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Pharmacological Study

Anti-oxidant studies and anti-microbial effect of *Origanum vulgare* Linn in combination with standard antibiotics

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Abstract

Background: *Origanum* is one of the over 200 genera in the Lamiaceae (mint family), and this genus includes culinary, fragrant, and medicinal properties. The plant is reported to contain anti-microbial properties, but it lacks combination studies with that of synthetic antibiotics. **Aim:** To investigate the anti-oxidant and anti-microbial interaction studies of *Origanum vulgare* with standard drugs against *Bacillus* species of bacteria and *Aspergillus niger*. **Materials and Methods:** The anti-oxidant properties of phenolic, non-phenolic fractions of chloroform extract and volatile oil were evaluated by free radical-scavenging, hydrogen peroxide radical-scavenging assay, reducing power, and metal chelating assays. **Results:** The minimum inhibitory concentration and fractional inhibitory concentration index were determined which demonstrates the behavior of volatile oil, phenolic, and non-phenolic fractions of volatile oil with that of ciprofloxacin and fluconazole. The IC_{50} value for volatile oil was found to be 15, 30, and 30 μg/ml and that of phenolic fraction was 60, 120, and 120 μg/ml for free radical-scavenging, hydrogen peroxide-scavenging, and metal chelating assays respectively. Non-phenolic fraction was found to act antagonistically along with ciprofloxacin against *B. cereus* and *B. subtilis*, while the phenolic fraction exhibited indifferent activity along with ciprofloxacin against both the bacterial strains. **Conclusion:** This combination of drug therapy will not only prove effective in antibiotic resistance, but these natural constituents will also help in preventing body from harmful radicals which lead to fatal diseases.

Key words: Anti-oxidants, fractional inhibitory concentration index, free radicals, minimum inhibitory concentration, *Origanum vulgare*

Introduction

The medicinal plants have always played a key role in the maintenance of world health by providing the best source of remedies for a variety of ailments.[1] Infectious diseases are the leading cause of death and disabilities worldwide. Food-borne infections have been one of the major public health concerns and they account for considerably high cases of illness.[2] The numbers of invasive fungal and bacterial infections have dramatically increased in both developed and developing countries.[3] Due to indiscriminate and improper use of antibiotics, most of the clinically important micro-organisms have become resistant to not only single drug but also multidrug therapy.[4] Plants readily synthesize substances for defense against the attack of insects, herbivores, and micro-organisms.[5] The rate of emergence of antibiotic-resistant bacteria is not matched by the rate of development of new antibiotics to combat them.[6] Antibiotics obtained from herbal origin are having fewer side effects than those of synthetic ones and are having great therapeutic potential to heal infections.[7,8] Sometimes, a single antibiotic will not be effective, hence a chemically compatible combination each having antibiotic effect may give the desired effect, and by complex formation which is more effective in inhibition of micro-organism either by causing lyses of cell wall or by inhibiting its formation.[9]

Free radicals cause the oxidation of bio-molecules viz. protein, amino acids, lipid, and DNA which lead to cell injury and death. The use of anti-oxidant-rich diets leads to a limited incidence of cardio and cerebrovascular diseases and protects the body from free radicals.[10] Oxidative reactions limit the shelf life of fresh and processed food stuffs and are a serious concern in the food industry. Synthetic anti-oxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes. However, due to their unstable and highly volatile nature, safety, stability, and

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carcinogenicity still are the issues regarding the use of synthetic antibiotics in food industry as well as healthcare industry.[11] Natural anti-oxidants are not only safe but also effective. Hence, there is need of identification of new sources of anti-oxidants, especially of plant origin.

The volatile oil obtained from the aerial part of *Origanum vulgare* (Linn.) has shown activity against different fungal, yeast, viral, and bacterial species.[12,13]

The present work aimed at studying the *in-vitro* anti-oxidant effects and nature of interaction between phenolic, non-phenolic fractions of chloroform extract and volatile oil isolated from *O. vulgare* with that of synthetic anti-microbials.

Materials and Methods

Biological materials and chemicals

The freeze-dried leaves of *O. vulgare* belonging to family Lamiaceae were procured from Aum Agreefresh Pvt. Ltd., Vadodara, Gujarat, India and were identified by the same company. The specimen voucher was deposited in the Pharmacognosy section of Department of Pharmaceutical Sciences, with voucher no. Pg 11/06.

