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ANTIMICROBIAL EVALUATION OF SOME ESSENTIAL OIL COMBINATIONS AGAINST FOOD BORNE PATHOGENS

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ABSTRACT

Deterioration of food can be caused by various factors such as physical, chemical, enzymatic or microbiological changes. Microbiological spoilage is caused by bacteria, fungi and yeast. Due to increasing side effect of chemicals in food, consumers demand for some natural alternative to these chemicals. One of these alternatives is essential oils derived from plants. So, in the present study five commercial essential oils, having medicinal values and are used in food industries too were studied alone and in combinations. There antibacterial action was seen against four gram positive (*Micrococcus sp., Lactobacillus sp., Staphylococcus sp., Bacillus sp.*) and four gram negative bacteria (*Klebsiella sp., E. coli, Pseudomonas sp., Salmonella sp.*) isolated and identified using conventional methods from twenty different spoiled food samples. Out of this eight bacterial isolates, one gram positive (*Micrococcus yunnanensis*) and one gram negative bacteria *(E. coli)*, who showed best results in antibacterial assay were taken as models to study the different combinations of essential oils. The combinations of oils were studied using MIC method. Out of all the combination tested best results were given by Rosemary and Eucalyptus oil combination for both *E. coli* and *Micrococcus yunnanensis*.

Keywords: Essential oils combinations, Antimicrobial, Food spoilage

INTRODUCTION

One third of all food produced in the world is lost to spoilage. This is equivalent to 1.3 billion tons per year. Food loss, from farm to fork, causes considerable environmental and economic effects. In spite of the modern improvements in food hygiene and food production techniques, food safety is an increasingly important public health issue [1]. It has been estimated that as many as 30% people in the industrialized countries suffer from food borne diseases each year, and in 2000 at least two million people died of diarrheal diseases worldwide [2].

Preservatives are the substances, which are used to prevent food spoilage from microorganism. This will inhibit the growth of micro-organisms like bacteria and fungi. The control of food spoilage and pathogenic micro-organisms is achieved mainly by chemical control but the use of synthetic chemicals is limited due to a number of undesirable aspects which include carcinogenicity, teratogenicity, acute toxicity and the requirement of extended degradation periods with the consequent development of environmental pollution problems. The awareness of modern consumers about these problems has resulted in a "green" consumer profile that demands the absence of synthetic chemicals in food preservation accompanied by the requirement for extended shelf life in the majority of food products in conjunction with the scientific community and agro-industries and pharmaceutical industries to search for natural compounds that may satisfy consumer requests [3]. There is therefore scope for new methods of making food safe which have a natural or 'green' image. One such possibility is the use of essential oils (EOs) as antibacterial additives [4, 5].

EOs also called volatile or ethereal oils, are aromatic oily liquids obtained from plant flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. Application of EOs as natural food preservatives is an intensively searched area. In foodstuffs, higher concentrations of the EOs are usually required for the antimicrobial action than *in vitro* because the food matrix can represent a physical hurdle [6]. Regarding that higher amount of essential oils are required for food preservation, their application can negatively influence taste or odor [7]. To avoid this unwanted side effect, several EOs can be combined with each other. Combinations of EOs seem to be good alternatives for future issues in food protection. Essential oil as a whole is strong antimicrobial product then its major components are alone [8]. So, in the

present study some of the important essential oils and their combinations will be briefly studied, to find out the best combination of oils in terms of its MIC.

MATERIALS

Essential oils: Oils used for study:

Cinnamon oil, Eucalyptus oil, Lemongrass oil, Rosemary oil, Clove oil.

METHODS

1. Collection of food samples: Different spoiled food samples were used to isolate micro organisms. Food samples were collected aseptically by means of sterilized sampling devices into sterile containers from Solan district of Himachal Pradesh and samples of perishable foods were refrigerated immediately. Labeling was done as to the type of food, the place and the time of sampling. Each sample was sealed [9].

