Antimicrobial Potential of *Origanum vulgare (l.)* against Food Borne Pathogens

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Abstract

All the extracts of *Origanum vulgaris* showed sensitivity against most of the test bacterial cultures and also against microbes isolated from food samples but did not showed good antifungal results against *Aspergillus niger* and *Rhizopus oryzae.* Among all the extracts ethanolic extract showed maximum antimicrobial effect against all the standard bacterial cultures except *Enterococcus faecalis* ATCC 29212. Thus, it *Origanum vulgaris* can be used as potent inhibitor for the growth of microorganisms responsible for food spoilage and infections.

Key word :- Origanum vulgaris extracts ,Antimicobial Potential and Food Borne, Pathogens

INTRODUCTION

The genus *Origanum* (oregano) is significant in the family Lamiaceae and comprises of around 900 species of annual, perennial and shrubby herbs, widespread throughout the world (Bayder *et al*., 2004; Kordali *et al*., 2008). The genus includes some important culinary herbs, including Turkish wild oregano (*O. vulgare*) and sweet marjoram (*O. majorana* L), commercially available and exportable plants with appreciable market values (Baytop, 1999**;** Esen *et al*., 2007). *Origanum* plants are extensively used for the flavoring of alcoholic beverages, food products and in perfumery due to their spicy fragrance (Olivier, 1994**;** Filippo-D-Antuono *et al*., 2000). Besides their commercial importance, such plants have been used, for long, as condiments and spices for foods like salads, soups, sausages and meats (Baydar *et al*., 2004; Sagdic and Ozcan, 2004). Their use for the treatment of various diseases was also in practice, being sudorific, expectorant, stomachic, antiseptic, stimulant, and emmenagogic (Ozcan, 1998). Both academia and the food industry have been interested in the biological properties of *Origanum* extracts and essential oils due to their antimicrobial and antioxidant potential (Dorman and Deans, 2000; Aligiannis *et al*., 2001; Ozcan and Erkmen, 2001; Sagdic and Ozcan, 2004). Antimicrobial activity of *Origanum marjorana* has been observed against a wde range of microorganisms including bacterial and fungal strains. Nisha *et al*., 2010, showed the antimicrobial potential of different extracts of *Origanum marjorana* against *E. Coli*, *Bacillus subtilis*, *Mucor hiemetis* and *Aspergillus niger. (*Ashraf *et al*., 2011) showed that the constituents contained in Chloroform extract of Origanum vulgare 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.

MATERIALS AND METHODS

Herbal Materials

The leaves *Origanum vulgare (L.)* are used as herbal material for present study. These materials were collected from local area and place of work was Department of Microbiology, School of life science, Singhania University. Fig 3.1 shows the parts of plants used for the antimicrobial screening.

Bacterial Culture

Bacterial strains collected from culture banks

Ten bacterial strains (6 Gram neagtive and 4 Gram positive) and 2 fungal strains for the present work were collected from American type culture collection (ATCC), in the form of lyophilized culture. The cultures used were *Escherichia coli* ATCC11840*, Escherichia coli* ATCC 12632*, Pseudomonas flourescens* ATCC 13525*, Klebsiella pneumoniae* ATCC 9621*, Klebsiella aerogenes* ATCC 31488*, Proteus mirabilis* ATCC 29245*, Streptococcus lactis* ATCC 8043*, Staphylococcus aureus* ATCC 12600*, Bacillus cereus* ATCC 12826*, Enterococcus faecalis* ATCC 29212*, Aspergillus niger* ATCC 26603 and *Rhizopus oryzae* ATCC 8993.These strains used as standard test microorganism.

Bacterial strains isolated from food samples

Food sample collected from University canteen and road side was used for isolation. From each sample 25 g was aseptically weighed and macerated and 225 ml of sterile distilled water was added and shake for 1hr in incubator shaker at 37^0C. Serial dilution was carried out using sterile distilled water as diluents. From each dilution 50 µl was spreaded using the spread plate methods. Enriched sample were then streaked on nutrient agar media. Isolated colonies were subcultured on independent plates for further morphological and biochemical screening of microorganisms. The following Bacteria are isolated and identified from food samples. These strains used as isolated test microorganism.

1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Bacillus cereus*4. *Pseudomonas aeruginosa* 5. *Salmonella sp*

Preparation of herbal plants

All samples were washed in 50 µg/mL hypochlorite solution, sliced and air-dried at 50°C in a hot air oven. The final moisture content determined by gravimetrically method was 5-8% (dry basis). Dried samples were ground to powder using a mechanical grinder, and kept separately in air tight containers in dry condition until use.

