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Evaluation Of Synergistic Antimicrobial Activity and Antioxidant Activity of blend of Essential Oil contains Fennel, Coriander, Ajowan and Caraway

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Abstract: *In the present study evaluation of synergistic antimicrobial activity and antioxidant activity of blend of essential oil contains cardamom, coriander, fennel, caraway and ajowan were carried out. The essential oil was extracted from dried seed of cardamom, Coriander, Fennel, caraway and Ajowan were blend in sunflower oil used as base. The antibacterial activity and minimum inhibitory concentration (MIC) of essential oil against several pathogens was evaluated. The minimum inhibitory concentration (MIC) values of oils were determined by micro broth dilution assay. The results showed that the gram positive and gram negative strains of bacteria had different sensitivities to essential oils. The potential synergetic effect of blend of essential oil with a common antibacterial, Ciprofloxacin, was also studied. Ciprofloxacin and extracted essential oil in combination inhibited the growth of gram positive and gram negative bacteria. Extracted essential oil showed synergistic activity with ciprofloxacin against test strains. Antioxidant activity of the essential oils extracted were carried out using DPPH, H_2O_2 and Nitric oxide scavenging assay at 100 µg/mL and 200 µg/mL and compared with the standard Ascorbic acid. In the present study, extract blend of essential oil showed a significant effect in inhibiting DPPH, reaching up to 76.84% at concentration of 200 µg/mL. The blend of extract showed a significant effect in inhibiting H_2O_2, reaching up to 70.04 % at concentration of 200 µg/mL. The extract showed maximum activity of 72.80 % at 200 µg/mL, where as ascorbic acid at the same concentration exhibited 94.96% inhibition in Nitric oxide scavenging assay.*

Keywords: *Essential oil, antibacterial activity, MIC, antioxidant activity and synergistic activity.*

I. Introduction

Essential oils are mixtures of compounds obtained from spices, aromatic herbs, fruits, and flowers and characterized by their aroma. Essential oils and their main components have many applications in popular medicine, food, beverages, preservation, cosmetics as well as in the fragrance and pharmaceutical industries. The antimicrobial properties of essential oils have been known for a long time, and various researches have been conducted into their antimicrobial activities using various bacteria and fungi. Considering the increased pathogen resistance, investigations into the antimicrobial activities, mode of action and potential uses of essential oils and its components have gained a new impulse[1].

Cardamom, Fennel, Coriander, Caraway and ajown is produced from cultivated or wild plants in the mountainous regions of southern India. These has been used in the traditional Chinese medicine and Indian Ayurvedic medicine for thousands of years, mainly for treating respiratory diseases, fevers and digestive complaints. A steam distillation adequate common techniques is use for extraction of essential from each seed.

The cardamom essential oil is obtained by steam distillation from the ripe and dried seeds. It is a colorless or very pale yellow liquid with an aromatic, penetrating, slightly camphoraceous odor and a persistent, pungent, strongly aromatic taste. The major components of the cardamom oil are 1, 8-cineole (21-41%) and α-terpinyl acetate (21-35%). The cardamom oil also contains the following components: α- terpineol (0.8-6.2%, and to 11.5% in oil from Pakistan), limonene (1.7-3.7%), sabinene + β-pinene (0.3 2.4%), borneol (0.1-1.2%), linalool (0.4-8.7%), linalyl acetate (1.6-2.4%), nerol (0.6-1.6%), geraniol (1.1-3.7%), neryl acetate (0.8- 1.2%), farnesol (up to 12.5% from the total isomers), nerolidol (0.2-6.7%), isosafrole (3.8%) and other minor compounds. Trace constituents like unsaturated aliphatic aldehydes and α-terpinyl acetate may be important for the typical aroma of the oil[2,3].