2. Isolation and Identification of micro organisms: The isolation from food was done by first Serial dilution method and then grown on nutrient agar plates. The isolated colonies were identified by the traditional methods:

a. Bacteriological Analysis: It was done using selective media [10].

b. **Morphological Characterization:** All isolated microbes were characterized using morphological characterization and Gram staining [11, 12].

c. Biochemical Characterization: Biochemical Characterization was done by IMViC reactions i.e. Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test, Nitrate Reduction test, Lactose, Sucrose, Dextrose fermentation Reaction test by standard method [11, 12].

3. **Antibacterial activity**: Antibacterial activity was measured using Agar well diffusion method. On the Mueller Hinton agar plate's four wells were made of equal size. One well was positive control in which antibiotic was poured and other three wells were for test sample which were essential oils. Three different percentages of oil in DMSO i.e. 30 %, 40% and 50% were tested. On each plate fresh bacterial inocula was spread evenly. After filling wells the plates were incubated for 24 h at 37^{o}C. The experiment was performed in triplicate [13].

4. **Determination of minimal inhibitory concentration (MIC):** Investigation of the combined effects of essential oils was done by Checkerboard method. The Checkerboard method was performed using 96-well microtitre plates [14]. As models only two bacteria (one gram positive and one gram negative) showing maximum antibacterial activity was taken for further studies. Along the x axis one oil concentration was reduced to half in each subsequent well, whereas

other oil concentration was fixed at 50 µl. The bacteria tested were 80 µl in which well. Antibiotic was used as positive control and DMSO was used as negative control. The plate was incubated at 37^{o}C for 24 hrs after Readings were taken in ELISA reader. The combination mixture which showed maximum inhibition at lower concentration was taken as MIC.

RESULTS AND DISCUSSION

Most of the bacteria grow well at room temperature. They grow on fruits, vegetables or other foods and turn it to mushy or slimy in nature and even produce bad odor. This bad odor is produced because of the waste products generated by bacteria.

Morphological Characterization: A total of 36 isolates were isolated from 20 different food samples, which were then characterized according to their color, texture, shape, margin, arrangement, staining characteristics and morphology (Table1). Out of these 36 isolates 14 isolates were found to be gram negative rods, 16 were gram positive rods and 6 were gram positive cocci.

Biochemical Characterization: Isolates characterized by morphological characterization were then further characterized on the basis of biochemical analysis (Table 2). The biochemical tests revealed that the bacteria isolated from samples are *Bacillus, Klebsiella, Pseudomonas, E. coli, Lactobacillus, Staphylococcus, Micrococcus* and *Salmonella* sp. (table 2).

Antibacterial activity: Antimicrobial activity of the five essential oils (Lemon grass, Clove, Eucalyptus, Rosemary and Cinnamon) was tested against four gram positive (*Micrococcus sp., Lactobacillus sp., Staphylococcus sp., Bacillus sp.*) and four gram negative bacteria (*Klebsiella sp., E. coli, Pseudomonas sp., Salmonella sp*.) isolated from different types of food samples. Various food borne pathogens including *E. coli* O157:H7, *Salmonella Typhimurium*, *S. aureus*, *L. monocytogens*, *Campylobacter* and others are documented to show sensitivity against Essential oils [15]. Most of the essential oils showed best results at 40% concentration and with increase in concentration from 40% the inhibition zone size decreased. As it is clear from Table 3 and 4 as all the essential oils showed maximum antibacterial activity against *E. coli* (gram negative bacteria) and *Micrococcus sp*. (gram positive bacteria). Maximum zone of inhibition (28±0.5mm) was obtained against *E. coli* by cinnamon oil whereas; Rosemary oil at 40% conc. showed 28±0.4mm zone against *Micrococcus sp*. Based on these studies *E. coli* and

Micrococcus sp. were selected for further studies. **These two bacteria were identified as *E. coli* and *Micrococcus yunnanensis* on the basis of molecular studies.** Antibacterial property was shown by all the essential oils for most of the bacteria. Cinnamon oil also gave good results and lemon grass oil gave results for gram positive bacteria, best being in case of *Bacillus sp.* and *Micrococcus sp.* Overall results showed that gram positive bacteria are more susceptible for essential oils then the gram negative bacteria (table 3, 4).

