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DETERMINATION OF ANTI MICROBIAL ACTIVITY OF ESSENTIAL OILS IN DIFFERENT CONCENTRATIONS

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ABSTRACT

Preservatives are added to pharmaceuticals to ensure safety and shelf life. A number of Essential oil components are identified as effective antimicrobials. The antimicrobial activity is assessed of pure oils. Oils were evaluated based on Refractive index, Specific gravity and Antimicrobial activity. In present study Syrups were prepared using Peppermint, Rose and Orange oils in different concentrations and antimicrobial activity was determined by using same syrups and compared with antimicrobial activity of pure oils determined by using same concentrations as used in syrups at the end of 7, 14 and 28 days. It was found that pure oils showed higher activity than syrups and only peppermint oil used in concentration of 2% showed antimicrobial activity after 28 days against *S. aureus.* Syrups containing Rose oil showed activity against *E. coli* in concentrations of 1% and 2%, against S. *aureus* only 2% syrup showed activity. All oils were found to be more active against Gram +ve microorganisms than Gram –ve microorganisms.

INTRODUCTION: Food products are perishable by nature and require protection to prevent spoilage during their preparation, storage and distribution to give them desired shelf life. Several techniques such as heating, refrigeration and addition of antimicrobial compounds are being currently used. Within the disposable arsenal of preservation techniques, the food industry investigates more and more the replacement of traditional food preservation techniques by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products. An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives. Recent approaches are increasingly directed towards possibilities offered by biological preservation. Originally added to change or improve taste, spices and herbs can also enhance shelf-life because of their antimicrobial nature. One such possibility is the use of essential oils (EOs) as antimicrobial additives [1-4].

Essential Oils: An **essential oil** is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20-70%) compared to others components present in trace amounts. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. The use of essential oils as antimicrobial agents, there synergistic and antagonistic effects and potential as biological preservatives has been discussed [5-7, 10].

The oils from Mint and Peppermint were found to be more effective against Gram positive bacteria [8]. (Leopold *et al*. 2009). Studies on Antimicrobial activities of different forms of Peppermint viz., aqueous, infusion, decoction, juice and essential oil against 11 different species of Gram-negative bacilli has also been done [9]. The aim of present study was to determine antimicrobial activity of essential oils namely Peppermint Oil, Rose Oil and Orange Oil, in different concentrations (0.5%, 1.0%, 2.0%) added to Paracetamol syrup during formulation (**Table 3**) and testing antimicrobial activity of oil in syrup after 7, 14 and 28 days of preparation by using agar diffusion method and to determine whether any linear relationship is seen or not with increasing concentration of essential oil in syrup formulations.

MATERIALS AND METHODS:

Essential Oils: Oils were obtained from Loba Chemicals. Oils were evaluated based on Refractive Index, Specific Gravity (**table 1**) and Antimicrobial Activity (**table 2**).

TABLE 1: PHYSICO- CHEMICAL EVALUATION

Sl. No.	Oil	Color	Odor	Taste	Refractive Index		Specific Gravity	
					As per USP	Lab	As per USP	Lab
1	Peppermint Oil	Light green	Characteristic	Pungent leaving a cooling sensation	1.459 - 1.465	1.33 - 1.35	1.459 - 1.465	1.54 - 1.59
2	Orange Oil	Yellowish	Characteristic	Characteristic	1.472 - 1.474	1.34 - 1.36	1.457 - 1.463	1.54 - 1.58
3	Rose Oil	Reddish Yellow	Characteristic	Soothing	1.457 - 1.463	1.33 - 1.35	1.472 - 1.474	1.53 - 1.59

Media: Nutrient agar media was prepared by dissolving 7 gm of Beef extract and 7 gm of peptone separately in water, both were mixed, 3.5 gm of sodium chloride added. Volume made up to 700ml and pH adjusted to 7.2- 7.4. Solution slightly warmed and agar added and autoclaved.

Microorganisms: *E. coli* (Gm –ve) and *S. aureus* (Gm +ve) *were* used in present study.

