Haryana Vet. (June, 2023) **62(1),** 101-105 ISSN 0033-4359 (P) **Research Article**

IN-VITRO EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM OREGANO (*ORIGANUM VULGARE*) AND LIME (*CITRUS AURANTIFOLIA*)

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Received: 12.08.2022; Accepted: 19.12.2022

ABSTRACT

The present study was envisaged to investigate *in-vitro* antimicrobial and antioxidant efficacy of oregano and lime essential oils for their possible application in food products. Antimicrobial potential of oils was measured by using zone of inhibition assay and Minimum Inhibitory Concentration (MIC) against foodborne pathogens including Gram positive and Gram negative organism whereas, antioxidant assay was performed using DPPH and ABTS radical scavenging activity. During evaluation of antimicrobial activity, it was found that both essential oils were effective against gram positive and gram negative organisms used in the study. The result of MIC values of oregano oil ranged from 300-3000 ppm whereas for lime oil it ranged from 1000 to 5000 ppm concentration. The *in-vitro* antioxidant activity was assessed using ABTS and DPPH radical scavenging activity of both the oils at five different concentrations ranged from 1000 to 10000 ppm. The DPPH values ranged between 37.96-78.46%, ranged to 29.46- 62.17%, whereas ABTS values ranged beetween 34.01-73.24%, 31.23-64.32% for oregano and lime oil, respectively at measured concentrations. It is concluded from the research that oregano and lime oils possess potent antimicrobial and antioxidant activity and can be used as a natural preservative in food products.

Keywords: ABTS, antimicrobial, DPPH, Lime, MIC, Oregano, Zone of inhibition

How to cite: Kumar, D., Mehta, N., Panwar, H., Chatli, M.K. and Malav, O.P. (2023). *In-vitro* evaluation of antibacterial and antioxidant activity of essential oils from oregano (*Origanum vulgare*) and lime (*Citrus aurantifolia*). *Haryana Vet.* **62(1)**: 101-105.

Due to harmful effect caused by chemical preservative there is paradigm shift of consumer preference towards natural preservative in food, which are safe and approved for human consumption (Alves-Silva *et al.*, 2013). Essential oils (EOs) can be a potential viable alternative to chemical preservative and it has been widely used these days in the nutritional, pharmaceutical, and agricultural industry. (Burt 2004 and Prabuseenivasan *et al.*, 2006). Essential oils (EOs) are extracted from plants and spices exhibit potent antimicrobial and antioxidant properties (Kumar *et al.*, 2017; Viuda-Martos *et al.*, 2010), which makes them interesting additives in food industry. In addition, most of them are classified as, Generally Recognized as Safe (GRAS). Oregano (origanum vulgare) essential oil is quite popular herb extracts that can be used as a meat preservative. Oregano (Origanum vulgare) EO shows broad-spectrum antimicrobial activity against most of food spoilage microorganisms (Bakkali *et al.*, 2008; Nedorostova *et al.*, 2009). Among the components which exhibit antimicrobial activity are carvacrol, thymol and -terpinene (Burt, 2004). It has been observed that thymol has an inhibitory activity against a wide range of bacteria, including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium*, whereas carvacrol is effective against pathogenic bacteria such as *S. typhimurium*, *E. coli*, *L. monocytogenes* and *Bacillus cereus* (Gaysinsky *et al.*, 2007). However, despite of high potential of oregano EO as natural antimicrobial agent in food, its application is limited because of its strong flavor and odor, which could adversely affect the organoleptic properties of the product (Hayouni *et al.*, 2008). These two major phenols *viz*. carvacrol and thymol, constitute about 78-82% of oregano oil and are primarily responsible for most of the antioxidant activity (Yanishlieva *et al.*, 1999).