The micro-organisms used for the anti-microbial studies of volatile oil and extract were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The bacterial strains used were *B. cereus* MTCC 6728 and *B. subtilis* MTCC 121 and fungal strain used was *A. niger* MTCC 404. The media used for the growth and maintenance of micro-organisms were nutrient agar (NA), for bacteria, Potato Dextrose Agar (PDA) for fungi (Hi-media, Mumbai, India). The organic solvents used for the extraction and fractionation of plant metabolites were of analytical grade.

Phosphate buffer was made from monosodium and disodium phosphate (Sigma-Aldrich, Mumbai, India). All other chemicals used like tris HCl, Di-Phenyl Picryl Hydrazyl (DPPH), Ferrozine, BHT, gallic acid, ascorbic acid, Ethylene Di-amine Tetra Acetic Acid (EDTA) in this study were of Hi Media.

Preparation of extracts

500 g of leaves of the drug was placed in a closed flask with chloroform and after 24 h, filtered and concentrated in a rotary vacuum to yield 12.5 g of paste-like extract.[14]

In order to separate the phenolic from non-phenolic fraction of the chloroform extract, a liquid-liquid extraction was done. In a separatory funnel, 2 g of the extract was diluted in 40 ml of chloroform and washed three times with 120 ml of 0.1 N sodium hydroxide. The chloroform phase was separated and concentrated to obtain the crude non-phenolic fraction. To further purify this fraction, 0.3 g of it was diluted in ethanol and centrifuged at 3600 × g at 10°C for 15 min. Ethanol was concentrated from the supernatant to obtain the purified non-phenolic fraction. The basic aqueous phase was acidified with 6N HCl to pH 3.0 and 40 ml of chloroform was added to extract the phenolic fraction. The phenolic fraction was dissolved in chloroform and separated by preparative Thin Layer Chromatography (TLC) on silica gel-G eluting with benzene-methanol 95:5. The phenolic fractions were localized with ultraviolet light and extracted from the silica gel by soxhlet, using the same solvent as in TLC.[14]

Extraction of volatile oil

Volatile oil was extracted from freeze-dried leaves (1 kg) by hydro-distillation method by using Clevenger's apparatus for 2.5 h. The yellowish oil (16.6 ml, yield = 1.66% v/w) obtained was separated from the aqueous phase and dried over anhydrous sodium sulfate and stored at 4°C until used.

GC-MS analysis of volatile oil

The oil sample was diluted with hexane in the ratio of 1:100 and used for the further analysis. The quantitative analysis was done with the help of chromatographer in gas phase (Agilent 7890A GC system) equipped with MS detector (5975C inert XL EI/CI MSD), HP-5MS capillary column (Agilent 19091S-433: 1548.52849 HP-5MS 5% Phenyl Methyl Silox) having dimensions 30 m × 250 µm × 0.25 µm. The column temperature was programmed from initial 80°C up to 300°C. The temperature of the injector was fixed to 270°C. The debit of gas (helium) vector was fixed to 1 ml/min and split injection with split ratio 50:1. The volume of injected sample was 2 µL of diluted oil in hexane (10%). The components were identified based on comparison of their relative retention time and mass spectra with those of standards, W9N08.L library data of the Gas Chromatography-Mass Spectrometery (GC-MS) system and literature data.

Anti-oxidant studies

The following assays were done to determine the anti-oxidant activity of volatile oil and phenolic and non-phenolic fractions of chloroform extract.

Reducing power assay

The reducing power was determined by the method of Athukorola *et al.*[15] Different concentration range (25-800 µg/ml) of volatile oil, phenolic, and non-phenolic fractions of chloroform extract were prepared in Di-Methyl Sulfoxide (DMSO). To 1 ml of diluted solutions of volatile oil, phenolic and non-phenolic fractions of chloroform extract, 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) were added and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (6 mM) and absorbance was measured at 700 nm. BHT was taken as the positive control.[16]

Free radical-scavenging assay

The reaction mixture (3.0 ml) consisted of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of the phenolic and non-phenolic fractions of chloroform extract and volatile oil, respectively, dissolved in DMSO up to a concentration range of 10-640 µl/ml and 1.0 ml of methanol. It was incubated for 10 min in dark, and then the absorbance was measured at 517 nm. BHT was taken as the positive control. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition } (\%) = (A0 - A1/A0) \times 100$$