S. No	Sample	Code	Morphology of colony	Simple staining	Gram staining	Suspects
1.	Apple	S1	Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
			Slimy, White, Somewhat translucent, raised growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli*
			Slimy, White, Somewhat translucent, raised growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli,*
3.	Egg	S3	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Pseudomonas sp.*
			Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
			Shiny, convex colonies with entire margins	Rods	Gram (-ve)	*Salmonella sp.*
4.	Curd	S4	Small ,creamy, whitish, convex colonies	Rods	Gram(+ve)	*Lactobacillus sp.*
5.	Milk	S5	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Pseudomonas sp.*
			Small ,creamy, whitish, convex colonies	Rods	Gram(+ve)	*Lactobacillus sp.*
			Soft, smooth, yellow growth	Cocci	Gram(+ve)	*Micrococcus sp., Staphylococcus sp.,*
			Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
6.	Ice cream	S6	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Pseudomonas sp.*
			Small ,creamy, whitish, convex colonies	Rods	Gram(+ve)	*Lactobacillus sp.*
			Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
7.	Bread	S7	Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
8.	Cooked food	S8	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Pseudomonas sp.*
			White, moist and glistening growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli,*
9.	Cucumber	S9	Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
			Abundant, Opaque, golden growth	Cocci	Gram(+ve)	*Micrococcus sp., Staphylococcus sp.,*
			Slimy, White, Somewhat translucent, raised growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli*
10.	Tomato	S10	Abundant, opaque, white waxy growth	Rods	Gram(+ve)	*Bacillus sp.*
			Slimy, White, Somewhat translucent, raised growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli*
			Abundant, Opaque, golden growth	Cocci	Gram(+ve)	*Micrococcus sp., Staphylococcus sp.*
			Abundant, opaque, white waxy growth	Rods	Gram(-ve)	*Pseudomonas sp.*
11.	Sugarcane juice	S11	Abundant, Opaque, golden growth	Rods	Gram(+ve)	*Bacillus sp.*
			Small ,creamy, whitish, convex colonies	Cocci	Gram(+ve)	*Micrococcus sp., Staphylococcus sp.,*
12.	Milk cream	S12	Small ,creamy, whitish, convex colonies	Rods	Gram(+ve)	*Lactobacillus sp.*
13.	Cheese	S13	Shiny, convex colonies with entire margins	Rods	Gram(+ve)	*Lactobacillus sp.*
14.	Meat	S14	Abundant, Opaque, golden growth	Rods	Gram (-ve)	*Salmonella sp.*
15.	Lady finger	S15	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Klebsiella sp., E. coli,*
16.	Ghee	S16	Small ,creamy, whitish, convex colonies	Cocci	Gram(+ve)	*Micrococcus sp. , Staphylococcus sp.*
			White, moist and glistening growth	Rods	Gram(-ve)	*Pseudomonas sp.*

17.	Honey	S17	Abundant, Opaque, golden growth	Rods	Gram(+ve)	*Lactobacillus sp.*
			White, moist and glistening growth	Rods	Gram(-ve)	*Klebsiella sp., E. coli,*
18.	Papaya	S18	Small ,creamy, whitish, convex colonies	Cocci	Gram(+ve)	*Micrococcus sp., Staphylococcus sp.*
19.	Capsicum	S19	Abundant, thin, white growth with medium turning green	Rods	Gram(-ve)	*Klebsiella sp., E. coli,*
20.	Buttermilk	S20	Abundant, Opaque, golden growth	Rods	Gram(+ve)	*Lactobacillus sp.*

Table 1: Morphological characterization of bacterial isolates isolated from spoiled food samples

