International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Antibacterial activity and DPPH scavenging antioxidant potential in *Origanum vulgare L.*

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Abstract

Origanum vulgare L. has been known as plant species with prominent biological properties for a long time. Antibacterial effect of methanolic extract from dried leaves and stem of *Origanum vulgare* L., which was collected from I.I.I.M, Srinagar Kashmir ,was studied against two Gram positive strains viz. *Staphylococcus aureus* MTCC- 96, *Bacillus subtilius* MTCC- 441 and four Gram negative strains viz. *Pseudomonas aeriginosa* MTCC- 1688, *Proteus vulgaris* MTCC- 321, *Escherichia coli,* MTCC- 443 and *Klebsiella pneumonia* MTCC- 3384 which are the most important food borne pathogens known to cause severe infections. The bioassay showed that *Origanum vulgare* L., exhibited strong antibacterial activity against all the above mentioned strains at all tested concentrations (50 and 25 mg ml^{-1}) particularly against *Bacillus subtilius* with mean zone of inhibition 18mm at concentration of 50 mg ml^{-1}. *Escherichia coli* showed the least activity with mean zone of inhibition of 10 .33 mm at the concentration of 25 mg ml^{-1}.The methanolic extract was also studied for DPPH free radical scavenging activity, where its antioxidant potential was also found to be appreciably strong (76.73% at concentration of 600µg/ml).The present study clearly indicates that the crude methanolic extract of *Origanum vulgare L.* from high altitude of Kashmir Himalaya (2350 m) shows significant antibacterial activity and DPPH free radical scavenging activity in concentration dependent manner.

Keywords: *Origanum vulgare*, antibacterial, free radical scavenging, agar well diffusion method .

Introduction

The use of herbal medicines for the treatment of diseases and infections is a safe and traditional way(Najafi and Deokule,2010).Moreover, volatile compounds obtained from plants have known antibacterial, antifungal and insecticidal effects (Cleff *et al*.,2010; Giordani *et al*., 2004;Trombeta *et al*., 2005). Antibiotic resistance has become a global concern (Westh *et al*., 2004) and medicinal plants have been extensively used for years due to their biological properties. Bacterial resistance to antibiotics is a major therapeutic problem and the pace at which new antibiotics are being produced is slowing(Russell ,2002). The increasing prevalence of multidrug resistant bacterial strains and the recent appearance of strains with reduced susceptibility to antibiotics raises the concern towards bacterial infections (Sieradzki *et al*.,

1999).Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infections(Iwu *et al*., 1999).

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. 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).

The aim of the current study was to analyse the antibacterial and antioxidant activity of *Origanum vulgare* L. growing in the Kashmir Himalayas so as to evaluate its effectiveness to inhibit the growth of various Gram positive and Gram negative pathogenic bacteria.

Materials and Methods

Plant material

The leaves and stem of *Origanum vulgare* L. were collected in June 2013 from I.I.I.M, Srinagar, Kashmir. 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 extract

The aerial parts of the plant were properly cleaned and dried under shade for one week. Dried and powdered plant material weighing 55 gms. was extracted with methanol using a soxhlet apparatus at 50-65^{0}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=weight of powder/weight of extract×100 .
It was then dried, labelled and stored at 4^0 c in storage vials for experimental use.

Antibacterial activity

Microorganisms tested

Microbial cultures of six different species of both Gram positive and Gram negative bacteria were used for determination of antibacterial activity. The bacterial strains used were *Pseudomonas aeriginosa, Proteus vulgaris, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilius.* All the bacterial strains were sub-cultured at 37°C on Mueller- Hinton agar (Hi-media) slants every fifteen days and stored at 4°C.

Antibacterial activity assay

The antibacterial activity of methanolic extract of *Origanum vulgare* L. was determined by agar well diffusion method as adopted by Perez *et al*.,1990. Each microorganism was grown overnight at 37°C in Mueller-Hinton Broth. Ten microlitres (10µL) of standardized inoculum (0.5 Mac-Farland) of each test bacterium was inoculated on molten Mueller-Hinton agar, homogenized and poured into sterile Petri dishes. The Petri dishes were allowed to solidify inside the laminar hood. A standard cork borer of 5mm in diameter was used to make uniform wells into which was added 50mgml^{-1} and 25mgml $^{-1}$methanolic extract of *Origanum vulgare L.* diluted in DMSO. Standard antibiotic Ofloxin (OF)(30µg/disc) was used as positive control and DMSO as negative control. The plates were then

incubated at 37 ± 1°C for 24h. As the bacteria grows it forms a turbid layer except in the region where the concentration of antibacterial agent is above the minimum inhibitory zone and a zone of inhibition is seen (Fig.1).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.