Where, A0 is the absorbance of control and A1 is the absorbance of test.[16]

Hydrogen peroxide radical-scavenging

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Phenolic and non-phenolic fractions of extract and volatile oil (10-320 μg/ml) in DMSO were added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was taken as positive control. The percentage of hydrogen peroxide-scavenging was calculated as follows:

% Scavenged (H_2O_2) = (A0 – A1/A0) × 100

Where, A0 is the absorbance of control and A1 is the absorbance of test.[16]

Metal chelating activity

The chelation of ferrous ions was estimated using the method of Dinis *et al.*[17] 0.1 ml of the extract (phenol, non-phenolic fractions) and volatile oil in the concentration range of 10-640 μl/ml separately were added to a solution of 0.5 ml ferrous chloride (0.2 mM). The reaction was initiated by the addition of 0.2 ml of ferrozine (5 mM) and incubated at room temperature for 10 min and then the absorbance was measured at 562 nm. Disodium EDTA was taken as the positive control. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as:

[(A0 – As)/As] × 100

Where A0 was the absorbance of the control, and As was the absorbance of the extract or disodium EDTA (positive control).[16,18]

Anti-microbial studies

Minimum Inhibitory Concentration (MIC) determination and comparison of MIC determination by spectrophotometric and visual methods and growth curve

MIC was determined by modified method as described.[19,20] The concentration of stock solutions of phenolic and non-phenolic fractions were 10 mg/ml, and that of ciprofloxacin and fluconazole were 0.25 mg/ml in DMSO, respectively, for bacterial and fungal strains. 0.5 ml of phenolic and non-phenolic fractions and volatile oil were mixed with 0.5 ml of ciprofloxacin respectively. MIC of phenolic, non-phenolic fraction, volatile oil, and ciprofloxacin was determined using 2-fold serial dilution method. For determination of interaction effect of phenolic, non-phenolic fractions, and volatile oil, 0.5 ml of respective test samples were mixed with 0.5 ml of ciprofloxacin stock solution and 0.5 ml of fluconazole for bacterial and fungal strains respectively. MIC was determined using 2-fold serial dilution method. Tubes containing only bacterial suspensions and nutrient broth were used as positive control and negative control were the tubes with only nutrient broth.

Optical Densities (ODs) were measured at 35°C using Thermo Scientific 2000/2000 C nanodrop spectrophotometer, (Nano Drop products, Wilmington, USA) at 405 nm. OD of each replicate at before incubation (T_0) was subtracted from OD after incubation at 37°C (T_{24}) for bacterial cultures and at room temperature for fungal strains respectively. The adjusted OD of each control tube was then assigned a value of 100% growth. The percent inhibition of growth was thus determined using the formula:

Percent Inhibition = 1 – (OD of tube containing test solution/ OD of corresponding control tube) × 100.

The MIC is reported as the lowest concentration of test material which results in 100% inhibition of growth of the test organism. Visual MIC was determined by noting down the concentration of that first tube in which there is no appearance of turbidity after incubation of 24 h and it was compared with that of MIC determined by spectrophotometric method.

Fractional inhibitory concentration index determination

The FIC index (FICI) was calculated by dividing the MIC of the combination of phenolic fraction, non-phenolic fraction, volatile oil, and reference antibiotic respectively.[21]

FIC of vol. oil = MIC of vol. oil in combination with antibiotic drug/MIC of vol. oil.

FIC of phenolic fraction = MIC of phenolic fraction in combination with antibiotic drug/MIC of phenolic fraction.

FIC of non-phenolic fraction = MIC of non-phenolic fraction in combination with antibiotic drug/MIC of non-phenolic fraction.

FIC of antibiotic drug = MIC of antibiotic drug with particular fraction/MIC of drug.

FICI (vol. oil) = FIC of vol. oil + FIC of antibiotic drug.

FICI (phenolic fraction) = FIC of phenolic Ffraction + FIC of antibiotic drug.

FICI (non-phenolic fraction) = FIC of non-phenolic fraction + FIC of antibiotic drug.

