Pharmacogn Res. 2022; 14(3):328-332
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Physicochemical Properties and Inhibitory Effects of Oregano Oil against Uropathogenic

Rana Thamer Hadi Alkhafaji*, M. Jayashankar

ABSTRACT

Background: Natural oil is an aromatic by-product of highly volatile plants. It can be easily extracted from a variety of natural sources using a variety of distillation processes due to its volatility. Oregano oil is a substance derived from the oregano plant, also known as Origanum vulgare. **Objectives:** The purpose of this study was to determine the physicochemical properties of oregano oil and to relate them to its chemical composition. Moreover, to examine its antibacterial activity against urinary tract pathogens. **Materials and Methods:** Commercial natural oregano oil (purity ≥98%) was obtained from Bluray food products operating under the brand name Bluray Food Products. The physico-chemical characteristics of oregano oil were determined using different techniques. GC-MS was used to analyze the chemical properties of oregano oil as well as analysis of saponification value, peroxide value, p- Anisidine value, pH value, and organoleptic properties (color, odor, consistency, nature, and solubility) were performed. **Results:** According to the results, GC-MS identified 66 different chemicals in oregano oil, accounting for 100% of the overall composition. It was found that oregano oil has a saponification value of 187.94 mg KOH/g, a peroxide value of 6.22, and a P-Anisidine value of 3.645. In addition, the oil of oregano was found to have inhibitory activity against all uropathogenic bacteria tested. E. coli has an inhibitory zone of 29 mm, *P. aeruginosa* 27 mm, *K. pneumoniae* 20 mm, *P. mirabilis* 22 mm, *E. aerogenes* 21 mm, *E. faecalis* 21-mm, *A. baumannii 22*-mm, *N. gonorrhoeae* 24-mm, *S. aureus* 26 mm, and *S. epidermis* 20-mm. **Conclusion:** Based on these results, it can be concluded that oregano oil was found to have optimal antibacterial activities against all tested urinary pathogenic bacteria.

Keywords: Oregano oil, GC-MS, Antibacterial activity, Physicochemical properties.

Rana Thamer Hadi Alkhafaji*, M. Jayashankar

Department of Studies and Research in Microbiology, Mangalore University, Post Graduate Center, Kodagu, Karnataka, INDIA.

Correspondence

Ms. Rana Thamer Hadi Alkhafaji

Department of Studies and Research in Microbiology, Mangalore University, Post Graduate Center, Kodagu-571232, Karnataka, INDIA.
Email id: ranathamer37@gmail.com

History

- Submission Date: 14-05-2022;
- Review completed: 30-05-2022;
- Accepted Date: 25-06-2022.

DOI : 10.5530/pres.14.3.48

Article Available online
https://www.phcogres.com/v14/i3

INTRODUCTION

The term "natural oil" refers to a biochemical product or group of biochemical products that are produced in the cytoplasmic fluid and are present in the space between cells in the form of minute droplets. It has a strong, highly volatile odor and is composed of aromatic or non-aromatic compounds.[1] Natural oil is an aromatic product from highly volatile plants that, due to its volatility, can be easily extracted from a variety of natural sources using a range of distillation methods, the most common of which is the steam distillation method.[2]

The natural oil is organic, highly concentrated, volatile, soluble in alcohol, colorless, hydrophobic, less viscous than oil, less dense than water, has a watery texture, and is highly aromatic, with specific aromas from the plants that supply those.[3] The chemical properties of natural oils vary greatly depending on temperature, soil conditions, altitude, and country of origin.[4] Oregano oil is a product made from the oregano plant, Origanum vulgare is a common herb. It contains a higher concentration of naturally occurring plant beneficial chemicals.[5] Oregano oil is a natural oil extracted from *O. vulgare* L. It is used worldwide as a raw material for medicinal and health products. Previous studies have indicated that more than 50% of oregano oil is composed of phenolic compounds (mainly carvacrol and thymol). This oil also contains sesquiterpene, terpenes, terpineol alcohol, flavonoids, and other compounds.[6-7] Oregano oil has many properties that deserve further characterization and confirmation. The chemical components of oregano can inhibit the growth of a wide variety of microorganisms in the laboratory. It will be particularly important to characterize the conditions under which its components can combat human infection.[8] The purpose of this study was to determine the physicochemical properties of oregano oil and to relate them to its chemical composition. Moreover, to examine its antibacterial activity against urinary tract pathogens.