Table 2: Biochemical characterization of bacterial isolates

Code	Indole	MR	VP	CU	NR	Lactose	Sucrose	Dextrose	Identified bacteria
S1a	-	-	-	-	+	-	A	A	*Bacillus sp.*
S1b	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S2a	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S2b	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S3a	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S3b	-	-	+	-	+	-	A	A	*Bacillus sp.*
S4a	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S5a	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S5b	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S5c	-	-	-	-	-	-	-	-	*Micrococcus sp.*
S5d	-	-	+	-	+	-	A	A	*Bacillus sp.*
S6a	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S6b	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S6c	-	-	+	-	+	-	A	A	*Bacillus sp.*
S7a	-	-	+	-	+	-	A	A	*Bacillus sp.*
S8a	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S8b	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S9a	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S9b	-	-	+	-	+	-	A	A	*Bacillus sp.*
S9c	-	+	-	-	+	A	A	A	*Staphylococcus sp.*
S10a	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S10b	-	-	+	-	+	-	A	A	*Bacillus sp.*
S10c	-	+	-	-	+	A	A	A	*Staphylococcus sp.*
S10d	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S11a	-	-	+	-	+	-	A	A	*Bacillus sp.*
S11b	-	-	-	-	-	-	-	-	*Micrococcus sp.*
S12	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S13	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S14	-	+	-	-	-	-	-	-	*Salmonella sp.*
S15	+	+	-	-	+	AG	A	AG	*E. coli*
S16a	-	+	-	-	+	A	A	A	*Staphylococcus sp.*
S16b	-	-	-	+	+	-	-	-	*Pseudomonas sp.*
S17a	-	-	-	-	+	+	+	+	*Lactobacillus sp.*
S17b	-	+	-	+	+	AG	AG	AG	*Klebsiella sp.*
S18	-	-	-	-	-	-	-	-	*Micrococcus sp.*
S19	+	+	-	-	+	AG	A	AG	*E. coli*
S20	-	-	-	-	+	+	+	+	*Lactobacillus sp.*

A= Acid, G= Gas, AG= Acid + gas

Table 3: Antibacterial activity of the essential oils against gram negative bacteria

Oils	*E. coli*				*Pseudomonas sp.*				*Klebsiella sp.*				*Salmonella sp.*			
	Zone of inhibition (mm) Concentration (%)															
	30	40	50	A	30	40	50	A	30	40	50	A	30	40	50	A
Rosemary	18± 0.1	25± 0.5	18± 0.3	29± 0.5	-	20± 0.5	17± 0.3	30± 0.5	18± 0.5	20± 0.5	17± 0.2	34± 0.3	11± 1	25± 0.4	16± 0.3	33± 0.5
Eucalyptus	20± 0.5	21± 0.4	22± 0.5	29± 0.5	15± 0.3	20± 0.2	-	32± 0.5	15± 0.6	18± 0.2	12± 0.5	33± 0.7	18± 0.2	18± 0.5	20± 0.7	35± 0.5

Clove	**10± 0.5**	**21± 0.1**	**13± 0.3**	**29± 0.4**	**-**	**-**	**18± 0.5**	**33± 0.5**	**11± 0.3**	**18± 0.1**	**17± 0.5**	**32± 0.5**	**-**	**12± 0.2**	**10± 0.8**	**35± 0.5**
Lemon grass	**-**	**-**	**-**	**30± 0.7**	**-**	**-**	**-**	**31± 0.3**	**-**	**-**	**-**	**33± 0.5**	**10± 0.5**	**-**	**-**	**33± 0.4**
Cinnamon	**25± 0.7**	**27± 0.1**	**28± 0.2**	**30± 0.5**	**22± 0.4**	**25± 0.2**	**23± 0.5**	**31± 0.6**	**19± 0.4**	**27± 0.3**	**25± 0.2**	**33± 0.7**	**25± 0.5**	**27± 0.6**	**22± 0.8**	**35± 0.4**

A= Antibiotic,-= no activity.

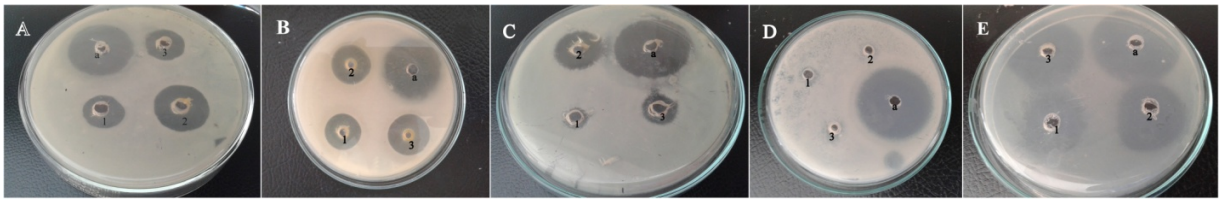

Figure 1: Antibacterial activity of Essential oils against *E. coli.* [A= Rosemary oil, B= Eucalyptus oil, C= Clove oil, D= Lemon grass oil, E= Cinnamon] [1=30% of oil, 2= 40% of oil, 3=50% of oil, a= antibiotic].