Lime essential oil has potential application in food, pharmaceutical and cosmetic industries due to its chemical and sensory characteristics (Rao & McClements, 2012). It is generally used in products such as beverages, baked goods, sweets, desserts and ice cream (Gamarra *et al.*, 2006). Lime essential oil is a complex mixture of different chemical constituents divided mainly into three major classes: terpenes (75%), oxygenated complexes (12%) and sesquiterpenes (3%). Lime essential oil is extremely volatile in nature, and encapsulation strategy possibly, is a viable alternative to deliver slow release of its bioactive compounds. The major constituents responsible for lime oil (Citrus aurantifolia) antimicrobial effects are limonene, -terpineol and -terpinen (Talei and Meshkatalsadat 2007). So, the aim of the research work was to evaluate the antimicrobial and antioxidant potential of oregano and lime essential oils to use them as natural preservative in food and foodproducts.

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MATERIALS AND METHODS

Source of Oregano and Lime essential oils

Steam distillated Oregano oil was purchased from Zane Hellas in UK. As per specifications provided by supplier, essential oil of oregano was Greek in origin and was having more than 89% of Carvacrol and Less than <2% Thymol, whereas cold pressed Lime oil was purchased from Naissance, originated in Peru. Various physiochemical properties of oregano and lime essential oil are depicted in Table 1. All the reagents and chemical used in the study were of analytical grade.

Bacterial strains and growth conditions

Nine pure freeze dried cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India *viz*. *Escherichia coli* (MTCC 2991), *Salmonella enterica* (MTCC 3231), *Listeria monocytogenes* (MTCC 657) and *Bacillus cereus* (MTCC 6728) *Staphylococcus aureus* (MTCC 7443), *Shigella flexneri*, *Yersinia enterolitica* (MTCC 3238), *Pseudomonas aeruginosa* (MTCC 9544), *Vibrio parahaemolyticus* (MTCC 451), These cultures were rejuvenated and stock cultures were made and are being maintained in the department by regular passaging.

Antimicrobial activity estimation

Antimicrobial potential of oregano and lime essential oil was tested against nine entero-pathogenic bacterial cultures which were cultured into fresh broth media and brought into log phase of growth by incubating them at 37° C for 24 hours before being tested. The antibacterial efficacy was evaluated using well diffusion method. Petri plates were decanted with sterilized nutrient agar and left untouched for 36 hours and thereafter wells of 10 mm diameter were bored in the agar plates using sterile cork borer. The plates were inoculated with premeasured 100 l of each bacterial cultures by spread plating. Then 100 l of oregano and lime oil was poured into each of the wells and the plates were again incubated at 37° C for 24 hours. The petri plates were observed for any appearance of zones of bacterial growth inhibition around the wells containing oregano and lime essential oil. Diameters (mm) of these zones were measured and are depicted in Fig. 1 and 2, respectively.

Minimum inhibitory concentration (MIC)

Identical concentrations of log phase bacterial cultures were set by adjusting their absorbance at 600 nm. Concentrations of Oregano and Lime essential oil were adjusted with the help of DMSO. In the Microtiter plates, 100 l of each bacterial culture were added into 30 l of oil dilution and 170 l of nutrient broth. The plates were preserved for incubation at 37° C for 24 hours. Absorbance of samples were evaluated at 600 nm to observe growth inhibition. The inhibition in growth was also confirmed by streaking the samples on nutrient agar plates and observing for any bacterial growth after 24 h of incubation at 37° C.

Table 1. Physiochemical properties of Oregano and Lime essential oil

Physico chemical properties	Oregano Oil	Lime Oil
1. Specific Gravity	0.9350-0.9650	0.845 - 0.875
2. Refractive Index	1.5015-1.5155	1.470 - 1.490
3. Main constituent by GC	Carvacrol 89.36% +	Limonene 57.70% +

Table 2. Minimal Inhibitory Concentration (ppm) of Oregano and lime essential oils against nine food spoilage microorganisms

Sr. No.	Test Microorganisms	MIC of Oregano Oil (ppm)	MIC of Lime Oil (ppm)
1.	Staphylococcus aureus	1000	5000
2.	Bacillus cereus	700	1000
3.	Escherichia coli	3000	5000
4.	Shigella flexineri	500	3000
5.	Pseudomonas aeruginosa	3000	5000
6.	Listeria monocytogenes	300	3000
7.	Yersinia enterocolitica	300	3000
8.	Salmonella enteric	1000	5000
9.	Vibrio parahaemolyticus	700	5000