Fennel is widely cultivated and used as a culinary spice is produced from cultivated or wild plants in the mountainous regions of southern India. It has been used in the traditional Chinese medicine and Indian Ayurvedic medicine for thousands of years. It have mainly antimicrobial, digestive properties.. It is a colorless or very pale yellow liquid with an aromatic, penetrating, slightly camphoraceous odor and strongly aromatic taste. The major components of the fennel oil are Anethol (50-60%). Estragole(10-20%), pinene(5-10%), Fenchone(1-2%), Limone(1-5%), Anisaldehyde(1-3%).

Coriander (*Coriandrum sativum* L.) essential oil is among the most used essential oils worldwide. It has widely used in aroma therapy. Presently it possesses antimicrobial, antioxidant properties also. Linalool is the major component which is present about 60-70% and Pinene, Limonene, Terpiene, Camphor, Terpinol, Geraniol, Geranyl Acetate also ssome other component also present.

Ajowan oil traditionally was used as diuretic, carminative, and antihelmentic which contain 40-50 % of thymol. Some biological effects of ajowan such as antiviral, anti-inflammatory, antifungal, antipyretic, antifilarial, analgesic, antinociceptive and antioxidant activity have been confirmed. Limonene and Terpinol the other major component also present inthe ajowan oil in 10-30%.

Caraway which is one of the oldest aromatic and medicinal plants known. In addition, 41 volatile compounds were identified in the seed essential oils of the three caraway ecotypes, the main ones being carvone (61.58–77.35%) and limonene (16.15–29.11%)[4,5].

II. Materials And Methods

Plant material

The seeds of Cardamom, Fennel, Coriander, Caraway and ajown were obtained from trade market, humidity 8%.

Extraction of essential oil[6, 7]

Extraction of essential oil from cardamom and fennel

The air-seeds of cardamom and fennel were ground separately in an attrition mill to a size of 0.15-0.25 mm and the extract obtained by a 1 dm^3 volume C2H2F4 (1,1,1,2-tetrafluorethane) laboratory-extractor (Nenov, 2006) under following conditions (continuous flow and evaporation of solvent): pressure 0.5 MPa; temperature 18-20oC and extraction time 60 min. The physico-chemical properties were measured according to Russian Pharmacopoeia (1990).

Extraction of essential oil from coriander

Plant material was oven dried at 50°C for 24 h to reduce water content. Extracts were prepared by blending preserved plant material (approximately 200 g dry weight) in 99% ethanol and petroleum ether (1:3 w/v ratio). After 24 h, the mixture was filtered through Whatman filter paper (No.1) using a Buchner funnel. The solvent was removed with a rotary vacuum evaporator at 40°C (30 mmHg). The oil was stored in dark vials at 4°C before analyzing. The waste or residue after extracted by petroleum ether of plant materials was repeated once with ethanol, called secondary extraction, similar to the above condition.

Extraction of essential oil from caraway

Essential oil from caraway seeds were extracted by a modified method of Bligh and Dyer. Thus, 1 g air-dried seed was fixed in boiling water for 5 min and then ground manually using a mortar and pestle. A chloroform/methanol/hexane (LabScan Ltd.) mixture (4:3:2, v/v/v) was used for total lipid extraction (Marzouk and Cherif, 1981). After washing with water of fixation and decantation during 24 h at +4◦C, the organic phase containing total lipids was recovered and dried under a stream of nitrogen. The residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) and stored at −20 ◦C for further analyses. Total lipid extraction was made in triplicate.

Essential oil extraction from Ajowan

The Ajowan seeds were ground and the resulting powder was subjected to hydro distillation for 3 hours in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (12).

The extracted oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C for gas chromatography (GC) and GC/ mass spectrometry (MS) analysis for conformation.