Table 4: Antibacterial activity of essential oils against gram positive bacteria

Oils	***Staphylococcus sp.***				***Lactobacillus sp.***				***Bacillus sp.***				***Micrococcus sp.***			
	Zone of inhibition (mm) Concentration (%)															
	30	**40**	**50**	**A**	**30**	**40**	**50**	**A**	**30**	**40**	**50**	**A**	**30**	**40**	**50**	**A**
Rosemary	**20± 0.2**	**18± 0.3**	**16± 0.5**	**32± 0.1**	**13± 0.5**	**20± 0.5**	**18± 0.5**	**33± 0.5**	**20± 0.2**	**22± 0.3**	**18± 0.1**	**31± 0.5**	**22± 0.4**	**28± 0.4**	**24± 0.3**	**31± 0.4**
Eucalyptus	**14± 0.1**	**18± 0.7**	**15± 0.6**	**31± 0.1**	**15± 0.5**	**20± 1**	**12± 0.6**	**33± 0.5**	**15± 0.1**	**18± 0.5**	**12± 0.3**	**30± 0.8**	**16± 0.6**	**20± 0.5**	**18± 0.4**	**31± 0.2**
Clove	**10± 0.5**	**17± 0.8**	**14± 0.5**	**30± 0.1**	**10± 0.3**	**15± 0.4**	**13± 0.6**	**35± 0.4**	**12± 0.3**	**18± 0.5**	**12± 0.4**	**31± 0.5**	**12± 0.2**	**20± 0.1**	**15± 0.5**	**30± 0.1**
Lemon grass	**-**	**12± 0.4**	**-**	**30± 0.1**	**-**	**-**	**-**	**35± 0.5**	**11± 0.2**	**14± 0.2**	**10± 0.7**	**30± 0.5**	**12± 0.3**	**25± 0.1**	**16± 0.5**	**30± 0.5**
Cinnamon	**21± 0.5**	**23± 0.5**	**25± 0.5**	**32± 0.5**	**18± 0.2**	**25± 0.5**	**24± 0.5**	**35± 0.5**	**18± 0.1**	**25± 0.5**	**22± 0.2**	**30± 0.5**	**21± 0.4**	**28± 0.2**	**26± 0.5**	**31± 1**

A= Antibiotic, -= no activity

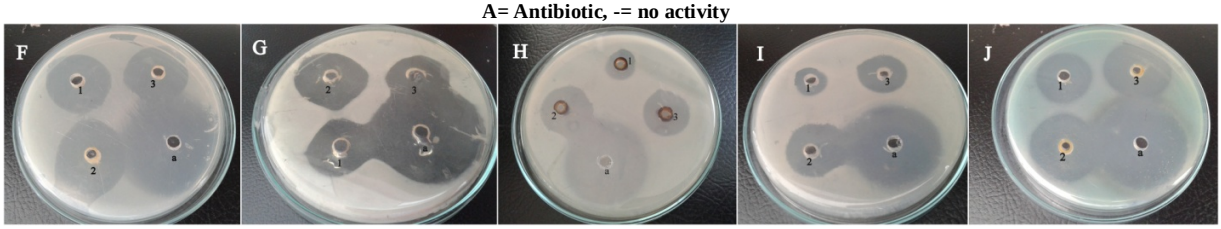

Figure 2: Antibacterial activity of Essential oils against *Micrococcus yunnanensis.* [A= Rosemary oil, B= Eucalyptus oil, C= Clove oil, D= Lemon grass oil, E= Cinnamon], [1 = 30% of oil, 2 = 40% of oil, 3 = 50% of oil, a= antibiotic].