MATERIALS AND METHODS

Oregano Oil Sample

Commercial natural oregano oil (purity ≥98%) was obtained from Bluray food products operating under

Cite this article: Alkhafaji RTH, Jayashankar M. Physicochemical Properties and Inhibitory Effects of Oregano Oil against Uropathogenic. Pharmacog Res. 2022;14(3):328-32.

the brand name Bluray Food Products. It was extracted by distillation with water and steam for 6 hr by a Clevenger apparatus at 100°C.

GC-MS Analysis

GC-MS (Thermo Scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000) using column (Agilent DB 5MS (30 meter X 0.25 mm) s) was used to analyze oregano oil. With oregano oil, 10mg was reconstituted in 1000 µL hexane and injected (1 µl) for GC-MS analysis as the stationary phase. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The split ratio was 30:1 and the injected quantity was 1 µL. The program temperature conditions were: the oven temperature was maintained initially at 60°C hold for 5 min ramp at 10°C to 240°C Ramp to 300°C hold for 5 min. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage was adopted at 1823.5 V. The ion source was established at a temperature of 230°C, the maximum temperature was set at 250°C, and the quadrupole rod temperature was employed at 150°C, with the maximum temperature of 200°C. The mass range for this scanning was 50.0- 550.0 amu. All components were identified by matching the recorded mass spectra with the standard mass spectra provided by the NIST11.L database.

Saponification Value

Saponification is the hydrolysis of fats or oils to extract glycerol and the resulting fatty acid salt under simple conditions.[9] 1g of oregano oil was taken into 250ml clear round bottom flask. 10ml of ethanol/ether mixture (2:1v/v) and 25ml of 0.5N alcoholic KOH were added to it. The mixture was refluxed for 1hr. Then the contents were cooled to room temperature. Simultaneously another flask without adding oil was processed as blank. The reaction mixtures were titrated against 0.5N HCL using a 2-3 drops phenolphthalein indicator. From the difference between the titer values of the blank flask and the flask containing oil, the amount of KOH (in mg) that was used by 1g of oil during the reflux reaction was calculated and reported as saponification value.

Peroxide Values

Peroxide value is commonly used as an indicator to measure peroxides in the early stages of oxidation in fats and oils.[10] 500µL sample (450mg) was reacted with a 3ml acetic acid-chloroform solvent mixture. 3 volumes of glacial acetic acid were mixed with two volumes of chloroform). A saturated KI solution (50µL) was added and allowed to stand for 1 min in dark, and then 3ml of water was added. 0.01 N sodium thiosulphate pentahydrate was filled in a 25ml burette. Sodium thiosulphate pentahydrate solution was slowly titrated to the liberated iodine solution, with vigorous shaking until the yellow color was almost gone. Starch solution (50µL) was added as an indicator and titration was continued by shaking vigorously to release all iodine from the CHCI3 layer until the blue color disappears. One blank was conducted with each sample determination.

P- Anisidine Value

Anisidine value (AV) determines the quantity of the aldehyde in an oil or fat, in particular, those that are unsaturated.[11] 500µL of the fat solution was taken in a test tube. Isooctane (500µL) taken in a separate test tube served as blank. Samples and blank were reacted with P-Anisidine reagent (100µL) and mixed thoroughly. Absorbance at 350nm was recorded after 10 min of incubation.

Organoleptic Characterization

Within the scope of essential oil assessment and testing, the term organoleptic refers to using our five senses to observe the physical properties of essential oil: Color, Odour, Consistency, Nature (Viscosity), Solubility, and pH.

Agar Well Diffusion Method

The antibacterial activity of oregano oil was screened against uropathogen isolates by the agar well method described by.[12-14] All the bacterial cell suspensions were prepared and grown on tryptone broth and cultures were incubated for 24 hr at 37°C. The cell suspensions of all the cultures were adjusted to 1-2x 106cells/mL as follows: *E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis, E. aerogenes, E. faecalis, A. baumanni, N. gonorrhoeae, S. aureus,* and *S. epidermis* were inoculated on Soya bean casein digested agar plates 90 mm. Test compounds: sample (20µl), and Standard Ciprofloxacin (20µl) were added to the 5mm well on agar plates. The treated plates with *E. coli, P. aeruginosa, K. pneumoniae, P. mirabilis, E. aerogenes, E. faecalis, A. baumanni, N. gonorrhoeae, S. aureus,* and *S. epidermis* were incubated in an incubator at 37°C for 24 hr. After incubation, the treated plates were observed for a zone of inhibition around the wells as shown in Table 1.