Antioxidant activity

DPPH free radical scavenging assay

DPPH(1,1- Diphenyl-2-picrylhydrazyl) assay is one of the most extensively used method for determining the antioxidant potential of any biological sample (Brand-Williams *et al*., 1995 , Yen *et al*., 1994). DPPH is a purple stable free radical which is reduced to yellow coloured complex DPPH-H (1,1-diphenyl-2-picrylhydrazine) by the compounds which are capable of donating hydrogen or electron. 100 µl of different concentrations (100-600 µg/ml) of plant extract was added to 1 ml of 0.5 mM DPPH solution in methanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes under dark conditions. The absorbance of the mixture was read at 517 nm in a spectrophotometer against methanol (Brand-Williams *et al.,* 1995). The decrease in absorbance indicates increase in DPPH free radical scavenging potential. The percentage inhibition was calculated by the following equation.

$$\text{DPPH free radical Scavenging (\%)} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Where, $Abs_{control}$ is the absorbance of control and Abs_{sample} is the absorbance of sample.α-tocopherol was used as standard antioxidant and served as positive control.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Ahmad *et* al., 2010a).

Results and Discussion

Antibacterial activity

The methanolic extract of *Origanum vulgare* L. exhibited varying degree of antibacterial activity against the tested bacterial strains (Table- 1). The bacterial strains used were clinical and laboratory isolates. All these bacterial species are known to cause serious human infections. From clinical point of view, *Klebsiella pneumonia* causes neonatal nosocomial infection (Martinez *et al*., 1999). *Escherichia coli* cause's septicemias and can infect the gall bladder, surgical wounds, skin lesions and the lungs (Ghanni,1998).*Staphylococcus aureus* causes dermatitis and sialadenitis ,*Proteus vulgaris* causes bacteremia, sepsis and urinary tract infections (Black,1996; Mastroeni, 2002).*Bacillus subtilis* is known to cause disease in severely immune compromised patients (Oggioni *et al*.,1998) and *Pseudomonas aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections (Todar, 2004).All assayed bacteria were sensitive to the methanolic extract of *O. vulgare* presenting large growth inhibition zones. The results shown in Table - 1 depict that the methanolic extract of leaf and stem exhibited strongest antibacterial activity against *Bacillus subtilius* at both the concentrations of 50mgml^{-1}and 25 mg ml $^{-1}$ used, having mean zones of inhibition 18.00 mm and 14.66 mm respectively. Relatively modest antibacterial activity was observed against *Proteus vulgaris* (12.00 mm zone of inhibition) at concentration of 50mgml^{-1} and by *Escherichia coli* (10.33 mm zone of inhibition) at concentration of 25 mgml^{-1} followed by *Proteus vulgaris* with mean zone of inhibition 10.66mm at concentration of 25 mg ml^{-1}.

Many screening reports using disc diffusion techniques have established antibacterial activity

Table 1. Antibacterial activity exhibited by methanolic extract of *Origanum vulgare* L. against selected bacterial strains.

S No.	Bacterial strain used	Zone of inhibition (mm)		
		50 mg ml^{-1}	25 mg ml^{-1}	Positive control (Ofloxin)
1.	*Staphylococcus aureus* MTCC- 96	16.33±2.64	13.00 ± 1.52	29.33± 1.74
2.	*Bacillus subtilius* MTCC-441	18. 00±2.00	14.66 ± 2.00	36.33 ±1.52
3.	*Proteus vulgaris* MTCC-321	12.00 ± 1.74	10.66±1.52	25.00± 3.05
4.	*Pseudomonas aeriginosa MTCC- 1688*	16.66 ± 2.00	13±2.51	29.66 ± 0.57
5.	*Klebsiella pneumonia* MTCC- 3384	14.33± 2.51	12.00 ±1.00	27.00 ± 1.74
6.	*Escherichia coli* MTCC-443	13.66±1.52	10.33± 3.05	24.00 ± 1.52

Figure 1: Showing Zones of inhibition against (A) *P.aeriginosa* (B) *P .vulgaris* (C) *S. aureus*(D) *K.pneumoniae* (E) *E. coli* (F) *B. subtillus at* various concentrations of plant extract.