Determination of MIC: The combination effect of oils on *E. coli* and *Micrococcus yunnanensis* (taken as models for further studies) was studied by Checkerboard method (Table 5(i) to 9(ii). Out of all the combinations of oils with rosemary oil (where rosemary oil concentration was changed and other oils concentration was fixed to 50µL) best results were seen with Eucalyptus oil for both *E. coli* and *Micrococcus yunnanensis*. For *E. coli* when 6.25µL of Rosemary oil was mixed with 50µL of Eucalyptus oil the MIC value was 0.125 and in case of *Micrococcus yunnanensis* MIC was 0.098 when Rosemary oil was 12.5µL and Eucalyptus oil was 50µL [Table 5(i) and 5(ii)]. From Tables 6(i) and 6(ii) it was concluded that for Eucalyptus oil

the best combination was with Rosemary oil (other oils concentration fixed to 50 µL and Eucalyptus oil concentration changed). For *E. coli* MIC was 1.102 when Eucalyptus oil was used at concentration of 12.5µL with rosemary oil (50µL) and for *Micrococcus yunnanensis* the best MIC of 1.131 was shown when Eucalyptus oil was 6.25µL and Lemon Grass oil was 50 µL. Lemon grass oil combinations showed better results than the oil when used alone. When lemon grass oil concentration was changed and other oils concentration was fixed, it gave best result for *E. coli* (MIC= 1.066) with clove oil at6.25 µL conc. and for *Micrococcus yunnanensis* (MIC=0.886) with cinnamon oil (Table 7 i and 7 ii) at conc.12.5µL conc. In case of clove oil (table 8 i and 8 ii), its best combination for *E. coli* was with cinnamon oil (MIC=1.444) at 50µL. For *Micrococcus yunnanensis* clove oil at 12.5µL concentration gave MIC of 0.521 with Eucalyptus oil. Clove oil gave better result alone then in combination with other oils. Again better results for *Micrococcus yunnanensis* (MIC= 0.299) was seen by clove oil alone at 25µL concentration. The best Cinnamon oil combination for *E. coli* (MIC=0.852) was with Eucalyptus oil at 25µL concentration and for *Micrococcus yunnanensis* MIC=0.833 was shown with clove oil at 6.25µL. Cinnamon oil alone gave good results for both bacteria, MIC=0.192 at 25µL for *Micrococcus yunnanensis* and 0.343 for *E. coli* at 12.5µL as shown in tables 9(i) and 9(ii).

Out of all the combinations studied best of all was Rosemary and Eucalyptus oil combination for both the bacteria. The concentration of essential oils used can be effectively being reduced when mixed by other oils clear from the results given in tables 5 to 9. This practice can reduce the undesirable effects. The combination therapy of oils is much effective on gram positive bacteria then on gram negative bacteria, the result [tables 5 to 9] of this study confirm these already known findings [16].

Table 5 (i): Determination of MIC of rosemary with other oils for *E. coli.*

Concentration of other oils (50µL)	Rosemary oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Eucalyptus	0.431	0.279	0.248	0.245	0.125	0.458
Lemon grass	0.592	0.365	0.289	0.366	0.438	0.445
Clove	0.596	0.408	0.533	0.634	0.751	0.799
Cinnamon	0.673	0.455	0.743	0.817	0.922	0.914
Rosemary oil alone	1.417	1.304	1.186	1.224	1.262	1.313
Control	0.100	0.098	0.100	0.099	0.100	0.100

Table 5 (ii): Determination of MIC of rosemary with other oils for *Micrococcus yunnanensis:*

Concentration of other oils (50µL)	Rosemary oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Eucalyptus	0.376	0.294	0.127	0.098	0.122	0.113
Lemon grass	0.673	0.294	0.334	0.399	0.437	0.44
Clove	0.658	0.46	0.566	0.668	0.718	0.919
Cinnamon	0.792	0.464	0.737	0.940	0.937	0.985
Rosemary oil alone	1.482	1.379	1.263	1.188	1.253	1.246
Control	0.093	0.095	0.092	0.093	0.094	0.094

Table 6 (i): Determination of MIC of Eucalyptus oil with other oils for *E. coli:*