Table 3. DPPH and ABTS Radical Scavenging Potential of Oregano essential oils (Mean±S.E.)*

Conc. (ppm)	DPPH (% Radical Scavenging Activity)	ABTS (% Radical Scavenging Activity)
1000	37.96±0.25	34.01±0.24
2000	55.46±0.35	51.23±0.28
3000	66.27±0.45	55.24±0.32
5000	72.23±0.37	66.17±0.62
10000	78.46±0.39	73.24±0.42

Table 4. DPPH and ABTS Radical Scavenging Potential of Lime essential oils (Mean±S.E.)

Conc. (ppm)	DPPH (% Radical Scavenging Activity)	ABTS (% Radical Scavenging Activity)
1000	29.46±0.23	31.23±0.42
2000	39.17±0.27	43.17±0.39
3000	48.17±0.31	52.43±0.52
5000	56.14±0.64	60.14±0.47
10000	62.17±0.39	64.32±0.43

Antioxidant activity assay

The antioxidant activity of both oils was measured using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) and 2, 2[1]-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS)

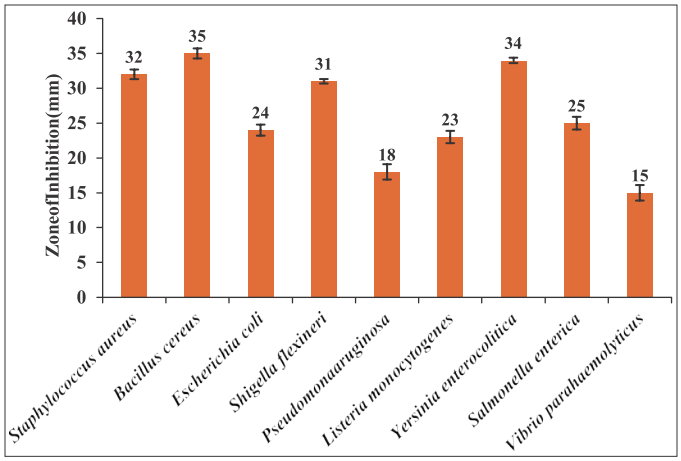

Fig. 1. Zone of Inhibition (mm) of Oregano oil against food spoilage microorganisms