Results and Discussion

GC-MS analysis of volatile oil and anti-oxidant studies

GC-MS analysis of volatile oil indicates that total 35 compounds were characterized and quantified. The major component of volatile oil *Origanum vulgare* is carvacrol (86.5%), followed by β-cymene (7.2%), γ-terpinene (0.642%), 3-cyclohexen-1-ol (0.565%), δ-cadinene (0.421%), β-bisabolene (0.400%).

Anti-oxidant activity

Reducing power assay

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic anti-oxidant action.[22] In the reducing power assay, the presence of anti-oxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. It was found that the reducing powers of extract fractions and volatile oil also increased with the increase of their concentrations. Not only volatile oil, but also phenolic and non-phenolic fractions showed better anti-oxidant activity than that of ascorbic acid. It was observed that BHT and volatile oil at the concentration

of 200 µg/ml show absorbance of 0.711 and 0.753, respectively, which shows that reducing power of volatile oil is equivalent to BHT at this concentration. Phenolic fraction is able to reduce at the concentration of 400 µg/ml while non-phenolic fraction exhibited negligible effect and did not show significant reducing activity [Table 1, Figure 1].

Free radical-scavenging assay

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical-scavenging ability of various samples.[23] DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as anti-oxidants and therefore radical scavengers.[24] It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. IC_{50} for free radical-scavenging activity are reported in Table 1. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity.[18,23,25]

Volatile oil obtained from hydrodistillation of *Origanum vulgare* leaves consist of 86.58% of carvacarol, 7.25% p-cymene, 0.565% 4-terpeniol, 0.106% methyl eugenol. All these

Table 1: Anti-oxidant effects of volatile oil, phenolic and non-phenolic fractions of chloroform extract

Reducing power assay				
C (µg/ml)	Standard BHT (absorbance)	O (absorbance)	P (absorbance)	NP (absorbance)
25	0.104±0.11	0.151±0.32	0.023±0.17	0.01±0.21
50	0.150±0.21	0.172±0.15	0.101±1.41	0.049±0.45
100	0.303±0.34	0.41±0.83	0.257±0.33	0.134±0.19
200	0.711±0.51	0.753±0.74	0.680±0.18	0.189±0.75
400	1.234±1.09	1.403±0.63	0.943±0.27	0.25±0.12
800	1.763±1.11	1.912±0.98	1.313±0.34	0.293±0.37
Free radical-scavenging assay				
C (µg/ml)	Standard BHT % inhibition	O % inhibition	P % inhibition	NP % inhibition
10	55.8±1.11	47.2±0.75	33.3±0.10	3.1±0.78
20	57.2±0.32	52.7±0.23	40.9±0.17	5.4±0.65
40	73±0.15	59.4±0.44	48.1±0.22	8.1±0.34
80	79.7±0.14	64±0.78	53.1±0.27	11.7±0.12
160	81±1.21	77.4±0.31	62.6±0.44	15.7±0.61
320	84.2±0.41	81.5±0.14	64.4±0.28	21.6±1.56
640	87.3±0.76	84.6±1.20	68.4±0.31	29.2±0.86
Hydrogen peroxide radical-scavenging assay				
C (µg/ml)	Standard ascorbic acid % scavenging	O % scavenging	P % scavenging	NP % scavenging
10	48.7±0.23	42.4±0.11	31.7±0.21	4.8±0.15
20	51.2±0.87	46.3±0.56	37.5±0.37	6.3±0.19
40	58±0.67	55.1±0.49	41.4±0.24	10.7±0.31
80	67.3±0.14	63.4±0.31	47.3±0.29	13.1±0.26
160	73.6±0.42	69.2±0.19	58±0.10	20±0.18
320	88.2±0.38	80±0.50	69±1.31	23.9±0.33
Metal chelating activity				
C b (µg/ml)	Standard (EDTA)	O (% chelation)	P (% chelation)	NP (% chelation)
10	48.7±0.21	39.9±0.34	15±0.34	8.2±0.60
20	68.9±0.11	47±0.64	24.3±0.78	13.8±0.91
40	76.7±0.19	54±0.32	38±0.21	20.3±0.67
80	87.3±0.31	63.3±0.78	47±0.17	29.7±0.46
160	91.6±0.56	72.4±0.15	57.7±0.16	34.7±0.33
320	93.8±1.88	77.1±0.55	64.6±0.36	43.8±0.21
640	98.5±0.76	84.6±0.17	69.7±0.48	53.7±0.38
Calculation of IC_{50}				
Assay	**O**	**P**	**NP**	**Standard**
Free radical-scavenging	15	60	-	5
H_2O_2 radical-scavenging	30	120	-	15
Metal chelating	30	120	480	15