TABLE 2: ZONE OF INHIBITION FOR PURE OILS

Oil in concentration used in syrups	*S. aureus*	*E. coli*
P1 (0.5%)	25mm	23mm
P2 (1.0%)	38mm	36mm
P3 (2.0%)	42mm	38mm
O1 (0.5%)	18 mm	16mm
O2 (1.0%)	24mm	23mm
O3 (2.0%)	30mm	28mm
R1 (0.5%)	25mm	26mm
R2 (1.0%)	35mm	34mm
R3 (2.0%)	38mm	36mm

General Syrup Preparation Method:

1. Prepare a mixture of water, 1, 2 propylene glycol, glycerol and sorbitol.
2. Slowly add oil to the above solution.
3. Dissolve Kollidon 25 and sodium saccharin in it.
4. Slowly add acetaminophen and keep stirring till drug is dissolved.

Evaluation of Syrups: Syrups were evaluated for pH and viscosity

1. pH of syrups.
2. Viscosity of Syrup without oil: Viscosity of syrup was determined using Brookfield Viscometer using spindle no. 61.

TABLE 3: FORMULA FOR SYRUPS

Ingredients	P 1 (0.5%)	P 2 (1.0%)	P 3 (2.0%)	R 1 (0.5%)	R 2 (1.0%)	R 3 (2.0%)	O 1 (0.5%)	O 2 (1.0%)	O 3 (2.0%)	Syrup without oil
Acetaminophen	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sorbitol	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sodium Saccharin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Kollidon 25	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Glycerol	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
1, 2- Propylene Glycol	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Peppermint Oil	0.5	1.0	2.0	-	-	-	-	-	-	-
Rose Oil	-	-	-	0.5	1.0	2.0	-	-	-	-
Orange Oil	-	-	-	-	-	-	0.5	1.0	2.0	-
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Note: P: Peppermint oil, R: Rose oil, O: Orange oil.

Antimicrobial activity study: To establish antimicrobial activity media above mentioned was added in Petri plates and activity for pure oils and syrups were evaluated by Diffusion method. Pure Oils were diluted with n- Hexane in same concentration as were used in syrups.

RESULTS AND DISCUSSION: Oils were evaluated generally and based on Refractive Index, Specific Gravity and Antimicrobial Activity. Results are shown in Table 1 and 2. It was found that pure oils were more active against Gram +ve microorganisms than Gram –ve microorganisms. Stable syrups were prepared using different Essential oils as shown in Table 3 and Antimicrobial activity of pure oils and Syrups were evaluated. pH of syrups prepared was determined and it was found to increase with time as shown in **Table 4(a)** and viscosity of plain syrup determined using Brookfield viscometer is shown in **Table 4(b)**.

Out of the 9 syrups prepared only syrup containing Peppermint oil (2%) showed Antimicrobial activity at the end of 28 days against *S. aureus*. {Shown in **Table 5 (a) and (b)**} Syrups containing Orange oil did not show any activity against any of the Microorganisms used during the time duration of study. Syrups containing Rose oil in concentrations of 1% and 2% showed activity against *E. coli* for 14 days and against *S. aureus* syrup containing 2% oil showed activity.

TABLE 4 (a): PH OF SYRUPS

Syrup	pH at day of preparation	pH at 28 days
Syrup without oil	4.30	3.92
P1 (0.5%)	4.32	4.03
P2 (1.0%)	4.33	3.86
P3 (2.0%)	4.50	3.88
O1 (0.5%)	4.45	4.08
O2 (1.0%)	4.72	4.09
O3 (2.0%)	4.80	4.21
R1 (0.5%)	4.70	3.98
R2 (1.0%)	4.75	3.90
R3 (2.0%)	4.82	3.85

TABLE 4 (b): VISCOSITY OF PLAIN SYRUP

Syrup	RPM	Spindle No.	Viscosity (cps)
Syrup at day of preparation	6	61	366.0
Syrup at 28th day	6	61	579.6