RESULTS

Physicochemical Characteristics of the Oregano Oil

The physicochemical characteristics of oregano oil were determined using different techniques. Analysis of saponification value, peroxide value, p- anisidine value, pH value, and organoleptic properties (color, odor, consistency, nature, and solubility) were performed according to Torres-Alvarez *et al*.

Gas Chromatography - Mass Spectrometry (GC–MS) Analysis

The oregano oil sample was subjected to GC-MS analysis and a total of 66 separated peak compounds were identified in oregano oil accounting for 100% of the whole composition shown in Figure 1. In this study, the mass of the compounds and fragments recorded were matched with the NIST database for the identification of probable compounds present in the oregano oil sample. The most abundant ingredient major content found in oregano oil was (7.76%) Benzene, 1-methyl-2- (1-methyl ethyl). While, (2.56%) 1, 4-Cyclohexadiene, 1-methyl-4- (1-methyl ethyl). As well as (15.26%) 1, 6-Octadien-3-ol, 3, 7-dimethyl. Bicyclo rated (3.17%), [2.2.1] heptane-2-ol, 1, 7, 7-trimethyl-, (1S-endo).While, (22.23%) Phenol, 2-methyl-5-(1-methyl ethyl), (2.33%) Dodecane, 2, 6, 11-trimethyl-, (2.83%) Bicyclo [5.2.0] nonane, 2-methylene-4, 8, 8-trimethyl-4-vinyl-, (2.84%) Octadecanoic acid, 3-[(1-oxododecyl) oxy] -1, 2-propanediol ester, (4.90%) Dodecanoic acid, 1, 2, 3-propane triol ester, (4.17%) Methylenebis (2, 4, 6-triisopropyl phenyl phosphine), and (3.12%) 1, 4-Epoxynaphthalene-1(2H)-methanol, 4, 5, 7-tris (1, 1-dimethyl ethyl)-3, 4-dihydro. Furthermore, some studies have agreed with our results where some studies indicated more than 50% of oregano oil consists of phenolic compounds (primarily carvacrol and thymol). The oregano oil also contains sesquiterpene, terpinene, terpineol alcohol, flavonoids, and other compounds.[6-7]

The results of the physicochemical properties of oregano oil are shown in Table 1. The saponification values of oregano oil revealed 187.94 mg KOH/g, this value is relatively higher than that of beeswax (93 mg KOH/g), which is commonly used for soap making.[15] This justifies that the oregano oil used in this study would be of good use in the production of soap.

Additionally, the peroxide value of oregano oil appears at 6.22. This fact suggests that the oil under study is highly susceptible to rancidity at room temperature. Therefore, the high values contribute to an increase in the rancidity rate.[16]

Table 1: Physicochemical properties of oregano oil.

Sample	Oregano oil				
	Sample weight in (gms)	Blank titration value (ml)	Sample titration valu e (ml)	Blank - Sample (ml)	Values
Characterizations	**Saponification value (mg KOH/g)**				
	1	35	28.3	6.7	187.94
	Peroxide value(mEq/kg)				
	0.45	0.2	3.0	2.8	6.22
	P- Anisidine value				
	0.16	0.302	0.788	0.486	3.645
Characterizations	**Organoleptic properties**				
color	Odor	Consistency	Nature	Solubility	pH
Golden brown	terpene woody	Homogenous	viscous	chloroform	3.52