Concentration of other oils (50µL)	Eucalyptus oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.116	1.236	1.254	1.102	1.166	1.178
Lemon grass	1.411	1.408	1.152	1.159	1.148	1.172
Clove	1.527	1.301	1.345	1.363	1.348	1.286
Cinnamon	1.659	1.607	1.624	1.921	1.974	1.222
Eucalyptus oil alone	1.488	1.377	1.238	1.013	1.805	1.728
Control	0.101	0.095	0.101	0.099	0.100	0.101

Table 6(ii): Determination of MIC of Eucalyptus oil with other oils for *Micrococcus yunnanensis:*

Concentration of other oils (50µL)	Eucalyptus oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.155	1.144	1.156	1.231	1.195	1.190
Lemon grass	1.481	1.395	1.177	1.176	1.131	1.165
Clove	1.465	1.413	1.272	1.266	1.227	1.217
Cinnamon	1.795	1.56	1.739	1.826	1.881	1.908
Eucalyptus oil alone	1.116	1.006	1.103	1.174	1.156	1.178
Control	0.093	0.095	0.092	0.093	0.093	0.093

Table 7 (i): Determination of MIC of Lemon grass oil with other oils for *E. coli:*

Concentration of other oils (50µL)	Lemon grass oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.044	1.612	1.36	1.291	1.197	1.19
Eucalyptus	1	1.583	1.215	1.151	1.128	1.132
Clove	1.408	1.241	1.175	1.102	1.066	1.105
Cinnamon	1.327	1.255	1.235	1.374	1.398	1.359
Lemon grass oil alone	1.224	1.135	1.187	1.297	1.306	1.375
Control	0.101	0.099	0.101	0.099	0.100	0.101

Table 7 (ii): Determination of MIC of Lemon grass oil with other oils for *Micrococcus yunnanensis:*

Concentration of other oils (50µL)	Lemon grass oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.932	1.566	1.382	1.253	1.195	1.179
Eucalyptus	1.881	1.553	1.301	1.159	1.170	1.196
Clove	1.271	1.148	1.068	0.994	0.997	1.006
Cinnamon	1.185	1.135	0.918	0.886	0.913	0.933
Lemon grass oil alone	1.332	1.282	1.319	1.363	1.393	1.379
Control	0.093	0.092	0.092	0.094	0.094	0.094

Table 8 (i): Determination of MIC of Clove oil with other oils for *E. coli:*

Concentration of other oils (50µL)	Clove oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.669	1.807	1.501	1.261	0.902	0.895
Eucalyptus	1.474	1.941	1.364	1.282	1.179	1.165
Lemongrass	1.511	1.323	1.137	0.954	0.805	0.612

Cinnamon	1.56	1.444	1.455	1.329	1.288	1.193
Clove oil alone	0.757	0.748	1.000	1.108	1.176	1.253
Control	0.100	0.097	0.101	0.099	0.102	0.102

Table 8 (ii): Determination of MIC of Clove oil with other oils for *Micrococcus yunnanensis:*

Concentration of other oils (50µL)	Clove oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.384	0.957	0.946	0.827	0.824	0.822
Eucalyptus	1.182	0.657	0.633	0.521	0.577	0.484
Lemongrass	1.256	1.061	0.803	0.649	0.629	0.558
Cinnamon	1.361	1.174	1.213	1.258	1.132	1.116
Clove oil alone	0.69	0.485	0.299	0.348	0.913	1.01
Control	0.092	0.092	0.092	0.093	0.094	0.094

Table 9 (i): Determination of MIC of Cinnamon oil with other oils for *E. coli:*

Concentration of other oils (50µL)	Cinnamon oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.631	1.22	0.912	0.811	0.807	0.541
Eucalyptus	1.515	1.225	0.852	0.983	0.734	0.429
Lemongrass	1.629	1.284	1.123	0.925	0.774	0.674
Clove	1.66	1.49	1.401	1.327	1.337	1.337
Cinnamon oil alone	0.698	0.569	0.513	0.343	0.348	0.361
Control	0.100	0.101	0.101	0.099	0.101	0.101

Table 9 (ii): Determination of MIC of Cinnamon oil with other oils for *Micrococcus yunnanensis:*