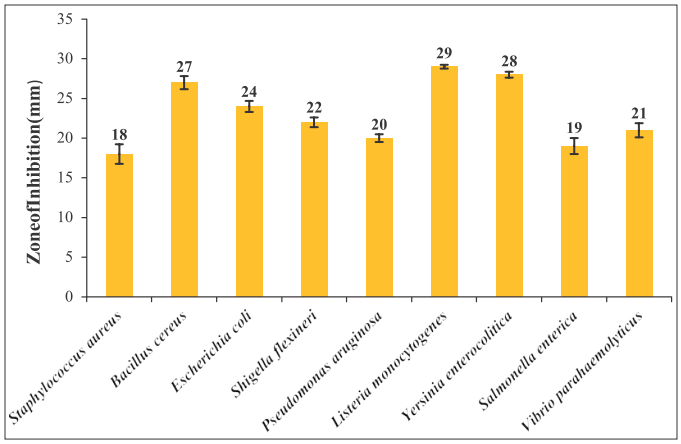

Fig. 2. Zone of Inhibition (mm) of Lime oil against food spoilage microorganisms

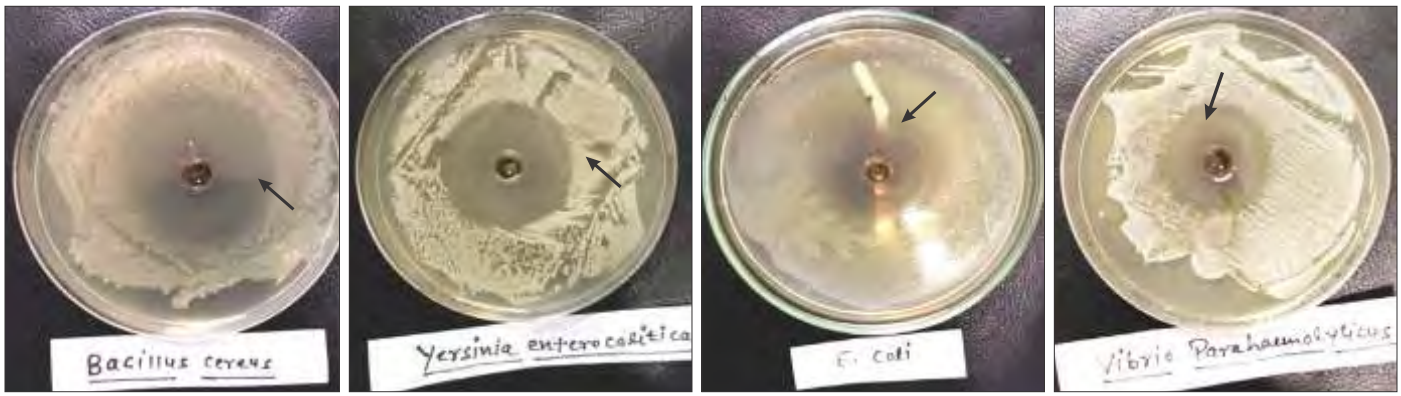

Fig. 3. Zone of inhibition of Oregano oil against (A) *Bacillus cereus* (B) Yersinia enterocolitica and Lime oil (C) Listeria monocytogenes (D) Vibrio parahaemolyticus food spoilage organism

radical scavenging assay. DPPH radical scavenging activity of Oregano and Lime was evaluated using a methanolic solution of the "stable" free radical, DPPH•. The Blois method (1958) was used for studying the effect of various oil concentrations on DPPH• radicals with slight modifications. A solution of DPPH• in methanol (0.15 mmol/L) was prepared. Different oil concentrations were diluted in methanol, thereafter 200 l of each dilution was mixed with 50 l of DPPH• solution in a 96-well microtiter plate. The mixture was allowed to stay at room temperature in dark for 30 min. The radical scavenging activity was measured as a decrease in absorbance of DPPH at 517 nm.

ABTS cation decolorization assay was performed on various concentrations of oregano and lime essesntial oil diluted in methanol. ABTS radical cation was freshly prepared by mixing 14 mM ABTS with equal volume of 4.95 mM potassium persulphate andmixture was kept for 24 hours at room temperature. The ABTS radical cation was used for the assay after dilution with Phosphate Buffer Saline (PBS) appropriately. To 50 l of various concentrations of the oils, 150 l of ABTS solution was added. After 1 min incubation at room temperature, change in absorbance was measured at 732 nm. The cation scavenging activity was measured same as with DPPH. The antioxidant activity was calculated as a percentage of inhibition according to the following equation:

$$\% \text{ Radical Inhibition} = \frac{\{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}\}} \times 100$$

Statistical analysis

Data was statistically analysed using 'SPSS-16.0' (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran, 1994). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have total six number of observations (n=6). The mean values were reported along with standard error. The statistical significance was estimated at 5% level ($P<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Antimicrobial activity and MIC of essential oils from Oregano and Lime

Antimicrobial activity was performed for oregano

and lime essential oil by well diffusion method against nine foodborne pathogens. The Results obtained for MIC (ppm) of both oils against the above mentioned organisms is presented in Table 2.