Determination of antibacterial activity[8,9]

Antimicrobial activity of the essential oils extracted was determined against reference microbial strains. The used test microorganisms and their origins are listed in Table 2. The strains are deposited in the Microbial Culture Collection. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of essential oils extract were determined by serial broth dilution method in accordance with CLSI reference method (CLSI Standards, 1990) A stock solution to be tested was prepared by diluting the respective cardamom extract sample in DMSO (Sigma-Aldrich Co.). Antimicrobial activity of the extract was determined in concentration of 2 % (w/v). The antibacterial activity of the seed extracts was carried out by disc diffusion assay that was used to screen (Kumar *et al.*, 2001; Gulluce *et al.*, 2003). Muller Hinton agar (MHA) plates were swabbed with the

respective broth culture of the organisms (diluted to 0.5 McFarland Standard with saline) and kept for absorption to take place. Sterile 6 mm diameter filter paper discs were impregnated with 2% w/v of seed extract that dissolved in sterile dimethylsulfoxide (DMSO). Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ciprofloxacin (5 µg/ml) was used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested. The plates were incubated overnight at 37°C. The antimicrobial activity was evaluated by measuring the zone expressed as mm of inhibition against test organism. Six discs per plate and three plates were used, and each test was run in triplicate.

Preparation of bacteria strain

Four different bacteria were used. Two species of Gram positive bacteria, *S. aureus, B. Subtilis* and two Gram negative bacteria, *E. coli, P. aeruginosa* were obtained. Bacteria were sub-cultured on nutrient agar at 37°C prior to being grown in nutrient broth overnight. All overnight (ON) cultures were standardized by matching to the McFarland 0.5 turbidity standard using sterile saline to produce approximately $1.5x10^8$ colony forming units (cfu) per ml.

Determination of minimum inhibitory concentration (MIC) [10]

The minimum inhibition concentration (MIC) values were also studied for the bacteria which were determined as sensitive to the extracts in disc diffusion assay. The inoculated bacteria as prepared from 24 h nutrient broth cultures and suspensions were adjusted to 0.5 McFarland turbidity standards. Extracts dissolved in DMSO were first diluted to the highest concentration (50 µg/ml) to be tested, and then serial two fold dilutions were made in a concentration range from 6.25 µg/ml to 50 µg/ml. The least concentration of each extract showing a clear of inhibition was taken as the MIC levels.

Test bacterial strain was grown in nutrient broth for 16-18 h at 37°C on rotary shaker. Cells were harvested by centrifugation (8,000 g, 15 min, 4°C). Bacterial pellet thus obtained was given three washings with sterile phosphate buffer saline (0.1 M, pH 7.2) and finally suspended in the same buffer. Two-fold serial dilutions of black tea-extract or antibiotic were made in sterile nutrient broth. Each dilution was inoculated with 10 µl of 1:100 diluted overnight grown test bacterial cultures and incubated at 37°C. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was taken as minimum inhibitory concentration (MIC). A loopful from the highest dilution streaked on nutrient agar plates which did not show any bacterial growth after overnight incubation was taken as minimum bactericidal concentration (MBC). Nutrient medium (20 ml/ 100 ml flask) with and without boiled black tea extract (concentration equivalent to MIC for the test-strains) in duplicates was inoculated with 20 µl of 1:100 diluted overnight grown test bacterial cultures. Samples withdrawn at intervals were used for determining bacterial viable counts by spread plate on nutrient agar plate.

Assay of antioxidant enzymes

***DPPH radical-scavenging activity*[11]**

The DPPH assay was carried out as described by Hsu *et al.* with some modifications. A volume of 1.5 mL of 0.1 mmol/L DPPH solution was mixed with 1.5 mL of various concentrations (10 to 500 µg/mL) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula: DPPH radical scavenging activity (%)=$(A_{control}-A_{test})/A_{control}\times100$. Where, $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of samples. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the graph after plotting inhibition percentage against extract concentration.

Nitric oxide scavenging activity[12]

Nitric oxide was generated from sodium nitroprusside and was measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (10 mmol/L) in phosphate buffer saline (PBS) was mixed with different essential oil extract and incubated at 25 °C for 150 min. The samples were added to Griess reagent (1% sulphanilamide, 2% $H_3PO_4$and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive

control. All the tests were performed in triplicate and the graph was plotted with the mean values. The percentage of inhibition was measured by the following formula: Radical scavenging activity (%)= ($A_{control}$-A_{test})/$A_{control}$×100. Where $A_{control}$ is the absorbance of the control (without extract) and A_{test} is the absorbance in the presence of the extract/standard.