Preparation of extracts

Because of the limitation of solubility of fine particles of the spices, the highest preparable concentration was 500 µg/ml.

Hot Water Extraction

30 g of the sample were batch extracted by dissolving it in 100 ml boiled distilled water, and shaken for 30 minutes at 100°C in a shaking water bath. Crude extracts were centrifuged at 10000×g for 30 minutes and then vacuum filtered using a 47 mm glass microfibre filter to obtain the clear extracts. The extracts were filtered again using 0.45 µm filter to obtain the sterile extracts. The hot water extracts were kept in sterile glass bottle and stored frozen at -40°C

Solvent Extraction

Thirty Gram of shade dried, powder of plant materials were filled separately in the thimble and extracted successively with 150 ml of ethanol, methanol, chloroform and ethyl acetate separately using a Soxhlet extractor for 4-8 h. Extract was concentrated using vaccum rotary evaporator and frozen at -80°C before freeze drying. After complete
solvent evaporation, solvent extract was weighed and preserved at 4^{o}C in airtight bottles

until further use. Stock solutions of solvent extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 500 µg/ml

Determination of the Antimicrobial Activity

Preparation of the Inoculums

For the preparation of the inocula, test organisms were taken from the stock culture and were grown separately in 50 ml Trypticase Soy Broth and incubated at 37°C for 24 hours on an orbital shaker at 200 rpm. Each test organism suspension was subsequently streaked out on Trypticase Soy Agar and incubated at 37°C for 24 hours. A single colony was transferred to Trypticase Soy Agar slants and incubated at 37 °C for 24 hours. These stock cultures were kept at 4°C. For use in experiments a loop of each test organism was transferred in 50 ml Trypticase Soy Broth, and incubated separately at 37°C for 18-20 hours. This culture was used for the antibacterial assays.

Determination of Zone of Inhibition

The antimicrobial assay was performed by agar well diffusion method for extracts. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^{8} cfu/ml) and poured into the Petri plate (Hi-media). For agar well diffusion method, wells were prepared in the plates with the help of a cork-borer (0.85 cm). 25 µl, 50 µl, 75 µl and 100
µl of the test compound were introduced into separate wells along with controls. The plates were incubated overnight at 37^{0}C. Microbial growth was determined by

measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented. Media are made up to the final concentration is used in the usual methods

of microbiological assay, but with addition of 1.5 percent of agar. During preparation of the plates strict asepsis is unnecessary; normal cleanliness and the use of oven dried apparatus have so far avoided all trouble from contaminating organisms and it has also been found unnecessary to sterilize either test or standard solution.

Preservative agent

The standard preservative used in the present study was benzoic acid (1%) 20 µl quantity.

RESULT AND DISCUSSION

Antimicrobial activity of aqueous extract of Origanum vulgare (L.)

The aqueous extract showed no inhibition against any bacterial strain but it showed zone of inhibition of 14 mm and 13 mm diameter for *Aspergillus niger ATCC 26603* and *Rhizopus oryzae ATCC 8993* respectively (Table 1, Table 2).

Antimicrobial activity of methanolic extract of Origanum vulgare (L.)

No zone of inhibition was observed for methanolic extract of *Origanum vulgare* (L.)against *Proteus mirabilis* ATCC 29245. Maximum inhibition was 25 mm against *S. aureus* ATCC 12600 (Fig 4.30). High zone of inhibition was also observed against *E. coli* ATCC 12632, *P. flourescens* ATCC 13525, *E. faecalis* ATCC 29212, *E. coli* ATCC 11840, *Klebsiella pneumonia* ATCC 31488, *S. lactis* ATCC 8043 (24 mm, 22 mm, 21 mm, 21 mm, 20 mm, 20 mm respectively). Moderate zone of inhibition was observed against *Rhizopus oryzae ATCC 8993* (13 mm) (Table 1).

High antimicrobial potential was observed against *S. aureus* and *B. cereus* (25 mm and 20 m) (Table 2). Medium zone of inhibition of 15 mm, 13 mm and 11 mm was observed against *E. coli* (isolated from rice), *P. aeruginosa* (isolated from burger) and *E. coli* (isolated from burger) respectively. This extract *of Origanum vulgare (L.)*was not sensitive for *Salmonella sp* isolated from food sample (Table 2).

Antimicrobial activity of ethanolic extract of Origanum vulgare (L.)