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	3.881	3.817	4.008	986513	0.62	alpha.-Pinene
2	4.070	4.008	4.133	476300	0.30	Camphene
3	4.509	4.450	4.667	1122763	0.70	beta.-Pinene
4	4.732	4.675	4.775	168232	0.11	alpha.-Phellandrene
5	4.888	4.833	4.925	1491708	0.94	Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-
6	4.980	4.925	5.217	12353958	7.76	Benzene, 1-methyl-2-(1-methylethyl)-
7	5.070	5.050	5.142	252013	0.16	1,4-Methano-1H-Cyclopropa[d]pyridazine, 4.4a,5,5a-tetrahydro-6,6-dimethyl-, (1.alpha.
8	5.438	5.325	5.517	4086682	2.56	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
9	5.847	5.783	5.883	298462	0.19	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-
10	5.959	5.883	6.250	24307221	15.26	1,6-Octadien-3-ol, 3,7-dimethyl-
11	6.663	6.617	6.742	161267	0.10	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-
12	6.960	6.883	7.042	5041646	3.17	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-
13	7.109	7.042	7.225	2682343	1.68	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
14	7.284	7.233	7.342	513980	0.32	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-
15	7.967	7.908	8.008	310603	0.20	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-
16	8.096	8.050	8.175	246080	0.15	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
17	8.225	8.183	8.292	251360	0.15	Tetradecane, 2,6,10-trimethyl-
18	8.453	8.325	8.517	629746	0.40	Heptadecane
19	8.604	8.517	8.675	6347235	3.99	Phenol, 2-methyl-5-(1-methylethyl)-
20	8.746	8.675	8.833	35407565	22.23	Phenol, 2-methyl-5-(1-methylethyl)-
21	8.977	8.892	9.025	2499251	1.57	Phenol, 2,3,5,6-tetramethyl- $$ Durenol $$ Phenol, tetramethyl
22	9.066	9.025	9.375	3715895	2.33	Dodecane, 2,6,11-trimethyl-
23	9.551	9.492	9.617	198795	0.12	10-Heptadecen-8-ynoic acid, methyl ester, (E)-
24	9.681	9.617	9.750	463423	0.29	Phenol, 2,3,5,6-tetramethyl-
25	9.809	9.758	9.917	161313	0.10	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)-
26	10.162	10.075	10.258	176972	0.11	Heneicosane $$ n-Heneicosane $$ Heneicosane
27	10.318	10.258	10.383	195370	0.12	Eicosane
28	10.455	10.383	10.533	4505771	2.83	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-
29	10.704	10.625	10.775	937347	0.59	1H-Cyclopropa[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.b
30	11.152	11.050	11.208	389635	0.24	Tetradecane, 2,6,10-trimethyl-
31	11.262	11.208	11.333	639059	0.40	Eicosane
32	11.389	11.333	11.417	497167	0.31	1H-Cyclopropa[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.al
33	11.471	11.417	11.550	4130839	2.59	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-
34	11.604	11.558	11.642	208352	0.13	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
35	11.690	11.642	11.758	335984	0.21	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
36	11.805	11.758	11.858	163538	0.10	Heptadecane
37	12.399	12.342	12.433	326283	0.20	1H-Cyclopropa[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4
38	12.484	12.433	12.550	1465537	0.92	Caryophyllene oxide
39	12.914	12.858	12.967	160702	0.10	Heneicosane
40	13.100	12.975	13.158	785862	0.49	tau.-Cadinol
41	13.767	13.700	13.825	600528	0.38	Eicosane
42	14.238	14.175	14.292	161765	0.10	Eicosane
43	15.238	15.117	15.300	308552	0.19	Heneicosane
44	15.667	15.517	15.717	226641	0.14	Heneicosane
45	16.020	15.958	16.092	624189	0.39	Eicosane
46	16.154	16.092	16.217	1027522	0.65	Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-
47	16.412	16.292	16.592	983247	0.62	Dibutyl phthalate
48	17.088	16.992	17.150	170577	0.11	Limonen-6-ol, pivalate
49	17.338	17.158	17.392	228721	0.14	Eicosane
50	17.703	17.533	17.767	254461	0.16	Dotriacontane
51	18.063	17.992	18.100	341640	0.21	Octadecane, 1-chloro-
52	18.282	18.225	18.375	173640	0.11	Cycloisolongifolene, 9,10-dehydro-
53	18.461	18.375	18.708	884340	0.56	Hexadecanoic acid, butyl ester
54	19.916	19.800	20.033	527361	0.33	Dotriacontane
55	20.188	20.067	20.350	821883	0.52	Octadecanoic acid, butyl ester
56	24.435	24.325	24.475	376284	0.24	Dodecanoic acid, 1,2,3-propanetriyl ester
57	24.676	24.475	24.800	2256820	1.42	Dodecanoic acid, 1,2,3-propanetriyl ester
58	24.936	24.800	25.075	2871808	1.80	Dodecanoic acid, 1,2,3-propanetriyl ester
59	26.675	26.567	26.783	255931	0.16	Trimethyl(4-tert.-butylphenoxy)silane
60	26.928	26.800	27.000	199898	0.13	1,2,4-Triazol-3-amine, 5-(1,3,5-trimethyl-4-pyrazolyl)amino-
61	27.212	27.133	27.258	205884	0.13	Hexadecanoic acid, 2-[(1-oxotetradecyl)oxy]-1,3-propanediyl ester
62	27.488	27.258	27.567	2768612	1.74	Hexadecanoic acid, 2-[(1-oxotetradecyl)oxy]-1,3-propanediyl ester
63	27.693	27.567	27.817	4516935	2.84	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester
64	28.024	27.817	28.500	7806517	4.90	Dodecanoic acid, 1,2,3-propanetriyl ester
65	29.236	29.133	29.367	6644321	4.17	Methylenebis(2,4,6-triisopropylphenylphosphine)
66	32.322	32.192	32.517	4962863	3.12	1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro-
				159267712	100.00	

Figure 1: Chemical composition of the oregano oil.

