole used as standard drug. All the values given are the mean value of three reading.

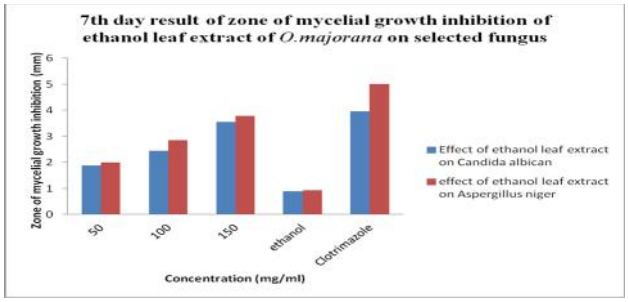

Fig 4: The antifungal activity of ethanolic extract (diameter of the inhibition zone, mm) of *Orignum majorana* on two different human pathogenic fungi.

4. DISCUSSION

The leaf extracts of *Origanum majorana* were screened for its phytochemical analysis and antifungal activity. The solvents used for the leaves extraction were Ethanol and Petrolium-ether. The extract was tested against infectious diseases causing by fungal *pathogens* such as *Aspergillus niger, Candida albican* using the potato dextrose agar well diffusion method. The Ethanol extract of *Origanum majarona* (Table-3) showed more activity against fungus like *Aspergillus niger* and the zone of diameter 2, 2.85, 3.78 mm for the concentrations 50,100,150 mg/ml respectively where as on Petroleum ether extract, (Table-2) showed the zone of diameters are 1.2, 1.85, 2.2 mm for the concentrations 50,100,150 mg/ml respectively. [21, 22, 23]

The Ethanol extract of *Origanum majorana* showed better activity against the fungus like *Aspergillus niger* with the zone of 3.78 mm followed by Petroleum ether extract showed zone of diameter 2.2 mm at highest test concentration. The Ethanol extract activity on *Candida albican* also more compare to Petroleum ether extract. In the present study the fungi on Ethanol and Petrolium ether extracts showed a varying degree of inhibition of the growth against tested organism. The results confirmed that presence of Antifungal activity in the shade dried extract of *Origanum majorana* against the human pathogenic organisms.

Preliminary phytochemical analysis of Ethanolic extract & Petroleum ether extract showed (Table-1, Figures-1 & 2) the presence of Glycosides, Flavonoids, Tannins, Alkaloids, Steroids & Terpenoids whereas the Petrolium ether extract revealed the Flavonoid, Tannins, Glycosides & Alkaloids as active phytochemical constituents.

5. CONCLUSION

The present study was carried out with a vision to setup standards that could be beneficial for detecting the authenticity of this vital medicinal plant. The antifungal activity of various plants has been reported by many researchers. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins, phenol and glycosides are producing a better opportunity for testing wide range of microorganism. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants. The wide spectrum of activity of *Origanum majorana* extracts has been documented earlier. This study evaluated the inherent antifungal activity of ethanolic extract of *Origanum majorana*. From the obtained results it can be concluded that although ethanol in itself has antifungal activity, ethanolic extract of *Origanum majorana* has a synergistic activity. Since *Origanum majorana* is easily available and well- tolerated, it can be incorporated into medications for topical fungal infection. However, further studies for its incorporation into oral preparations, safety and cost- effectiveness has to be conducted.

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