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Antioxidant and Antibacterial Studies on Essential Oils Used as Alternatives for Chemical Preservatives

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ABSTRACT

The present study mainly focused for the development of replacements for the synthetic chemical additives in food products and cosmetics. The essential oils of Tulsi (Ocimum sanctum), Lemongrass (Cymbopogon nardus), Garlic (Allium sativum), Marigold (Tagetes patula) and Rice (Ricebran oil from husk of Oryza sativa) were selected for the microemulsion preparation. High energy emulsification method achieved using Ultrasonication and the prepared microemulsions were tested for radical scavenging activity using DPPH method to determine the antioxidant levels and the antimicrobial properties by using agar diffusion and micro dilution methods on Pseudomonas aeruginosa. The zone of inhibition obtained was compared with that of control having only the antibiotic, it was found to be increased by 25% for antibiotic 1 (Ciprofloxacin) and by 26.67% for antibiotic 2 (Cotrimoxazole). The maximum antioxidant activity of 66.5% found in the TCM blend indicates that the antimicrobial and antioxidant activity can be exploited the TCM blend as the ideal food and cosmetic preservative.

Keywords: Essential oils, Antibacterial activity, *Pseudomonas aeruginosa*, Antioxidant, DPPH.

INTRODUCTION

Essential oils (EOs), also called volatile oils, are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. There are many studies carried out in the in vitro conditions, which evidence microbial activity of herbal extracts [1–3] and essential oils [4–6]. Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity [7]; cinnamon oil also possesses anti-diabetic property [8]. Anti-inflammatory activity has been found in basil [9]. Lemon and rosemary oils possess antioxidant property [10, 11]. Peppermint and orange oils have shown anticancer activity [12, 13]. EOs have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, and prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts [14, 15]. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidized [16]. The highest scavenging activity of DPPH radical was observed for clove and origanum EOs with the EC_{50} values of 35.7±1.2 and 46.8±0.4 µg/mL, respectively [17].

Among essential oils from roots, plai essential oil showed the highest DPPH radical scavenging activity, followed by turmeric and ginger essential oil, respectively [18]. Free radical scavenging activity (DPPH assay) and reducing

power of essential oil from Thymus longicaulis subsp. These results show the variations in the antioxidant activity with source of essential oils [19]. EOs have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [20, 21]. Lavender and cinnamon oils exhibited approximately 1–2 times higher activity against *S. aureus* and *E. coli* in comparison with results of others [22, 23]. The antimicrobial activity against most gram-positive and gram-negative bacteria was reported (24). A higher antimicrobial activity of EOs was observed on gram-positive bacteria than gram-negative bacteria [25]. Preservatives are added to cosmetics to maintain their microbiological purity during manufacture, packing, storage, but especially during the entire period of use. So that these natural compounds can successfully be used in the cosmetic industry as a preservative.

Antibacterial study

In this study, the effect of essential oils on the growth of *Pseudomonas aerugonosa* was tested. Different concentrations of essential oils and essential oil blends were prepared (Table 1) and they were serially diluted to about 2 % v/v. The zone of inhibitions obtained for these oils and their blends are as shown in the Figure 1. When the zone of inhibition obtained was compared with that of control having only the antibiotic, it was found to be increased by 25% for antibiotic 1 (Ciprofloxacin) and by 26.67% for antibiotic 2 (Cotrimoxazole). The antimicrobial efficiency of antibiotic (Ciprofloxacin) was also checked by mixing microemulsion in them. The results obtained were compared for the Marigold + Antibiotic, Blend + Antibiotic, Marigold microemulsion + Antibiotic and Blend microemulsion + Antibiotic. The comparison is clearly shown for *P. aeruginosa* in the Figure 2. This proves that, blend T (30 µL) C (30 µL) M (40 µl) is the best amongst them with diameter 17 mM.

Table 1. Concentrations of EOs and EO blends

Blend No.	Blend name	Conc. (µL)
Blend 1	TCM	30, 30, 40
Blend 2	TCR	30, 30, 40
Blend 3	GCM	30, 30, 40
Blend 4	TRM	30, 50, 20
Blend 5	CMR	30, 50, 20
Blend 6	GRM	30, 20, 50

The increase in efficacy of antibiotic disk Bacitracin mixed with different EO is estimated as 9 mM (Control), 10 mM (T), 12 mM (M), 9.5 mM (R), 9.5 mM (C), 10 mM (G). Effect of Bacterial Efflux Pump can be studied from this. The results obtained for the blends of essential oils also gave different results. Six blends were developed for this study, which are as follows in table 2.