The result for the p-anisidine values of oregano oil was shown with an anisidine value of 3.645. Aldehydic compounds in fats and oils react with p-anisidine, in the presence of acetic acid, to form yellowish reaction products. According to the method, the intensity of the yellowish compounds is not related only to the number of aldehydic compounds present, but also to their structure. A double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times, and p-anisidine determines the number of aldehydes (principally 2-alkenals and 2,4-denials) present in oils and fats.

In addition, the results of organoleptic characteristics of the oregano oil obtained (color, odor, flavor, solubility) are analyzed as shown in Table 1.

Antimicrobial Activity

The result of the antibacterial activity of oregano oil was tested by measuring the radius of the inhibition zones (mm) using the agar well diffusion method as a qualitative screening test. The oregano oil had antimicrobial activity against all of the bacteria tested, Figure 1-10. Here, the level of bacterial growth inhibition by *E. coli* has an inhibitory zone of 29 mm, *P. aeruginosa* had an inhibitory zone of 27 mm, *K. pneumoniae* has an inhibitory zone of 20 mm, *P. mirabilis* has an inhibitory zone of 22 mm, *E. aerogenes* has an inhibitory zone of 21 mm, *E. faecalis* has a 21-mm inhibitory zone, *A. baumannii* has a 22-mm inhibitory zone, *N. gonorrhoeae* has a 24-mm inhibitory zone, *S. aureus* has an inhibitory zone of 26 mm, and *S. epidermis* has a 20-mm inhibitory zone. This finding confirms the previous findings which revealed that the antibacterial activity of oregano oil against highly resistant bacterial isolates *E. coli*, *P. mirabilis*, *K. pneumoniae*, and *P. aeruginosa* implicated in urinary tract infections.[17] As well as, researcher found that oregano oil inhibited the growth of *bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus subtilis*, *S. aureus*, *corynebacterium xerosis*, *E. coli*, *K. pneumoniae*, *P. Vulgaris*, and *Mycobacterium smegmatis*, among other gram-positive and gram-negative bacteria.[18]

CONCLUSION

In this study, the natural oil of oregano (Origanum vulgare) was extracted by steam distillation by a Clevenger apparatus at 100°C for 6 hr and obtained from Bluray Food Products operating under the brand name Bluray Food Products. The main compounds of oregano oil were analyzed by GC-MS technique as well as the saponification value, peroxide value, p-anicidin value, pH value, and organoleptic properties (color, odor, consistency, nature, and solubility) were determined. Finally, the oil of oregano was found to have antibacterial activities against *E. coli* has an inhibitory zone of 29 mm, *P. aeruginosa* has an inhibitory zone of 27 mm, *K. pneumoniae* has an inhibitory zone of 20 mm, *P. mirabilis* has an inhibitory zone of 22 mm, *E. aerogenes* has an inhibitory zone of 21 mm, *E. faecalis* has a 21-mm inhibitory zone, *A. baumannii* has a 22-mm inhibitory zone, *N. gonorrhoeae* has a 24-mm inhibitory zone, *S. aureus* has an inhibitory zone of 26 mm, and *S. epidermis* has a 20-mm inhibitory zone. As a result, it can be said that oregano oil may be suitable in medical applications, especially as an antibiotic against harmful microorganisms. Moreover, it is useful in soap making and