O: Volatile oil, P: Phenolic fraction, NP: Non-phenolic fraction, C: Concentration (µg/ml), - : Note in this concentration range, ± indicates standard error mean, BHT: Butylhydroxytoluene, EDTA: Ethylene di-amine tetra acetic acid

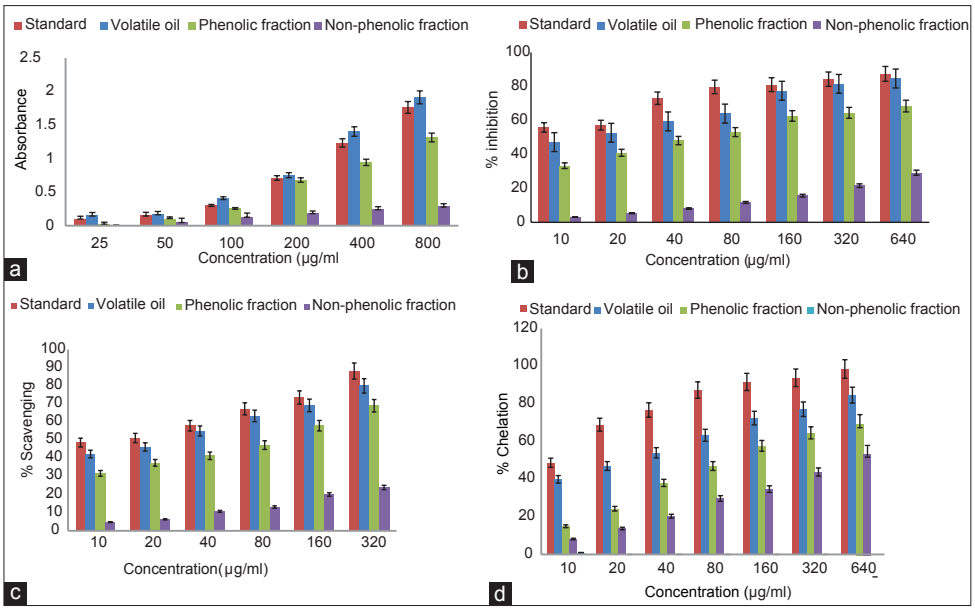

Figure 1: Anti-oxidant activity determination by (a) Reducing power assay (b) DPPH assay (c) Hydrogen peroxide radical scavenging assay (d) Metal chelating activity

phenolic compounds and terpenes are responsible for highest anti-oxidant activity of volatile oil IC_{50} = 15 µg/ml. Phenolic fraction of chloroform extract of leaves showed the activity at IC_{50} at 60 µg/ml which is less than that of BHT taken as standard (IC_{50} = 5 µg/ml) while the non-phenolic fraction did not show significant anti-oxidant activity [Figure 1].

Hydrogen peroxide radical-scavenging assay

Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water.[26,27] The ability of the volatile oil and fractions of chloroform extract to effectively scavenge hydrogen peroxide, determined according to the method of,[16] where they are compared with that of ascorbic acid as standard. The extract fractions and volatile oil were capable of scavenging hydrogen peroxide in a concentration-dependent manner. IC_{50} for scavenging of H_2O_2 were 120, 30 µg/ml, respectively, for phenolic fraction of chloroform extract and volatile oil while non-phenolic fraction had negligible anti-oxidant activity at this concentration range [Table 1, Figure 1].

Metal chelating activity

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry.[28] The transition metal, iron, is capable of generating free radicals from peroxides and may be implicated in human cardiovascular disease.[29] Because Fe^{2+} causes the production of oxyradicals and lipid peroxidation, minimizing its concentration affords protection against oxidative damage. In the presence of other chelating agents, the ferrozine complex formation is disrupted with the result that the red color of the complexes decreases. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, i.e., the activity was increased on increasing concentration from 10-640 µg/ml. It was reported that chelating agents are effective as secondary anti-oxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.[30] The IC_{50} for phenolic fraction and volatile oil were found to be 220 and 50 µg/ml while non-phenolic fraction showed negligible activity in this concentration range [Table 1, Figure 1].