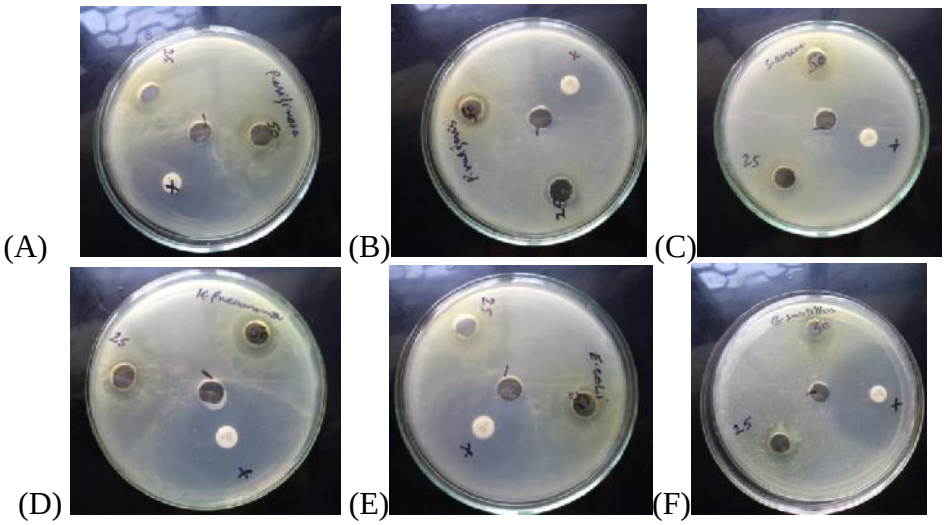
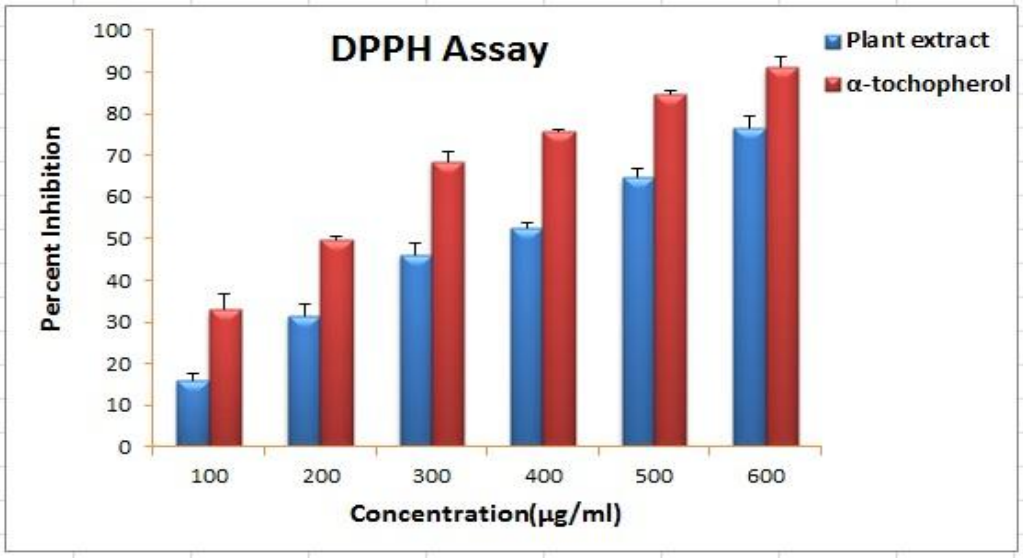


Table 2:DPPH activity at various concentrations of methanolic extract of *Origanum vulgare* L.(Values are ± S.D of three experiments).

Conc. (µg/ml)	Plant extract	α-tochopherol
100	16. 06± 1.54	32. 86 ± 3.68
200	31.43± 2.65	49.82 ± 0.71
300	46.25± 2.54	68.43 ± 2.59
400	52.59± 1.04	75.76 ± 0.58
500	64.68± 2.05	84.77 ± 0.84
600	76.73 ±2.56	91.00± 2.63
IC_{50} Value	**324.71**	**201.05**

Fig.2: DPPH Assay of methanolic extract of *Origanum vulgare L.*



of *Origanum vulgare* L. extracts against number of pathogens (Baydor *et al*., 2004) including *Bacillus brevis , Bacillus cereus, Bacillus subtilis, Escherechia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Staphylococcus aureus.* Viuda - Martos *et al*., 2008 reported antibacterial activity of *Origanum vulgare* L. against *Staphylococcus carnosus, Staphylococcus xylosus* and *Lactobacillus sakei.* Furthermore, Derwisch*et al*.,2010 has reported antibacterial activity of *Pseudomonas aeruginosa, Salmonella typhi* and *Micrococcus luteus* against *Origanum vulgare* L.
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 bacterial strains has not been reported , to the best of my knowledge, can prove to be a valuable antibacterial agent.