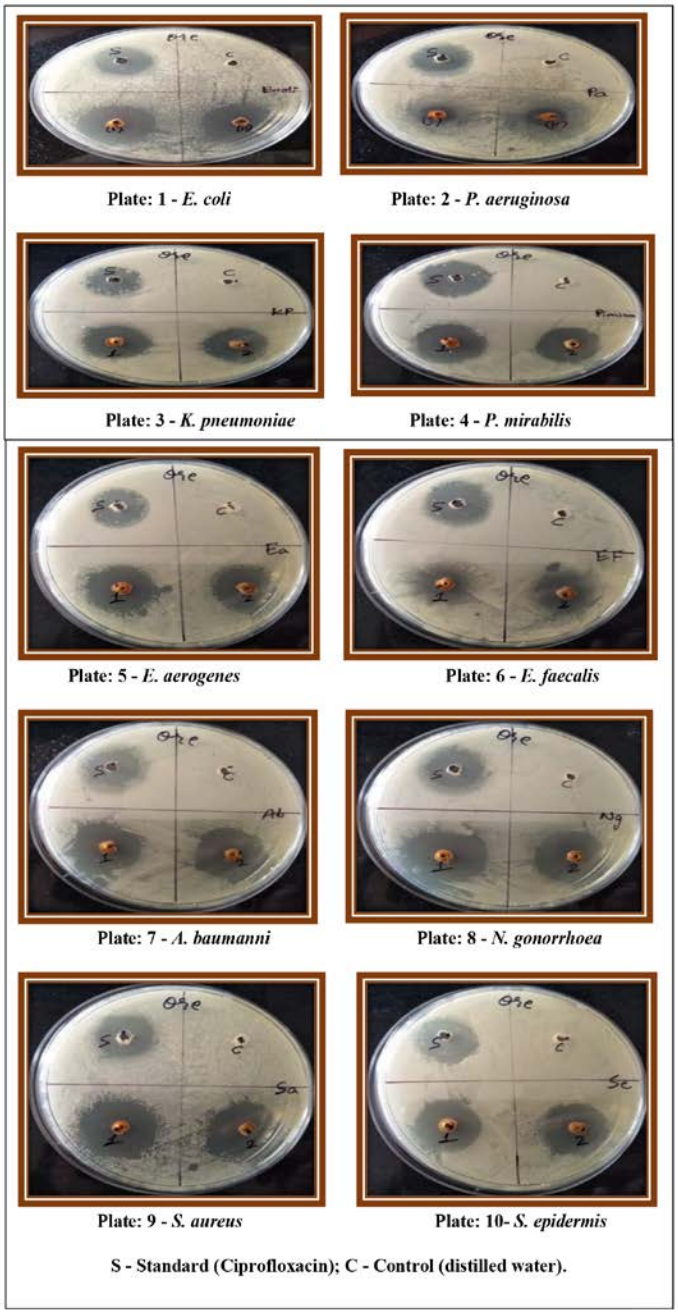

Plate: 1-10: Inhibitory activity of oregano oil against organisms tested.

companies/pharmaceutical industries due to its saponification value of 187.94, peroxide value of 6.22, and p-anicidin value of 3.645.

ACKNOWLEDGEMENT

The authors are grateful to the Research Guide Prof. M. Jayashankar, Department of Studies and Research in Microbiology, Mangalore University, Post Graduate Center, Kodagu, for the guidance and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas chromatography–Mass spectrometry.

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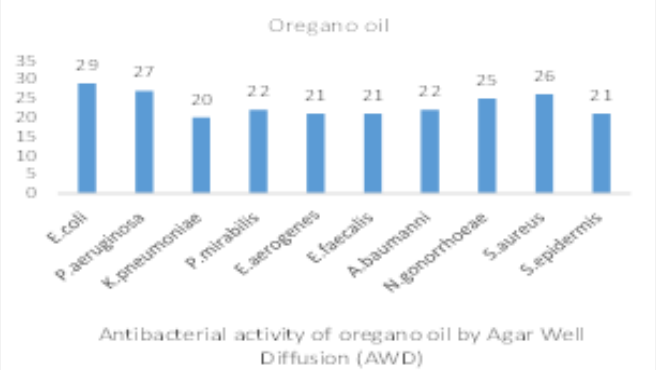
GRAPHICAL ABSTRACT



SUMMARY

This study revealed that the oregano oil evaluated have varying degrees of antibacterial activity. It found oregano oil has moderate antagonistic activity against uropathogen bacteria. It displayed the maximum growth inhibition zone at 29 mm and 27 mm against *E. coli* and *P. aeruginosa*, respectively and minimum zone of growth inhibition of 20mm against *K. pneumoniae*, and 21 mm against *E. aerogenes*, *S. aureus* and *E. faecalis*, respectively. While, GC-MS analysis detected 67 volatile chemical compounds in oregano oil.

Cite this article: Alkhafaji RTH, Jayashankar M. Physicochemical Properties and Inhibitory Effects of Oregano Oil against Uropathogenic. Pharmacog Res. 2022;14(3):328-32.