Hydrogen peroxide scavenging activity[13,14]

Scavenging activity of hydrogen peroxide by the plant essential oil extract was estimated using the method of Ruch*et al.* with little modification. 4 mmol/L solution of H_2O_2 was prepared in PBS (pH 7.4). Plant essential oil extract (4 mL), prepared in distilled water at various concentration was mixed with 0.6 mL of 4 mmol/L H_2O_2 solution prepared in PBS and incubated for 10 min. The absorbance of the solution was taken at 230 nm against a blank solution containing the plant extract in PBS without H_2O_2. Ascorbic acid was used as positive control. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation: H_2O_2 radical scavenging activity=($A_{control}$-A_{test})/$A_{control}$×100. Where $A_{control}$ is the absorbance of H_2O_2 radical+methanol; A_{test} is the absorbance of H_2O_2radical+sample extract or standard.

Statistical analysis

Results of the biochemical estimations are reported as means S.E.M. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA), Student's t-test was used for determining significance (Woolson, 1987).

III. Results And Discussion

The extract of the algae was used to carry out the anti bacterial activity the method used for the antibacterial activity was Agar Disc method. The results for the antibacterial activity were shown in the **Table 1.** The antimicrobial activity of essential oil may be influenced by some factors such as quality of the extraction of essential oil. The activity of the different essential oil collected from different seeds varied significantly among the various bacterial species studied. The cardamom extract demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria. The extract was less active against strains of *P. aeruginosa*, which belong to the group of the most resistible bacterial strains. The ability of *P. aeruginosa* to produce extracellular polysaccharides increased antimicrobial resistance. The results were shown in **Table 1, Figure2** and **Figure 3**.
The synergistic antimicrobial activity for the different combination of extracted essential oils was also carried out. When essential oils are combined (50:50) and tested, it was observed that it shown synergistic activity. Essential oil extracted from cardamom alone at 10%W/V tested against *E. coli, P. aeruginosa, S. aureus and B. subtilis* produced zone of inhibition (in mm) 24, 20, 22 and 24 respectively. When, Essential oil extracted from cardamom combined with essential oil extracted from coriander at 1:1 ratio, produced zone of inhibition in mm as 26, 24, 20 and 22 respectively. The results proved the synergistic activity when they were combined and tested. The synergistic antimicrobial activity of blend of essential oils extracted from cardamom, coriander, fennel, caraway and ajowan (1:1:1:1:1) were found to be remarkable when compared with standard. Essential oils also showed synergistic activity with ciprofloxacin and streptomycin (**Table 2**).

Table 1: The antibacterial activity of essential oils by Agar Disc method.

Sample	Concentration	Zone of inhibition(mm)			
		E. coli	***P. aeruginosa***	***S. aureus***	***B. subtilis***
CARDAMOM	5%W/V	10.0	14.0	12.0	12.0
(Essential oil)	10%W/V	24.0	20.0	22.0	24.0
CORIANDER	5%W/V	12.0	12.0	12.0	14.0
(Essential oil)	10%W/V	18.0	20.0	18.0	22.0
FENNEL (Essential	5%W/V	10.0	10.0	10.0	10.0
oil)	10%W/V	16.0	14.0	16.0	18.0
CARAWAY	10%W/V	10.0	10.0	10.0	10.0
(Essential oil)	10%W/V	14.0	16.0	14.0	14.0
AJAWAN	5%W/V	12.0	12.0	12.0	10.0
(Essential oil)	10%W/V	18.0	16.0	18.0	16.0
Ciprofloxacin	250 µg/ml	16.0	16.0	14.0	15.0
	500 µg/ml	30.0	30.0	28.00	30.0
Streptomycin	250 µg/ml	30.0	16.0	14.0	15.0
	500 µg/ml	32.0	32.0	30.0	32.0
Control	Distilled water	30.0	30.0	28.00	30.0

Table 2: The synergistic antibacterial activity of essential oils with ciprofloxacin and streptomycin by Agar Disc method.

