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In Vitro Antibacterial and Antifungal Activity of Methanol, Chloroform and Aqueous Extracts of *Origanum vulgare* and Their Comparative Analysis

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Abstract

The work reports antibacterial and antifungal activity of different solvent extracts of *Origanum vulgare*. The antimicrobial activity of methanol, chloroform and aqueous extracts were determined against nine different gram negative and gram positive bacterial strains and three fungal stains. The bacterial strains were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 9341), *Pseudomonas aeruginosa* (ATCC 33347), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Shigella flexneri* (ATCC 25929), *Salmonella para typhi A* (ATCC 9150) and *Proteus mirabilis* (ATCC 49565) and fungal strains were *Aspergillus flavus*, *Aspergillus nigar and Aspergillus pterus.* Agar well diffusion method was followed in this study. The comparative analysis of antibacterial activity reflects that among these three extracts, chloroform and methanol extracts shows promising result by exhibiting maximum antibacterial activity, whereas aqueous extract is not active against most of these strains. The analysis of antifungal activity reveals chloroform extract as most efficacious unlike methanol and Aqueous extracts.

Keywords: *Origanum vulgare*, Antibacterial Activity, Antifungal Activity

1. Introduction

Medicinal plants deeply connected with our lives especially those who not only hold culinary importance but also possess combative ability against number of microbes. They become cynosure in our homes, commercial and research sector. Their natural origin paves expedient outcomes, some in form of natural preservatives along with aiding our immune system to fight against pathogenic organisms. World Health Organization (W HO) supported the use of herbal medicines as safe therapy for the treatment of different diseases. Medicinal plants would be the paramount basis to find a range of drugs [1].

New drugs must be needed to eliminate the resistant microorganisms because a number of well known antibiotics cannot fight with resistant strains and become superseded [2]. Literature bear witness that this problem has proliferated to whole globe. As, the occurrence of fluoroquinolone resistant gram negative *Bacilli* colonizing community dwelling people with spinal cord dysfunction [3], ciprofloxacin-resistant gram negative *Bacilli* association with serious infections in children [4] are common examples. The resistant gram negative and gram positive species are associated with increased mortality and morbidity, prolonged hospitalization and increased costs. Screening of synthetic compounds and plant extracts determining their antibacterial and antifungal potential holds immense worth, as they may serve as a solution to eradicate antibiotic resistant microbes.

Antibacterial derived from plants are not related with undesired effects as the synthetic drugs and can be successfully employed to heal many infectious diseases [5]. About 80% populations of the developed countries use herbal medicines. A number of important drugs like quinine (antimalarial), vincristine (antitumor drug) and digitalis (heart regulator) were extracted from medicinal plants. The utilization of plant extracts and phytochemicals with identified biological activity may be of huge significance in therapeutic treatments [6-10].

The plant selected in this study is *Origanum vulgare* also commonly known as oregano belongs from genus

Origanum of lamiaceae family (mint family). This plant is located in hunza valley lies in karakoram ranges. Few of the important medicinal plants of this region are Artemisia maritime, Chenopodium ambrosioides, Ephedra gerardianam, Astragalus macropterus, Corydalis Adiantifolia, Sonchus asper (L)Hill, Hippophae rhamnoides L, Tamarix arceuthoides Bge, Salix acmophylla Boiss, Atriplex crossifolia C.A.Mey, Aquilegia pubiflora Wall. ex Royle, Primula veris L, Lonicera periclymenum L, Galium boreale L, Lactuca decipiens (H. and T) Clark [11]. The plant selected for antibacterial assay may have great potential for industrial applications [12-15].

Oregano has been recognized as one of the most used vegetable all over the world with abundant occurrence in East Europe, in the Middle Asia and South and North America [16,17]. The volatile oil of oregano has been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis and urinary tract disorders [18]. Carvacrol is a major active component of oregano and has potential uses as a food preservative [19]. Other chemical constituents include limonene, gammacariofilene, rho-cymenene, canfor, linalol, al-phapinene and thymol [20].

The present work has been designed to evaluate the potential of methanol, chloroform and aqueous extracts of the *Origanum vulgare* against nine different gram positive and gram negative pathogenic bacterial strains and three different fungal strains. The results obtained in this bioassay were compared and it was found that chloroform extract has high potential against tested bacterial and fungal strains.