Table 2. Determination of antimicrobial activity

Combined Extract code	Combined Extract name	Concentration	Zone of Inhibition
EC 1	TCM	30g, 30g, 40g	17 mm
EC 2	TCR	30g, 30g, 40g	14 mm
EC 3	GCM	30g, 30g, 40g	9 mm
EC 4	TRM	30g, 50g, 20g	12 mm
EC 5	CMR	30g, 50g, 20g	7 mm
EC 6	GRM	30g, 20g, 50g	5 mm

Antioxidant study

As per the results depicted in Table 3 and 4, it was clearly understood that the antioxidant activity of the combined extracts was high for TCM (66.50±2.56). The calculated % inhibition indicates that the compounds TRM and GRM showing less activity when compare to rest compounds while the compounds TCR and CRM showing almost an equal activity when compare to the standard ascorbic acid. Also the IC_{50} value found for TCM was almost equal to the standard, but it was slightly less for the compounds TCR and CRM, only a negligible variation found for TCM with the standard. From all these analysis, except the compounds GRM TRM and GCM rest all plant extracts were showed best antioxidant activities. Essential oil blend alone and combined plant extracts inhibitory results were coincided together for all tested blends (Table 3&4).

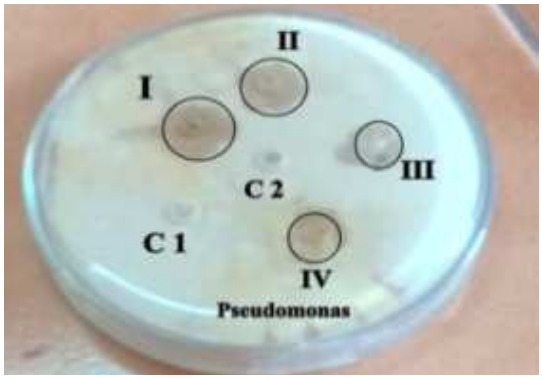

Figure 2. Agar well diffusion results for combined studies, C1- Control 1 (Distilled water), C2 Control 2 (DMSO), I. Blend microemulsion + Antibiotic (17 mm), II. Marigold microemulsion + Antibiotic (14 mm), III. Marigold + Antibiotic (9 mm), IV. Blend+ Antibiotic (12mm)

Table 3. Inhibitory percentage results of EO blend*

Combined plant extract	Antioxidant activity %	IC_{50} value
TCM	63.99±4.02	18.02 ug/ml
TCR	38.91±3.12	20.31 ug/ml
GCM	06.56±1.56	47.21 ug/ml
TRM	04.45±1.01	60.23 ug/ml
CRM	40.15±3.12	22.48 ug/ml
GRM	03.18±0.56	128.2 ug/ml
Ascorbic acid	62.56±6.41	18.23 ug/ml

**Data were expressed as mean ± SD (n = 4), statisti cally significant differences are at P < 0.05, ** Data were expressed as mean ± SD (n = 4)*

Table 4. Inhibitory percentage results combined plant extracts*

Combined plant extract	Antioxidant activity %	IC_{50} value
TCM	56.99±4.02	28.78 ug/ml
TCR	48.91±3.12	30.01 ug/ml
GCM	09.56±1.56	85.21 ug/ml
TRM	07.45±1.01	80.23 ug/ml
CRM	46.15±3.12	30.98 ug/ml
GRM	02.18±0.56	148.6 ug/ml
Ascorbic acid	58.56±6.41	24.23 ug/ml

**Data were expressed as mean ± SD (n = 4), statisti cally significant differences are at P < 0.05, ** Data were expressed as mean ± SD (n = 4)*

Preservative test for EO blends

Nearly 3-4 grams of freshly prepared wheat was taken from the flour mill. One was made as experimental set; while the other was the control.10 ml oil blend (a blend of Tulsi-4 ml, Marigold-3 ml and Rice bran oil-3 ml) was added to the experimental set, while water was added in the other one, to make it dough. It was left in open for around a fortnight. After 15 days, the one with oil blend showed lesser fungal growth, while the one with water had started growing fungus on it. It shows the efficacy of oil blends in preserving the food. The similar procedure was repeated for Marigold oil alone and its efficacy was tested as a preservative. Surprisingly, it alone gave a good result and helped in preserving wheat for longer period of time.