The MIC value (ppm) of oregano oil ranged between 300-3000 and it was found that oil was effective against both Gram Positive and Gram Negative organisms used in the study. Gutierrez *et al.* (2018) tested antimicrobial activity of various commercial essential oils against food borne pathogens and spoilage organisms associated with ready to eat vegetables and reported that in general, *Origanum vulgare* essential oil showed strong efficacy against Gram-positive bacteria than gram negative bacteria. These findings can be correlated to the fact that Gram-negative bacteria have an complex outer membrane which is rigid and rich in lipopolysaccharide (LPS), thereby limiting the diffusion of hydrophobic compounds through it, while this extra complex membrane is absent in Gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not rigid enough to resist small antimicrobial molecules, diffusion across the cell membrane. Similar finding has also been reported by (Zinoviadou *et al.*, 2009; and Bajera *et al.*, 2017). For oregano oil Maximum inhibition zone diameter was observed for *Bacillus cereus* (35±0.72) and minimum for *Vibrio Parahaemolyticus* (15±1.11). The results are in concordance with Chouhan *et al.* (2017), also reported that effectiveness of oregano oil is comparatively more for inhibition of Gram positive organisms than gram negative.

The Results obtained for MIC (ppm) of lime oil against the tested organisms is depicted in Table 2. MIC (ppm) of lime oil against targeted organisms was found in the range of 1000-5000. As observed, lime essential oil was effective against both Gram positive as well as Gram negative organisms. However, the value for Gram negative organisms was slightly higher than Gram positive. Similar results have been reported by Prabuseenivasan *et al.* (2006) who determined the antibacterial activity of lime oil against four gram-negative bacteria (*Scherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and two gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and found that tested lime oil was effective in inhibiting all the bacterial isolates. The major components of lime essential oil proved to be - pinene (12.6%), limonene (53.8%), - terpinene (16.5%), terpinolene (0.6%), -terpineol (0.4%) and citral (2.5%), which are very likely responsible for the good antimicrobial activity, in particular on Gram-positive bacteria (Costa *et al.*, 2014).The results of zone inhibition assay reveal that the maximum diameter was observed for Listeria monocytogenes (29±0.24) followed by Yersinia enterocolitica (28±0.37). A good correlation between MIC values and diameter of zones was observed.

The results for antioxidant ability (DPPH and ABTS) of Oregano and Lime essential oils are presented in (Table 3 and 4), respectively. DPPH free radical scavenging ability is because of hydrogen donating ability; the higher the number of hydroxyl groups, more the possibility of free radical scavenging ability (Chen and Ho, 1995). ABTS decolourization assay is a remarkable tool for decisive Antioxidant activity of hydrogen designating antioxidants. DPPH radical scavenging activity of both the oils was measured using method of (Blois, 1958). A solution of DPPH• (0.15 mmol/L) in methanol was used along with five different concentrations of oil (1000, 2000, 3000, 5000 and 10000 ppm). It was observed that there was an incremental trend of radical scavenging ability for ABTS and DPPH assay with increasing concentration of oil. Similar findings were reported by (Kanth *et al.*, 2018; Punya *et al.*, 2019) during study of radical scavenging activity of rosemary and thyme essential oil. The higher radical scavenging activity of these oils could be attributed to presence of active principles i.e. Carvacol and Limonene, respectively.

At 10000 ppm of essential oils concentration, Oregano and Lime oil showed 78.46% and 62.17% of DPPH, and 73.24% and 64.32% of ABTS radical scavenging activity, respectively. On the basis of MIC values, the concentration of Lime oil (0.7%) and Oregano oil (0.3%) that was found effective and corresponding concentration was having 56.14% and 66.27% of DPPH radical scavenging activity and 66.14% and 55.24% of ABTS radical scavenging activity for lime and Oregano Oil, respectively.

CONCLUSION

In the present study, *in-vitro* antimicrobial and antioxidant study of oregano and lime essential oil reveals that they possess potent antioxidant and antimicrobial potential against common food spoilage microorganism, having broad spectrum of activity against both Gram positive and Gram negative organisms. These can be a promising natural alternative to synthetic preservatives having toxic and carcinogenic effects. These results indicate that the oregano and lime essential oil have noteworthy antioxidant and antibacterial action and can be important for their use as food additive in foodstuffs, particularly meat products.

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