Concentration of other oils (50µL)	Cinnamon oil conc. (µL)					
	100	50	25	12.5	6.25	3.125
Rosemary	1.272	0.929	0.813	0.686	0.619	0.578
Eucalyptus	1.241	0.823	0.679	0.782	0.587	0.559
Lemongrass	1.335	1.031	0.771	0.668	0.575	0.543
Clove	1.368	1.105	0.963	0.856	0.833	0.874
Cinnamon oil alone	0.693	0.381	0.192	1.012	1.023	1.146
Control	0 .092	0.093	0.092	0.093	0.094	0.092

CONCLUSION

Klebsiella sp., E. coli, Bacillus sp., Lactobacillus sp., Pseudomonas sp., Micrococcus sp. and *Staphylococcus sp.* were the main food spoiling micro-organisms in food samples taken in this study. In the present study 36 isolates were collected from 20 different spoiled food samples, in which *Bacillus sp., Klebsiella sp., Lactobacillus sp., Pseudomonas sp.* and *Micrococcus sp.* were the main species responsible for spoilage. This study shows the best combination of two popular oils, rosemary and eucalyptus which was determined on the basis of MIC they showed for *E. coli* and *Micrococcus yunnanensis* isolated from spoiled food samples. This combination of oils should be further briefly studied for its potential to be used in food preservation and modern medicine. The essential oils derived from plants are important source of antimicrobials. As the method to study, bacterial strains and sources from which antimicrobials are derived are different, so it makes it difficult

to compare the results. The different antimicrobial has different mode of action. So, to achieve desired result different oils can be combined as shown in this study.

REFERENCES

[1] WHO. Food safety and foodborne illness. World Health Organization Fact sheet 237, revised January, Geneva; 2002.

[2] Burt S. Essential oils their antibacterial properties and potential applications in foods — a review, *Inter. J. Food Micro, 2014;* 94: 223-253.

[3] Lopez P, Sanchez C, Batlle R, Nerin C. Solid- and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains, Journal of Agricultural and Food Chemistry, 2005; 53: 6939–6946.

[4] Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae, International Journal of Food Microbiology, 2001; 67: 187–195.

[5] Yamazaki K, Yamamoto T, Kawai Y, Inoue N. Enhancement of antilisterial activity of essential oil constituents by nisin and diglycerol fatty acid ester, Food Microbiology, 2004; 21: 283–289.

[6] APHA (1998). Standard Methods for the Examination of Water and Wastewater, 18th Edn: American Public Health Association, Washington DC; 1998; 45-60.

[7] Prescott LM, Harley JP, Klein DA. Microbiology. 5th Edn: McGraw-Hill, New York; 2002; 1014.

[8] Holt JG, Krieg NR, Senath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology 9th Edn: Baltimore Md Williams and Wilkins; 1994.

[9] Sherman N, Cappuccino JG. Microbiology: a laboratory manual. 6th Edn: ISBN; 2005; 81(3):265-267.

[10] National Committee for Clinical Laboratory Standard. Performance Standards for Antimicrobial Disc Susceptibility Test. Approved Standard NCCLS Pubication, Villanova, PA, USA, M2-A-5; 1993.

[11] Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils, *Fitotera*, 2006; 77: 279-285.

[12] McAllister JC, Adams MF. Mode of Action for Natural Products Isolated From Essential Oils of Two Trees Is Different From Available Mosquito Adulticides, *J. Medl. Entomol, 2010;* 47: 1123-1126.

[13] Guenther E. The Essential Oils. D. Van Nostrand, New York, 1948.

[14] Nychas GJE. Natural antimicrobials from plants. In: Gould, G.W. Edn: New Methods of Food Preservation, Blackie Academic and Professional, London; 1995: 58– 89.

[15] Callaway TR, Carroll JA, Arthington JD, Edrington TS, Anderson RC, Ricke SC et al. Citrus products and their use against bacteria: potential health and cost benefits (chap. 17). In Watson R, Gerald JL, Preedy PR (Eds.), Nutrients, dietary supplements, and nutriceuticals: Cost analysis versus clinical benefits, New York, NY: Humana Press; 2011: 277-286.

[16] Van de Braak SAAJ, Leijten GCJJ. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union, CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam; 1999: 116.