TABLE 5 (a): ZONE OF INHIBITION FOR *E. COLI*

Syrup	07 Days	14 days	28 days
Syrup without oil	-	-	-
P1 (0.5%)	-	-	-
P2 (1.0%)	-	-	-
P3 (2.0%)	16mm	16mm	-
O1 (0.5%)	-	-	-
O2 (1.0%)	-	-	-
O3 (2.0%)	-	-	-
R1 (0.5%)	-	-	-
R2 (1.0%)	14mm	14mm	-
R3 (2.0%)	16mm	16mm	-

TABLE 5 (b): ZONE OF INHIBITION FOR *S. AUREUS*

Syrup	07 Days	14 days	28 days
Syrup without oil	-	-	-
P1 (0.5%)	-	-	-
P2 (1.0%)	-	-	-
P3 (2.0%)	18 mm	16mm	14mm
O1 (0.5%)	-	-	-
O2 (1.0%)	-	-	-
O3 (2.0%)	-	-	-
R1 (0.5%)	-	-	-
R2 (1.0%)	-	-	-
R3 (2.0%)	16mm	16mm	-

Images for Zone of Inhibition:

1. Peppermint oil syrup (2%) against S. aureus

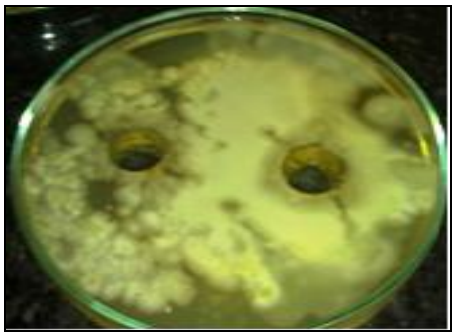

FIG. 1: 07 DAYS

FIG. 2: 14 DAYS

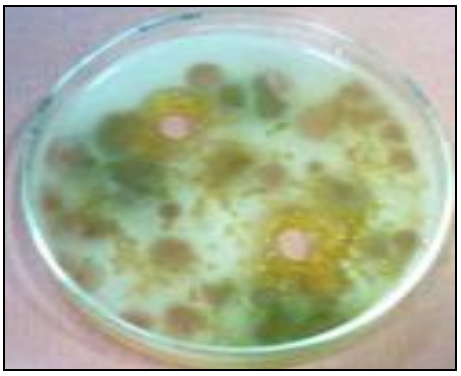

FIG. 3: 28 DAYS

2. Rose oil syrup (2%) against *S. aureus*

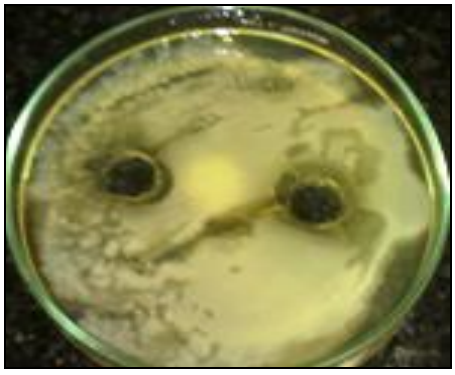

FIG. 4: 07 DAYS

FIG. 5: 14 DAYS

3. Peppermint oil syrup (2%) against *E. coli*

FIG. 6: 07 DAYS

FIG. 7: 14 DAYS

4. Rose oil syrup (2%) against *E. coli*

FIG. 8: 07 DAYS

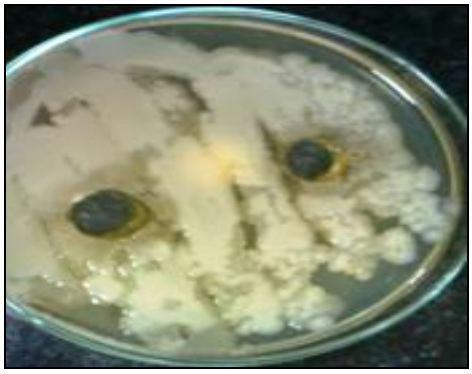

FIG. 9: 14 DAYS

CONCLUSION: From above study done it can be concluded that Pure Essential oils were found to have Antimicrobial activity against Gm +ve and Gm-ve and there was no proportional increase in Antimicrobial activity with increasing concentration of oils used in syrups.

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