Anti-microbial studies

MIC determination and comparison of MIC determination by spectrophotometric and visual methods and growth curve

MIC is that concentration at which absorbance at time initiation time (T0) and after 24 h incubation, (T24) becomes equal. The MIC of non-phenolic fraction, phenolic fraction, volatile oil, combination of non-phenolic fraction, phenolic fraction, and volatile oil, respectively, and ciprofloxacin in 1:1 ratio are shown in Tables 2 and 3 were at 0.312, 0.01953, 0.00122, 0.07800, 0.00488, and 0.00030 mg/ml as compared to MIC of ciprofloxacin at 0.00244 mg/ml against *B. subtilis* while against *B. cereus*, MIC of non-phenolic fraction, phenolic fraction, volatile oil, combination of non-phenolic fraction, phenolic fraction, and volatile oil, respectively, and ciprofloxacin in 1:1 ratio was found to be 1.25, 0.07800, 0.00244, 0.312, 0.00488, and 0.00061 mg/ml as compared to MIC of ciprofloxacin at 0.00970 mg/ml [Table 2]. MIC exhibited by non-phenolic fraction, phenolic fraction, volatile oil, combination of non-phenolic fraction, phenolic fraction, volatile oil, and fluconazole and fluconazole, respectively, was 2.5, 0.625, 0.00970, 0.07800, 0.01953, and 0.00244 mg/ml [Table 3]. The anti-microbial activities of phenolic compounds may involve multiple modes of action, for e.g. oils degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane,[31] damage membrane protein, interfere with membrane-integrated enzymes,[32] cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanism for energy production and metabolism, alter nutrient uptake, and electron transport [Figure 2].

Table 2: Minimum inhibitory concentration of volatile oil, phenolic, non-phenolic fractions of chloroform extract alone and in combination with ciprofloxacin against *Bacillus subtilis* and Bacillus cereus by microdilution method

	B. subtilis (% in)							*B. cereus* (% in)						
Conc. (mg/ml)	NP	NP+C	P	P+C	O	O+C	C	NP	NP+C	P	P+C	O	O+C	C
0.00015	5	13.3	30	51.6	73.3	96.6	68	3.3	8.3	13.3	59.3	68.3	87.3	43.4
0.00030	8.3	21.6	41.6	66.6	87	100	71.6	15	18.3	21.6	69	77.3	98	59.3
0.00061	10	35	53.3	76.7	95	>100	89	20	25	32.6	75	86	100	66.7
0.00122	25	45	61.6	85	100	>100	91.6	27.3	38	46.6	84.3	96.6	>100	75.6
0.00244	31.6	55	75	93	>100	>100	100	31	44.6	57	96	100	>100	89
0.00488	43.3	63.3	83.3	100	>100	>100	>100	36	54	65	100	>100	>100	99.3
0.00970	54	76	96.3	>100	>100	>100	>100	42.6	62.6	79.3	>100	>100	>100	100
0.01953	62.6	89.3	100	>100	>100	>100	>100	52	68	81	>100	>100	>100	>100
0.03900	74	93.3	>100	>100	>100	>100	>100	61.3	78.6	94	>100	>100	>100	>100
0.07800	81.6	100	>100	>100	>100	>100	>100	71	82.6	100	>100	>100	>100	>100
0.15600	90	>100	>100	>100	>100	>100	>100	82.3	92.6	>100	>100	>100	>100	>100
0.31200	100	>100	>100	>100	>100	>100	>100	83.6	100	>100	>100	>100	>100	>100
0.62500	>100	>100	>100	>100	>100	>100	-	90.6	>100	>100	>100	>100	>100	-
1.25000	>100	>100	>100	>100	-	-	-	100	>100	>100	>100	-	-	-
2.50000	>100	>100	>100	>100	-	-	-	>100	>100	>100	>100	-	-	-
5.00000	>100	>100	>100	>100	-	-	-	>100	>100	>100	>100	-	-	-
10.0000	>100	-	>100	-	-	-	-	>100	-	>100	-	-	-	-

NP: Non-phenolic fraction, P: Phenolic fraction, O: Volatile oil, S: Standard drug ciprofloxacin, % in: Inhibition, *B. subtilis: Bacillus subtilis*, *B. cereus: Bacillus cereus*

Table 3: Minimum inhibitory concentration of volatile oil, phenolic, non-phenolic fractions of chloroform extract alone and in combination with ciprofloxacin against *Aspergillus niger* by microdilution method