Sample	Concentration	Zone of inhibition(mm)			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
CARDAMOM + Ciprofloxacin	10%W/V	36.0	34.0	38.0	36.0
CARDAMOM + Streptomycin	10%W/V	36.0	32.0	32.0	34.0
CORIANDER + Ciprofloxacin	10%W/V	30.0	32.0	34.0	34.0
CORIANDER + Streptomycin	10%W/V	32.0	34.0	38.0	38.0
FENNEL + Ciprofloxacin	10%W/V	30.0	30.0	30.0	30.0
FENNEL + Streptomycin	10%W/V	36.0	34.0	36.0	38.0
CARAWAY + Ciprofloxacin	10%W/V	34.0	30.0	30.0	30.0
CARAWAY + Streptomycin	10%W/V	30.0	36.0	34.0	34.0
AJAWAN + Ciprofloxacin	10%W/V	34.0	32.0	32.0	30.0
AJAWAN + Streptomycin	10%W/V	38.0	36.0	38.0	36.0
CARDAMOM + CORIANDER	10%W/V	26.0	24.0	20.0	22.0
CARDAMOM + FENNEL	10%W/V	24.0	22.0	18.0	20.0
CARDAMOM + CARAWAY	10%W/V	20.0	22.0	20.0	24.0
CARDAMOM + AJAWAN	10%W/V	22.0	20.0	20.0	22.0
CORIANDER + FENNEL	10%W/V	22.0	24.0	22.0	20.0
CORIANDER + CARAWAY	10%W/V	20.0	22.0	24.0	20.0
CORIANDER + AJAWAN	10%W/V	20.0	20.0	20.0	22.0
FENNEL + CARAWAY	10%W/V	22.0	20.0	22.0	24.0
CARAWAY + AJAWAN	10%W/V	24.0	20.0	20.0	22.0
Blend of essential oil (Cardamom+caraway + Ajowan+fennel+coriander)	10%W/V	28.0	24.0	26.0	26.0
Ciprofloxacin					
Streptomycin	500 µg/ml	30.0	32.0	30.0	32.0
Control	Distilled water	30.0	30.0	28.00	30.0

Ciprofloxacin and Streptomycin was used for comparing antibacterial activity of different tea extract and studying synergistic activity with antibiotics. Differences were observed in the survival rates of bacteria with essential oils for the study of MIC. Both gram positive and gram negative bacteria was found to be more susceptible to growth inhibition by antibiotics when added with essential oils as the zones of inhibition were wider ranging from 30 to 38 mm on nutrient agar plates supplemented with 10% w/v essential oil extract as compared to the zones of inhibition on nutrient agar plates with antibiotic alone. Growth inhibition bacteria at concentration of ciprofloxacin and streptomycin (500 µg/ml) and essential oil extract (10 % w/v) were found more as compared the MIC of individual agent i.e. antibiotic or essential oils, which further confirm the synergistic activity. Synergistic microbial growth inhibition by essential oils extract and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and essential oils extract (**Table 2**).

Table 3: Minimum bactericidal concentration data of antibacterial activity for the essential oils.