Antioxidant activity

Studies conducted on free radical scavenging activity of medicinal plants have shown that efficiency of each plant species differs depending on the particular assay methodology, reflecting complexity of mechanisms involved on total antioxidant capacity (Matkowski *et al*., 2005). The DPPH method used for studying antioxidant activity of *Origanum vulgare L*. revealed that *Origanum vulgare* L. has a strong radical scavenging activity of 76.73% at concentration of 600µg/ml (Table 2).Cervato *et al*. (2000) showed that the antioxidant activities of extracts of oregano leaves (both aqueous and methanolic extracts) can inhibit all phases of lipid peroxidative process. The highest percentage of phenolic compounds present in the oregano oil (carvacrol and thymol) can be responsible for the highest ability to scavenger free radicals such as H^+, measured by DPPH method but a possible synergistic effect among oxygen containing compounds can be suggested too. The presence of available hydrogen atoms from phenol represents a good barrier against the primary oxidative process. These results indicate that the oregano essential oil could be in use as potential resource of natural antioxidants for food industry so that it is interesting to examine its application as natural antioxidant additive in some final food products. The IC 50 value of *Origanum vulgare L.* was found to be 324.71 (Fig. 2).

Conclusion

The present study was conducted to investigate the antibacterial and antioxidant activity of *Origanum vulgare L.* The bacterial strains used were all found to be sensitive to *Origanum vulgare L.* of promising

therapeutic agents that can be used in combating infectious diseases caused by drug- resistant microorganisms. Furthermore, *Origanum vulgare L.* proved to have a strong DPPH -scavenging antioxidant potential. Further study is needed to isolate, structurally characterize the pure compounds and evaluate their antimicrobial activity against multidrug resistant microbial strains.

References

Ahmed, N., H. Fazal, B.H. Abbasi, M. Rashid, T. Mahmood and N. Fatima.,2010a.Efficient regeneration and antioxidant potential in regenerated tissues of *Piper nigrum* L. *Plant, Cell, Tissue and Organ culture,* 102: 129–134.

Baydor, H., Osman S., Ozkan G. and Karadoa T.,2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*, 15 : 169 – 172.

Banso, A. and S.O. Adeyemo. ,2007.Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinera. Afr. J. Biotechnol*., 615:1785-1787.

Cleff, M., Meinerz, A.R., Xavier, M. ,Schuch, L.F. and Carlos, M.,2010. *Invitro* activity of *Origanumvulgare* essential oil against *Candida species*. *Braz. J. Microbiol*. , 41: 116-123.

Black, J. G., 1996. Microbiology: Principles and Application. p. 260.

Brand-Williams, W., Cuvelier, M. E., &Berset, C., 1995. Use of freeradical method to evaluate antioxidant activity. *Lebensm. Wiss. Technology*, 28: 25–30.

Cleff, M., Meinerz, A.R., Xavier, M. ,Schuch, L.F. and Carlos, M.,2010. *Invitro* activity of *Origanumvulgare* essential oil against *Candida species*. *Braz. J. Microbiol*. , 41: 116-123.

Cowan, M.M.,1999. Plant products as antimicrobial agents.*Clin.Microbiol.Rev*.,12:564-582.

Derwich, E,Z.Benziane, A. Boukir and R. Taouil.,2010.Phytochemical analysis and *in vitro* antibacterial activity of the essential oil of *Origanumvulgare*from Morocco. *American-EurasianJournal of Scientific Research*,52:120-129.

Duman, H., Baser, K.H.C, Aytec Z., 1998. Two new species and a new hybrid from Anatolia. *Turkish Journal of Botany*, 22 : 51 – 55.

Ghani, A .,1998. *Medicinal plants of Bangladesh*, pp. 78–83.

Giordani, R. ,P. Regli, J.Kaloustian, C. Mikai, L. Abou and H. Portugal., 2004.Antifungal effect of various essential oils against *Candida albicans*. Potentiation of Antifungal Action of Amphotericin B by essential oil from *Thymus vulgaris. Phytot.Res*.,18:990-995.

Ietswaart J.H.,1980. A taxonomic revision of the genus *Origanum* Labiatae, Leiden Botanical series, vol. 4 Leiden University Press, The Hague, Leiden.