2. Experimental

The part used are leaves of *Origanum vulgare* collected from hunza valley in August 2009. The leaves were then dried, homogenized and further subjected for extraction. The hot extraction method was followed to obtain methanol, chloroform and aqueous extracts of leaves of the plant.

2.1. Methanol Extract

The soxhlet extractor was used to afford different solvent extracts of the *Origanum vulgare*. The crude methanol extract was achieved by putting 10 g of the dried, homogenized plant in the porous thimble (made of tough filter paper) and 100 ml of the methanol in the bolt head flask of the extraction apparatus. The solvent was then boiled for one hour and methanol was rotary evaporated. The extract was then vacuum dried and yield of the methanol extract was 71.30%. The extract was then stored in refrigerator at 4˚C for further study.

2.2. Chloroform and Aqueous Extracts

The same method was adopted for the preparation of chloroform and aqueous extracts. The yield of chloroform extract was 6.20% and that of aqueous extract was 53.31%.

2.3. Antibacterial Activity

The antimicrobial activity of these crude extracts was determined against nine different gram positive and gram negative bacteria. Agar well diffusion assay was used to evaluate the antibacterial activity of these extracts [21]. The selected bacterial strains are *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 9341), *Pseudomonas aeruginosa* (ATCC 33347), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Shigella flexneri* (ATCC 25929), *Salmonella para typhi A* (ATCC 9150) and *Proteus mirabilis* (ATCC 49565).

Assay was conducted by using the Nutrient Agar. The fresh inoculum of these strains were prepared and diluted with sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5 Mc-Farland. A uniform bacterial lawn was developed by sterile cotton swabs. 8 mm sized borer was used to make the wells in the inoculated plates. Various dilutions of the dried plant extracts including 1000 ug/ml, 500 ug/ml, 250 ug/ml, 125 ug/ml and 62.5 ug/ml were prepared by using dimethyl sulfoxide (DMSO) as solvent. 200 ul of test extract and standard were then delivered to each well. DMSO was used as control in this antimicrobial study. Levofloxacin (125 ug/ml) a broad spectrum antibiotic effective against a number of gram positive and gram negative bacterial strains was used as standard. These plates were incubated at 37˚C for 24 hours. Antibacterial activity of the plant extracts was determined by measuring the diameter of zone of inhibition (mm) and presented by subtracting the activity of the control.

2.4. Antibfungal Activity

In vitro antifungal activity of the solvent extracts was tested against three fungi; *Aspergillus flavus*, *Aspergillus nigar and Aspergillus pterus* using poison plate method [22]. Potato dextrose agar (PDA) plates were prepared by using pour plate technique for each extract. A 2% concentration of each extract in DMSO as a solvent was used. A 2% solution of fluconazole was used as standard. A drug free control was included and plates were observed for growth after 48 h of static incubation at 30˚C and results are presented in **Table 4**. All of the plant extracts showed good to excellent antifungal activity.

3. Results and Discussion

The results of antibacterial activity of the methanol extract are presented in **Table 1**. The gram positive bacteria showed more susceptibility than gram negative to the methanol extract. The methanol extract is more active against *Staphylococcus aureus*, *Micrococcus luteus* as compared to all other bacterial strains. The extract has also shown pronounced effects against *Bacillus subtilis*. Among gram negative strains *Shigella flexneri* have shown competitive results. The findings are interesting for *Micrococcus luteus* as it is resistant towards the standard.

The **Table 2** presented the antibacterial activity of chloroform extract. The antibacterial activity was observed to be in dose dependent manner *i.e.*, 125 ug/ml showed more level of activity than 62.5 ug/ml against all the tested strains. Chloroform extract of Origanum *vulgare* was most active against gram negative bacteria, although it has shown significant effects against gram positive strains. The excellent results were shown against *Pseudomonas aeruginosa* and *Bacillus subtilis* in comparison to all the microorganisms tested. The extract has demonstrated considerable effect over *Mircococcus luteus* and *Salmonella typhi* by producing adequate zone of inhibition.

In **Table 3** it is obvious that the aqueous extract showed interesting activity against *Salmonella para typhi A* as the chloroform and methanol extract are inactive against this strain. It possesses activity against *Mircococcus luteus*, *Shigella flexneri*. The remaining tested bacterial strains are resistant towards the aqueous extract.