S.No	Code	Minimum Inhibitory concentration, MIC (µg/mL)					
		Gram (+)ve		Gram (-)ve			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*Negative control*	*Positive control*
1	Cardamom	4.0	3.0	4.0	3.0	-	+
2	Coriander	3.0	3.0	3.0	3.0	-	+
3	Fennel	4.0	4.0	3.0	4.0	-	+
4	Caraway	2.5	3.5	2.5	2.5	-	+
5	Ajowan	2.5	2.5	2.5	2.5	-	+
6	Blend of essential oil	2.5	2.5	2.5	2.5		
7	Blank					-	+
8	Ciprofloxacin	50	50	50	50	-	+

The bacterial suspensions were used as positive control; Extracts in broth were used as negative control

Graphical representation of antibacterial activity:

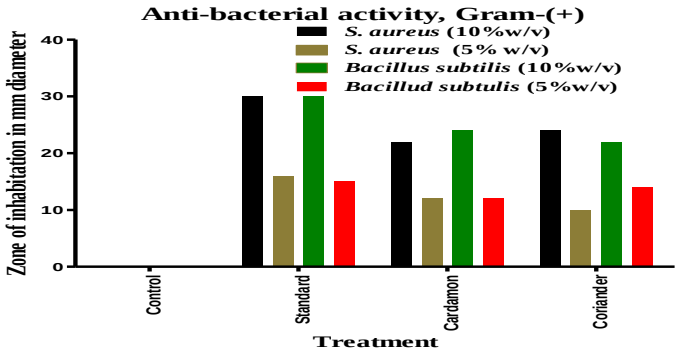

Figure 1: Anti-microbial activity against gram-(+) bacteria.

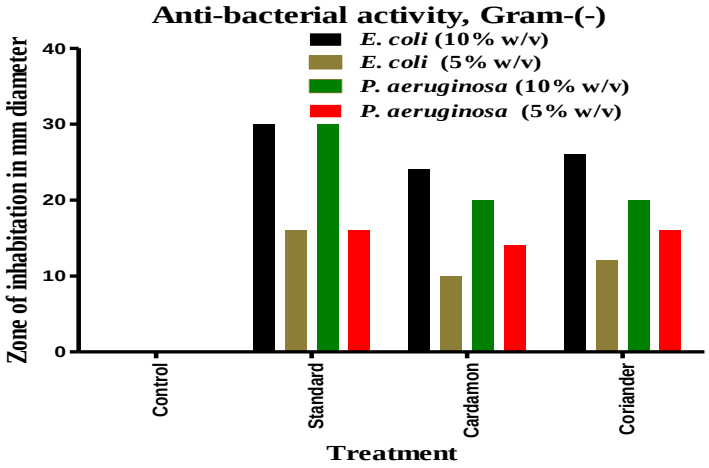

Figure 2: Antibacterial activity against gram-(-) bacteria.

Table 4: Antioxidant activity of essential oils with DPPH, H_2O_2 and Nitric oxide scavenging assay

Sample	Concentration	Antioxidant activity (% inhibition)		
		DPPH radical scavenging assay	*H_2O_2 radical scavenging activity*	*Nitric oxide scavenging assay*
CARDAMOM	100 µg/ml	36.37	35.11	31.39
CARDAMOM	200 µg/ml	66.22	60.72	62.25
CORIANDER	100 µg/ml	30.75	32.05	32.71
CORIANDER	200 µg/ml	62.33	60.93	60.39
FENNEL	100 µg/ml	38.37	39.33	30.32
FENNEL	200 µg/ml	76.84	70.04	72.80
CARAWAY	100 µg/ml	34.43	36.03	33.40
CARAWAY	200 µg/ml	70.67	72.27	72.60
AJAWAN	100 µg/ml	34.25	31.65	32.23
AJAWAN	200 µg/ml	68.37	62.77	61.30
CARDAMOM + CORIANDER	200 µg/ml	69.20	70.72	74.25
CARDAMOM + FENNEL	200 µg/ml	70.44	76.20	76.28
CARDAMOM + CARAWAY	200 µg/ml	72.21	75.34	78.76
CARDAMOM + AJAWAN	200 µg/ml	73.90	74.42	70.20
Blend of Essential oil	100 µg/ml	63.90	66.90	70.90
Blend of Essential oil	200 µg/ml	83.90	78.90	88.90
Ascorbic acid	100 µg/ml	60.34	56.08	62.38
Ascorbic acid	200 µg/ml	90.90	92.92	94.96