Iwu, M. W., Duncan, A. R., Okunji, C. O., 1999. New antimicrobials of plant origin. In: Perspectives on New Crops and New Uses. *ASHS Press, Alexandria,* VA: 457–462.

Jennifer, M. A .,2001. Determination of minimum inhibitory concentrations. *Journal of antimicrobial chemotherapy.* 48: 5-16.

Jonathan, S. G, Fasidi, I. O., 2003. Antibacterial activities of Nigerian edible macro fungi-*Lycoperdonpusilum* Bat. Ex and *Lycoperdongiganteus* Pers. *Afr. J. Biomed. Res*. 6, 85-90.

Komatis, M., N. Ifanti-Papatragianni and E. Melissari-Panagiotou.,1992.Composition of the essential oil of marjoram *Origanum majorana L.*. *Food Chemistry* ,452:117-118.

Martinez, A and Valencia, G .,1999. Manual de practicas de farmacognosia y Fitoquimia: Medellin: *Universidad antiquia; Marchafotiquimica*, 926: 59-65.

Mastroeni, P., 2002. Immunity to systemic *Salmonella* infections. *Curr. Mol. Med*. 2: 393-406.

Matkowski, A., P. Tasarz and E. Szypula.,2008. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, sub family Lamioideae. *J. Med. Plants Res*., 211:321-330.

Milos, M., J. Mastelic and I. Jerkovic.,2000.Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano *Origanum vulgare ssp. hirtum*. *Food Chemistry,* 711:79-83.

Najafi ,S. and S.S.Deokule.,2010.Pharmacognostic study of *Tylophoradalzellii*Hook.f. *Journalof Medicinal Plants Research*, 45:403-406.

Norrel, S. A. and K. E. Messley.,1997. Microbiology Laboratory Manual Principles and Applications.*Prentice Hall, Upper Saddle River. New Jersey*, pp: 85-90.

Oggioni, M.R; Pozzi ,G, Valensin P.E, Galieni ,P, Bigazzi, C.,1998. Recurrent septicemia in an immunocompromised patient due to probiotic strains of *Bacillus subtilis* . *J. Clin. Microbiol.* 36 1: 325–6.

Perez, C., Pauli, M., Bazerque, P. ,1990. An antibiotic assay by the well agar method. *Acta Biologiae et Medicine Experimentalis*15, 113–115.

Russell, A. D., 2002. Introduction of biocides into clinical practice and the impact on antibiotic resistant bacteria; *J. Appl. Microbial*. Symp. 92: 121–135.

Sahin, F., Gulluce, M., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., Agar, G., Ozer, H.,2004.Biological activities of the essential oils and methanol extracts of *Origanum vulgare* ssp.*vulgare* in the eastern Anatolia region of Turkey. *Food Control*, 15; 549 – 557.

Sieradzki, K., Roberts, R. B., Haber, S. W, Tomasz, A., 1999. The development of vancomycin resistance in a patient with methicillin resistant *Staphylococcus aureus* infection; *N. Engl. J. Med*., 340: 517–523.

Strycharz ,S. and K. Shetty.,2002. Response of oregano *Origanum vulgare L*.clonal lines to *Pseudomonas sp.* Z strain and polydye R-478 and implications for hyperhydricity prevention in tissue culture. *Process Biochemistry*, 353-4:227-235.

Todar's Online Textbook of Bacteriology. Textbookofbacteriology.net .,2004. Retrieved on 2011.

Trombeta ,D; Castelli, F; Sarpietro, M.G; Venuti, V; Cristani, M; Daniele, C; Saija, A; Mazzanti, G;and Bisignano , G.,2005.Mechanisms of antibacterial action of three monoterpenes. *Antim. Agents and Chemoth*, 49:2474-2478.

Viuda-Martos, M; Ruiz-Navajas,Y; Ferandez-Lopez , J.and Anjel Perez- Alvarez,J., 2008. Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *International Journal of Food Science& Technology*., 43 35: 526- 531.

Westh , H., Zinn, C.S; Rosdahl, V.T and Sarisa Study Group.,2004. An international multicancer study of antimicrobial consumption and resistance in *Staphylococcus aureus*isolates from 15 hospitals in 14 countries. *Microbiol. Drug Resist*, 102: 169-176.

Yen, G. C., and Duh, P. D. 1994. Scavenging effect of methanolicextracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agriculture and FoodChemistry*, 42 :629–632.