Concentration (mg/ml)	NP % in	NP+C % in	P % in	P+C % in	O % in	O+C % in	C % in
0.00015	3.4	51.7	8.6	34.4	45.8	68.9	13.7
0.00030	6.8	55.1	17.2	44.8	50	75.8	20.6
0.00061	13.7	60.3	21.7	56.5	67.9	89.6	31.0
0.00122	20.6	64.8	23.4	67.2	74.8	95.8	44.8
0.00244	31.0	68.6	33.4	72.4	83.7	100	51.7
0.00488	41.7	70	36.2	87.9	94.1	>100	62.1
0.00970	53.4	72.4	43.1	94.8	100	>100	75.8
0.01953	62.1	82.7	52.7	100	>100	>100	82.7
0.03900	63.7	91.3	69.3	>100	>100	>100	93.1
0.07800	71.3	100	70.6	>100	>100	>100	100
0.15600	74.1	>100	86.2	>100	>100	>100	>100
0.31200	81	>100	93.1	>100	>100	>100	>100
0.62500	84.4	>100	100	>100	>100	>100	-
1.25000	92.1	>100	>100	>100	-	-	-
2.50000	100	>100	>100	>100	-	-	-
5.00000	>100	>100	>100	>100	-	-	-
10.0000	>100	-	>100	-	-	-	-

NP: Non-phenolic fraction, P: Phenolic fraction, O: Volatile oil, S: Standard drug fluconazole, % in: Inhibition

FICI determination

FICI was calculated to describe standard drugs ciprofloxacin and fluconazole interactions with volatile oil, phenolic, and non-phenolic fractions, respectively, for bacterial and fungal strains. Synergy is defined as an FICI of ≤0.5. Indifference was defined as an FICI of ≥0.5 but of ≤4.0. Antagonism was defined as an FICI of >4.0.[33] Synergism was shown by volatile oil with an FICI of 0.312 and 0.282 against *B. cereus* and *A. niger* respectively. Non-phenolic fraction was found to act antagonistically along with ciprofloxacin against *B. cereus* and *B. subtilis* while phenolic fraction exhibited indifferent activity along with ciprofloxacin against both the bacterial strains [Table 4, Figure 2].

Conclusion

The anti-microbial interaction studies of *Origanum vulgare* and anti-oxidant evaluation of its chloroform extract is demonstrated for the first time against the pathogenic micro-organisms. These results suggest the possible exploitation of this plant in