Figure 4a. Control and Marigold, 4b.Control and experimental (with oil blend) set after 24 hours.

Figure 3. Control and experimental (with oil blend) set after 24 hours.

Wheat germ is a good source for vitamin D, E which helps in nourishing the skin and is gentle enough for dry and sensitive skin**.** 1 tea spoon of milk, overnight soaked almond paste, honey, aloevera gel and 3 drops of EO blend was added (Marigold-40 µl, Garlic-40 µl & Tulsi-20 µl). A control was prepared without oil blend; while other had ingredients and both preparations were kept for 24 hours. The controls got spoilt within 16 hours, while the experimental set survived for the entire day (Fig. 3, 4a and 4b).

MATERIALS AND METHODS

Essential oils and root extracts of Tulsi (*Ocimumsanctum*), Lemongrass (*Cymbopogannardus*), Garlic (*Allium sativum*), Marigold (*Tagetespatula*) and Rice (Ricebran oil from husk of *Oryza sativa*) was used for this study. High energy emulsification methods use mechanical energy capable of generating intense disruptive forces that breakup the oil and water phases and lead to the formation of tiny oil droplets [26]. A mixture of 5 ml ethanol (co-surfactant), 5 ml Tween 20 (surfactant), 80 ml Distilled water and 10 ml Marigold oil was taken in a beaker. In another beaker, while keeping everything else same, a blend is used (TCM). This was then allowed in a plastic beaker filled with ice and then kept under a sonication probe for 10 minutes, with a pulse 5 seconds, pulse interval 3 seconds, amplitude 35, and the temperature 30^{o} C. Sonication uses the probe that radiated ultrasonic waves to break down the macroemulsion by using gravitational forces. A cloudy clear solution indicates a microemulsion has formed.

Antimicrobial activity

Microemulsions prepared are tested for antimicrobial activities on *Pseudomonas aeruginosa*. From the agar well diffusion method the zones of inhibition obtained are measured in millimeter and compared with the zones obtained using essential oil/ blend alone without emulsion.

DPPH - Radical scavenging activity studies

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method of Blois [27] with a slight modification [28]. 4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) is mixed with 3.3 ml methanol. It is shielded from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was noted at 517nm as control. 50 µl of different concentrations of oil lends and standard compound were taken and the volume was made upto 150 µl using methanol. The samples were then diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 minutes at 517nm using methanol as blank on UV-visible spectrometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was

prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to Mensor [29].

The percentage inhibition of the DPPH radical was calculated using the following formula

$$I\ \% = \frac{A_0 - A}{A_0} \times 100$$

Where I = DPPH inhibition (%), A0 = absorbance of control sample (t = 0 h) and A = absorbance of a tested sample at the end of the reaction (t = 1 h).

Statistical Analysis

All results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA with Dunnett's post test was performed. GraphPad Prism version 6.07 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The 50% inhibitory concentration (IC_{50}) was calculated from the dose response curve obtained by plotting percentage inhibition verses concentrations.

CONCLUSION

In this study, the aqueous extracts of Marigold were effective against *P. aeruginosa.* Citronella Ethyl acetate root extract showed best result against *Pseudomonas* MIC value with15 mg/ml. Anna Herman *et al.* 2013 [31], were reported that tea tree oil inhibited the growth of all studied microbes; however its action against *P. aeruginosa* was weak which demonstrated the resistance of *P. aeruginosa* on action of tea tree oil [32, 33]. Marigold, Rice bran and Garlic showed almost similar result with average 13 mm zone of inhibitions, but Marigold lead amongst the three of them. Thus, stating the efficiency potential of Marigold as a preservative. The blend TCM was found to be the best one amongst the all prepared. Amongst the five essential oils, Tulsi along with Citronella oil showed remarkable antimicrobial effect against all the microbes used for this study. Whereas the Marigold oil showed a good anti-microbial activity against *P. aeruginosa*.

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