Maximum zone of inhibition observed for ethanolic extract of *Origanum vulgare (L.)* against microorganisms was 23 mm against *S. sureus* ATCC 12600 and *Bacillus cereus* ATCC 12826. Near to it *E. coli* ATCC 11840, *E. coli* ATCC 12632, *Klebsiella pneumoniae* ATCC 31488*, Klebsiella aerogenes* ATCC 9621, *Streptococcus lactis* ATCC 8043 also showed good sensitivity against the ethanolic extract of *Origanum vulgare (L.)* (19 mm, 22 mm, 19 mm, 19 mm, 21 mm respectively). No sensitivity was observed against *P. flourescens* ATCC 13525 and *Proteus mirabilis* ATCC 29245 (Table 1). Moderate antifungal activity was also observed against *Aspergillus niger* ATCC 26603, *Rhizopus oryzae* ATCC 8993 (15 mm and 12 mm) (Table 1).

The antimicrobial potential of *Origanum vulgare (L.)* was also observed against both standard cultures and microorganisms isolated from food samples. It was seen that *Proteus mirabilis* ATCC 29245 was resistant for all the extracts *of Origanum vulgare (L.).* This study is also supported by the work done by Ashraf *et al*. (2011), who showed that the methanolic, chloroform and aqueous extract of *Origanum vulgare (L.)* is not sensitive for *Proteus mirabilis.* The aqueous extract of *Origanum spp.* did not showed any antibacterial activity against any of the test bacterial strains (Nisha *et al*., 2010). It was also observed by Asraf *et al*. (2011) that the aqueous extract of *Origanum spp.* was not antimicrobial against *Pseudomonas aeruginosa, E. coli, Bacillus subtilis, Proteus mirabilis, Salmonella typhi* and *Staphylococcus aureus*. Though, it showed no activity against *Shigella flexneri*, *Salmonella paratyphi* and *Micrococcus luteus*. In contrast to these results in our experiment no activity was observed against *Salmonella sp.* isolated from food samples. Asraf *et al*. (2011) also showed antifungal potential of aqueous extract of *Origanum spp*. Our study supports this observation and shows antifungal activity of this extract against *Aspergillus niger and* Rhizopus oryzae.

The methanolic extract of *Origanum vulgare (L.)* showed high antimicrobial potential against almost all the bacterial strains used having highest zone of inhibition for *Staphylococcus aureus* ATCC 12600 (Ashraf *et al*., 2011). No inhibition was there for *Salmonella sp.* isolated from food sample. Antifungal activity was also observed for only *Rhizopus oryzae*.

The effect of ethanol extract as antimicrobial was efficient on all the investigated microorganisms. This result are in general within with the many studies which reported that, the marjoram ethanol extract can be used as an effective herbal against different pathogenic bacteria and fungi (Leeja *et al* 2007). It was also seen that with the increasing of marjoram ethanol extract concentration, the diameter of clear zone for all tested pathogens had significant also increased. The ethanolic extract of *Origanum vulgare (L.)* showed no inhibitory effect on *Pseudomonas flourescens* ATCC 13525. *Origanum vulgare (L.)* showed a strong growth inhibition effect against *E. coli* ATCC 12632, *E. coli* ATCC 11840, *Streptococcus lactis* ATCC 8043, *Klebsiella pneumoniae* ATCC 31488, *Klebsiella aerogenes* ATCC 9621, *S. aureus* ATCC 12600, *B. cereus* ATCC 12826, *Enterococcus faecalis* ATCC 29212 , *Aspergillus niger* and *Rhizopus oryzae*. This observation is completely concurrent with the observation confirmed by Bonjar, (2004), Mohamed *et al*. (2011),Verma *et al* (2013*)* Penalver *et al*. (2005) . The antimicrobial activity of ethanolic extract of *Origanum vulgare (L.)* is also supported by Nisha *et al*. (2010). Except *Salmonella* sp and *Pseudomonas aeruginosa* it was seen that this extract possess good antimicrobial potential against other bacterial strains isolated from food samples.The findings suggests that the chloroform extract of *Origanum vulgare (L.)*does not possess antimicrobial activity against *E. coli* ATCC 11840, *E. coli* ATCC 12632 and *S.aureus* ATCC 12600 (Ashraf *et al*., 2011). In contrast to these results Nisha *et al*. (2010), showed that the chloroform extract of *Origanum vulgare (L.)* was sensitive for *E. coli.* No antimicrobial activity was observed for ethyl acetate extract of *Origanum vulgare (L.).*

Against microbes isolated from food samples, this extract proved a moderate

antimicrobial agent showing zone of inhibitions in the range of 16 mm to 11 mm. No sensitivity was observed against *Pseudomonas aeruginosa* and *Salmonella sp* (Table 2).

Antimicrobial activity of Chloroform extract of Origanum vulgare (L.)