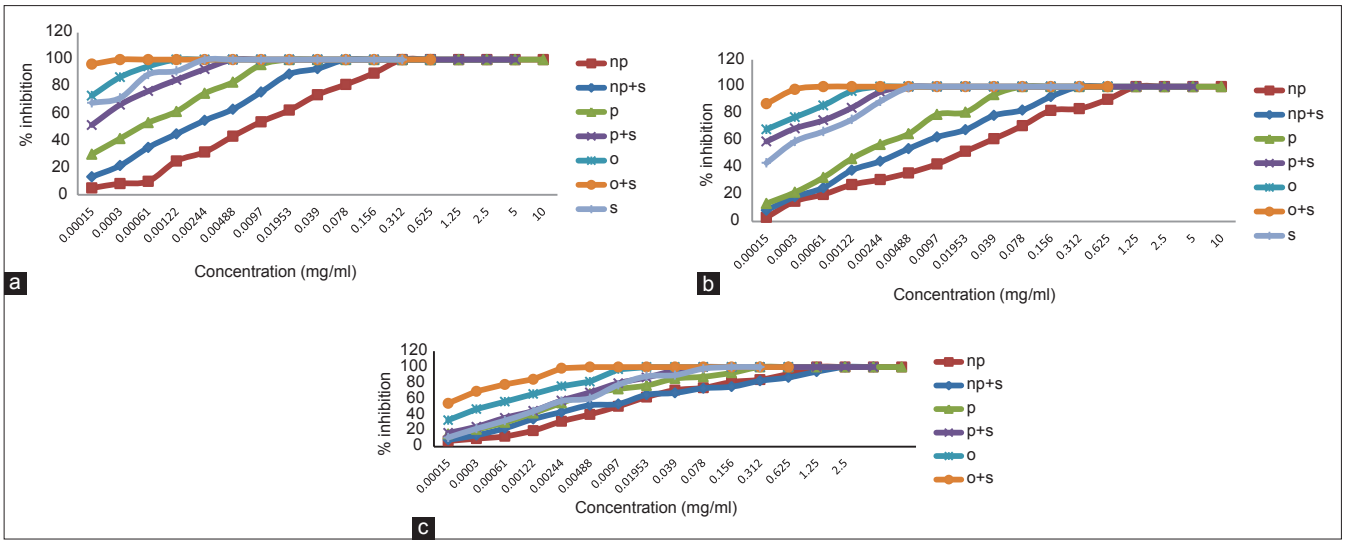

Figure 2: Growth curve (% inhibition against concentration in mg/ml) of (a) *B. subtilis* (b) *B. cereus* (c) *A. niger* in presence of phenolic, non-phenolic fractions and volatile oil alone and in combination with ciprofloxacin and fluconazole

Table 4: Fractional inhibitory concentration determination of volatile oil, phenolic, non-phenolic fractions of chloroform extract and standard antibiotic/anti-fungal drug and fractional inhibitory concentration index determination

Strain	FIC		FIC		FIC		FICI		
	O	S	P	S	NP	S	O	P	NP
Bacillus subtilis	5.000	2.500	0.249	2.000	0.250	31.967	7.500	2.249	32.217
Bacillus cereus	0.250	0.062	0.062	0.503	0.249	32.16	0.312	0.565	32.409
Aspergillus niger	0.251	0.031	0.031	0.250	0.031	1.000	0.282	0.281	1.031

O: Volatile oil, P: Phenolic fraction, NP: Non-phenolic fraction, S: Standard (ciprofloxacin for bacterial strain and fluconazole for fungal strain), FIC: Fractional inhibitory concentration, FICI: Fractional inhibitory concentration index

the management of the infectious diseases. Further purification of the extract may yield a novel anti-bacterial and anti-fungal drug. The almost equipotent activities of volatile oil compared to that of synthetic anti-oxidants and significantly high activity of phenolic fraction of chloroform extract suggests their usage in food industry in place of synthetic anti-oxidant which may prove to be carcinogenic.

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हिन्दी सारांश

ओरिगेनम वल्गेर के एण्टीऑक्सीडण्ट एवं एण्टीमाइक्रोबियल प्रभाव का प्रमाणित एण्टीबायोटिक्स के साथ अध्ययन

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ओरिगेनम, लेमियेसी परिवार के २०० फुलों में से एक है । इस कुल में सुगंधित और औषधीय द्रव्य शामिल है । ओरिगेनम अपने एन्टीमाइक्रोबियल प्रभाव के लिए जाना जाता है, पर अब तक सिंथेटिक एन्टीबायोटिक के संयोजन के साथ इसके प्रभाव का अध्ययन नही किया गया है । वर्तमान परिक्षण में ओरिगेनम वल्गेर का प्रमाणित एन्टीबायोटिक्स के साथ इसके एन्टीबायोटिक एवं एन्टीमाइक्रोबियल प्रभाव का अध्ययन बेसीलस कुल के बैक्टीरिया के विरुद्ध किया गया है । क्लोरोफार्म एक्स्ट्रेक्ट एवं बाष्पशील तैल के फिनोलिक एवं नॉनफिनोलिक अंश के एण्टीऑक्सिडण्ट प्रभाव को फ्री रेडिकल स्केवेन्जिंग, हाइड्रोजन पेरॉक्साइड, रेडिकल प्लैवीक ऐसे विरोध शक्ति एवं मेटल चेलिटिंग जैसे परीक्षणों के द्वारा परीक्षण किया गया । नॉनफिनोलिक अंश सिप्रोफ्लॉक्सॅसिन के साथ वी.सीरस एवं बी. सबटिलिस पर प्रतिवेधी की तरह कार्य करता है एवं फिनोलिक अंश सिप्रोफ्लॉक्सॅसिन के साथ अपरिवर्तित रुप में दोनों बैक्टीरिया के लिए कार्य करता है । यह संयोजन न केवल एन्टीबायोटिक प्रतिवेधकता में प्रभावी होगा बल्कि हानिकारक रेडिकल्स, जो घातक बीमारियों के कारण हैं उनसे भी शरीर की रक्षा करेगा ।