DPPH radical scavenging assay

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. DPPH is a stable free radical which produces deep purple colour in methanol solution. The principle of this assay is based on the reduction of purple coloured methanolic DPPH solution in the presence of hydrogen donating antioxidants by the formation of yellow coloured diphenyl-picryl hydrazine. As the absorbance decreases the more efficient, the antioxidant activity of the extract in terms of hydrogen atom donating capacity, the more antioxidant present in the extract, the more DPPH reduction will occur. It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to the capacity to be donors of

hydrogen atoms or electrons and to capture the free radicals. In the present study, extract showed a significant effect in inhibiting DPPH, reaching up to 76.84% (fennel) at concentration of 200 µg/mL. Our results suggested that different concentration have different activities and maximum activity was observed at 200 µg/mL concentration. The observed antioxidant of extracts may be due to the neutralization of free radicals (DPPH), either transfer of hydrogen atom or by transfer of an electron. The scavenging effect can be attributed to the presence of active phytoconstituents in them (**Table 4**).

H_2O_2 radical scavenging activity

Extract also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. Scavenging activity of H_2O_2 by the extract may be attributed to their phenolics, which can donate electrons to H_2O_2 thereby neutralizing it into water. The H_2O_2 scavenging activity was detected and compared with ascorbic acid. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It rapidly transverses cell membrane and once inside the cell interior, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. In the present study, extract showed a significant effect in inhibiting H_2O_2, reaching up to 70.04 % (fennel) at concentration of 200 µg/mL (**Table 4**).

Nitric oxide scavenging assay

In the present study, the extracts were investigated for its inhibitory effect on nitric oxide production. The present study revealed that the extract has a moderate activity in scavenging nitric oxide radical. Results shown that a significant decrease in the nitric oxide radical due to scavenging ability of extract and the standard ascorbic acid. The extract showed maximum activity of 72.80 % (fennel) at 200 µg/mL, where as ascorbic acid at the same concentration exhibited 94.96% inhibition. It is well known that nitric oxide play an important role in various inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis. Nitric oxide is a potent pleiotropic inhibitor of physiological process such as smooth muscle relaxation, neural signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (**Table 4**).

The synergistic antioxidant activity of different essential oils was carried out in combination of 50:50. It was observed that when cardamom were combined with coriander, fennel, caraway and ajowan, shown to have synergistic antioxidant activity. Coriander alone showed DPPH radical scavenging assay (% inhibition) at 200 µg/ml 66.22, H_2O_2 radical scavenging activity (% inhibition) at 200 µg/ml 60.72and Nitric oxide scavenging assay (% inhibition) at 200 µg/ml 62.25. But when Cardamom combined with coriander it shown DPPH radical scavenging assay (% inhibition) at 200 µg/ml 69.20, H_2O_2 radical scavenging activity (% inhibition) at 200 µg/ml 70.72 and Nitric oxide scavenging assay (% inhibition) at 200 µg/ml 74.25 (**Table 4**).

IV. Conclusion

The results showed that the gram positive and gram negative strains of bacteria had different sensitivities to essential oils. The essential oils demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria. Different extracts of essential oil exhibited good reducing power. In the present study, extract showed a significant effect in inhibiting DPPH, reaching up to 76.84% (fennel) at concentration of 200 µg/mL. The extract showed a significant effect in inhibiting H_2O_2, reaching up to 70.04 % (fennel) at concentration of 200 µg/mL. The extract showed maximum activity of 72.80 % (fennel) at 200 µg/mL, where as ascorbic acid at the same concentration exhibited 94.96% inhibition in Nitric oxide scavenging assay. But when all the extract was blend and used for antimicrobial as well as antioxidant activity, it shows significant effect compare to individual. In our present study it is proved that combination of some essential oil having more or similar biological activity than the regular synthetic medicine. This claim is a good indication for future aspect to herbal industries to formulate such combination of dosage form for certain common diseases for patient wand can reduce the use of synthetic medicine which have more contraindication effects.

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