The chloroform extract of *Origanum vulgare (L.)* showed zone of inhibitions in the range of 20 mm to 18 mm against microorganisms. No zone of inhibition was there in case of *E. coli* ATCC 11840, *E. coli* ATCC 12632, *Proteus mirabilis* ATCC 29246, *S. aureus* ATCC 12600 (Table 1).

This extract showed excellent antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella sp.* isolated from food samples (30 mm and 24 mm). Zone of inhibition of 16 mm and 15 mm was observed against *S. aureus* and *B. cereus*. *E. coli* was found to be resistant for this extract (Table 2).

Zone of inhibition of 18 mm and 10 mm was observed against *Aspergillus niger* ATCC 26603 and *Rhizopus oryzae* ATCC 8993 when treated with the chloroform extract of ***Origanum vulgare (L.)*** (Table 1).

Antimicrobial activity of Ethyl acetate extract of Origanum vulgare (L.)

No antimicrobial activity was observed by ethyl acetate extract of **Origanum vulgare (L.)** against any test microorganisms (Table 1, Table 2).

Table 1 Antimicrobial activity of *Origanum vulgare (L.)*showing Zone of Inhibition against microorganisms

S.No	Solvent	Conc (µl)	*E. coli* ATCC 11840	*E. coli* ATCC 12632	*P. flourescens* ATCC 13525	*K. pneumonia* ATCC 31488	*K. aerogenes* ATCC 9621	*P. mirabilis* ATCC 29245	*S. lactis* ATCC 8043	*S. aureus* ATCC 12600	*B. cereus* ATCC 1 826	*E. faecalis* ATCC 29212	*A. niger* ATCC 26603	*R. oryzae* ATCC 8993
1.	Aqueous	25	-	-	-	-	-	-	-	-	-	-	6	7
		50	-	-	-	-	-	-	-	-	-	-	8	10
		75	-	-	-	-	-	-	-	-	-	-	12	11
		100	-	-	-	-	-	-	-	-	-	-	14	13
2.	Methanol	25	13	15	12	9	9	-	11	16	8	11	-	5
		50	16	17	15	13	12	-	14	18	10	14	-	10
		75	19	20	18	17	15	-	17	22	14	18	-	13
		100	21	24	22	20	17	-	20	25	17	21	-	13
3.	Ethanol	25	11	16	-	10	11	-	14	14	15	6	10	6
		50	14	19	-	14	14	-	17	17	18	9	12	9
		75	17	21	-	17	16	-	19	20	21	10	14	12
		100	19	22	-	19	19	-	21	23	23	14	15	12
4.	Chlorofor m	25	-	-	9	11	7	-	10	-	11	9	11	5
		50	-	-	12	13	10	-	12	-	13	13	14	7
		75	-	-	18	15	15	-	16	-	16	17	18	10
		100	-	-	20	18	19	-	19	-	19	19	18	10
5.	Ethyl	25	-	-	-	-	-	-	-	-	-	-	-	-
		50	-	-	-	-	-	-	-	-	-	-	-	-
	acetate	75	-	-	-	-	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-	-	-	-	-
6.	10 % DMSO	20	-	-	-	-	-	-	-	-	-	-	-	-
7.	1 % Benzoic acid	20	14	14	13	12	12	13	14	15	16	15	12	12

Table 2 Antimicrobial activity of *Origanum vulgare (L.)* showing Zone of Inhibition against microorganisms isolated from food samples

S.No	Solvent	Conc (µl)	Bacteria isolated from Rice			Bacteria isolated from Burger		
			Escherichia coli	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella sp*
1.	Aqueous	25	-	-	-	-	-	-
		50	-	-	-	-	-	-
		75	-	-	-	-	-	-
		100	-	-	-	-	-	-
2.	Methanol	25	10	15	20	7	9	-
		50	12	17	23	8	11	-
		75	13	19	24	10	12	-
		100	15	20	25	11	13	-
3.	Ethanol	25	9	7	10	10	-	-
		50	12	8	12	11	-	-
		75	13	10	15	13	-	-
		100	15	11	16	14	-	-
4.	Chloroform	25	-	10	13	-	21	17
		50	-	12	15	-	24	21
		75	-	13	16	-	29	23
		100	-	15	16	-	30	24
5.	Ethyl acetate	25	-	-	-	-	-	-
		50	-	-	-	-	-	-
		75	-	-	-	-	-	-
		100	-	-	-	-	-	-
6.	10% DMSO	20	-	-	-	-	-	-
7.	1% Benzoic acid	20	21	19	19	22	18	18

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