Table 4 represent the antifungal studies. In Antifungal analysis, the chloroform, Aqueous and Methanol extracts activity were compared with standard Fluconazole. The chloroform extract exhibits most efficacious results against *Aspergillus flavus* and *Aspergillus pterus*. Its activity is against *Aspergillus niger* is quite low. The Aqueous and Methanol extracts do not exhibit reportable activity against these stains.

In this study the preparation of the solvent extracts was made by using both the organic solvent and water. From our investigation, it is concluded that the active antibacterial present in the leaves of *Origanum vulgare* are chloroform and methanol soluble. The active ingredients contained in extract of chloroform are quite efficacious against *Pseudomonas aeruginosa* and *Bacillus subtilis* along with activity against other strains. Whereas the active in Methanol extract was worthwhile against *Staphylococcus aureus*, *Micrococcus luteus*, *Shigella flexnari* along with other strains, which have shown considerable zone of inhibition. In antifungal analysis, the chloroform extract showed efficacious results.

Table 1. *In vitro* antibacterial activity of methanol extract of *Origanum vulgare*.

Bacterial Strains	Methanol Extract					Levofloxacin
	1000 ug/ml	500 ug/ ml	250 ug/ml	125 ug/ml	62.5 ug/ml	125 ug/ml
Escherichia coli	13	12	10	Nil	Nil	22
Shigella flexneri	20	15	11	10	Nil	29
Salmonella para typhi A	Nil	Nil	Nil	Nil	Nil	17
Salmonella typhi	Nil	Nil	Nil	Nil	Nil	28
Pseudomonas aeruginosa	15	12	11	10	Nil	21
Micrococcus luteus	24	19	15	14	12	Nil
Proteus mirabilis	Nil	Nil	Nil	Nil	Nil	Nil
Bacillus subtilis	17	15	12	Nil	Nil	24
Staphylococcus aureus	25	20	18	16	14	22

Table 2. *In vitro* antibacterial activity of chloroform extract of *Origanum vulgare*.

Bacterial Strains	Chloroform Extract					Levofloxacin
	1000 ug/ml	500 ug/ml	250 ug/ml	125 ug/ml	62.5 ug/ml	125 ug/ml
Escherichia coli	Nil	Nil	Nil	Nil	Nil	22
Shigella flexneri	13	12	11	10	9	29
Salmonella para typhi A	Nil	Nil	Nil	Nil	Nil	17
Salmonella typhi	16	13	12	11	10	28
Pseudomonas aeruginosa	23	20	18	17	13	21
Micrococcus luteus	17	14	13	10	10	Nil
Proteus mirabilis	Nil	Nil	Nil	Nil	Nil	Nil
Bacillus subtilis	22	16	15	14	11	24
Staphylococcus aureus	Nil	Nil	Nil	Nil	Nil	22

Table 3. *In vitro* antibacterial activity of aqueous extract of *Origanum vulgare*.

Bacterial Strains	Aqueous Extract					Levofloxacin
	1000 ug/ml	500 ug/ml	250 ug/ml	125 ug/ml	62.5 ug/ml	125 ug/ml
Escherichia coli	Nil	Nil	Nil	Nil	Nil	22
Shigella flexneri	14	13	12	11	10	29
Salmonella para typhi A	13	12	10	Nil	Nil	17
Salmonella typhi	Nil	Nil	Nil	Nil	Nil	28
Pseudomonas aeruginosa	Nil	Nil	Nil	Nil	Nil	21
Micrococcus luteus	14	12	10	9	Nil	Nil
Proteus mirabilis	Nil	Nil	Nil	Nil	Nil	Nil
Bacillus subtilis	Nil	Nil	Nil	Nil	Nil	24
Staphylococcus aureus	Nil	Nil	Nil	Nil	Nil	22

Table 4. *In vitro* antifungal activity of the solvent extracts of *Origanum vulgare*.

Sr. No	Fungal Strains	Plant Extracts			Fluconazole
		Aqeous	Chloroform	Methanol	
1	*Aspergillus flavus*	10 mm	23 mm	10 mm	37 mm
2	*Aspergillus niger*	9 mm	11 mm	-	23 mm
3	*Aspergillus pterus*	-	30 mm	-	36 mm

4. Conclusions

The constituents contained in Chloroform exhibit substantial activity against *Aspergillus flavus* and *Aspergillus pterus*. The results obtained confirm the therapeutic potency of Origanum *vulgare* used in traditional medicine. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation.

5. References

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