



FORMULATION AND EVALUATION OF AN AROMA THERAPY ROLL-ON USING MANDARIN ORANGE PEEL (CITRUS RETICULATA) ESSENTIAL OIL

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ABSTRACT:

Mandarin oranges (*Citrus reticulata*) are cultivated on a large scale in Southeast Asia, and their peel is an excellent source of essential oil, which is largely used in aromatherapy preparations. The aim of this study was to isolate and identify mandarin essential oil, prepare it as a roll-on aromatherapy product, and conduct a thorough physical analysis. Mandarin peels were hydro distilled to extract essential oil and then examined for physicochemical properties. Gas Chromatography-Mass Spectrometry (GC-MS) identified 18 chemical constituents of which the major one was limonene (28.58%). The product also contained lemongrass oil, menthol, coconut oil, and camphor. Physical characteristics like viscosity, stability, specific gravity (0.91 g/mL), and antibacterial activity were measured. The formulation inhibited *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus acidophilus*. The yield of the essential oil was 0.54%, and formulation M1 showed the maximum aroma intensity with a duration of almost five hours. In general, the roll-on aromatherapy satisfied all physical assessment criteria and showed significant antibacterial activities. This indicates that mandarin essential oil can be used successfully in topical aromatherapy use for sensory and therapeutic purposes.

Key words: *Citrus reticulata*, Aromatherapy, Antibacterial, Roll on, Bioactive compounds.

INTRODUCTION

Citrus reticulata, otherwise known as mandarin orange or tangerine, has been long valued for its fragrant property and medicinal use. Being one of the native species found in Southeast Asia, the citrus species has been utilized in most food and medical uses in various forms across the globe for many centuries⁽¹⁾. Aromatherapists are increasingly using essential oil made from the peels of mandarin oranges because of its numerous medicinal benefits and mild, gentle nature. *Citrus reticulata* essential oil is in higher demand due to the growing trend towards natural therapeutic treatments⁽²⁾. Limonene, linalool, and alpha-pinene are some of the main constituents of mandarin essential oil. Among the many bioactive compounds, limonene is the most important constituent and it contributes to the fresh characteristic aroma, often perceived in relation to mood enhancement, lower stress levels, and emotional balance⁽³⁾. The calming and soothing property of mandarin oil is the best for people looking for a natural remedy to reduce stress, minimize anxiety, improve sleep, and so much more among those who have sensitive skin⁽⁴⁾. It is gentle in nature, and therefore it finds wide application in therapeutic preparations meant for topical application so that patients can experience the therapeutic effects personally at the site of application⁽⁵⁾.

Administration through roll-on format for essential oils has become very popular in aromatherapy nowadays due to the accuracy, user-friendliness, and efficacy of the delivery system⁽⁶⁾. The roll-on container delivers precise and regulated amounts of essential oils to particular body parts, like the shoulders and back, or pulse points like the wrist and neck. Additionally, the roll-on form helps to evenly distribute oil to prevent discomfort from excess application⁽⁷⁾.

Therapeutic uses of mandarin essential oil are far beyond the improvement in emotional health. The oil, besides having tranquilizing and mood-elevating properties has previously been found to possess antimicrobial, anti-inflammatory, and digestive values⁽⁸⁾. Studies show that mandarin oil can be helpful in avoiding greasy skin, reducing conditions related to respiratory disorder, and enhancing digestion when it is applied in the course of aromatherapy therapy⁽⁹⁾. By adding mandarin essential oil to roll-on products, consumers can utilize them more effectively in a variety of emotional and physical conditions. Despite this increasing trend, further scientific

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research is required to better understand specific therapeutic properties of mandarin essential oil used in roll-on formulations⁽¹⁰⁾. Pharmacological effects and long-term safety profiles should be studied in order to achieve their true potential⁽¹¹⁾. Besides, consumer preference, formulation advancements, and the impact of mandarin oil when blended with other essential oils may create considerable knowledge that will help boost the effectiveness and appeal of roll-on aromatherapy⁽¹²⁾. Essential oil obtained from the peels of *Citrus reticulata* was formulated into a roll-on. Additionally, its physical properties, and aroma intensity were assessed.

MATERIALS AND METHODS

Materials

Mandarin orange was purchased from the local market of Chennai, Tamil Nadu, India. Lemon grass oil was procured from vedas oil Pvt, Ltd, Coconut virgin oil was purchased from vedas oil Pvt, Ltd, Camphor was purchased from BMR chemical Pvt, Ltd, Menthol was purchased from AOS Pvt, Ltd.

Plant Material

In this study, locally available ripe *Citrus reticulata* peels were employed. The peels from the fruit were hand-picked in such a way that pulp or seeds were excluded and cleaned thoroughly to free the peels from dust or dirt. The chopped pieces of size 2–3 cm was obtained with better surface area and increased distillation efficiency.

Hydro-Distillation Setup

A Clevenger apparatus was used for the extraction of essential oil from peels of *Citrus reticulata*. The setup includes a distillation flask, a condenser, and a collection flask (**Fig. 1**). The condenser is connected to the distillation flask, and the collection flask is attached at the opposite end to gather the condensed liquid. The apparatus is placed in a well-ventilated room to ensure effective heat dissipation and maintain safety during the experiment⁽¹³⁾.

Extraction Procedure

The distillation flask was filled with an anticipated 500 g of prepared *Citrus reticulata* peel. The plant materials were fully immersed by the addition of distilled water to the flask. The water-to-plant material ratio was kept at approximately 3:1 in order to ensure enough steam was produced. Distillation started when the flask was heated with an electric heating mantle. Water started to boil slowly, producing steam over the plant material and causing all of its volatile oils to evaporate. These steam-saturated vapors travel in the direction of the condenser, where cooling takes place⁽¹⁴⁾.

Collection and Separation

The receiving flask was used to collect the distillate liquid, which comprised a mixture of hydrosol and essential oil from orange peel. After the distillation is completed, which usually takes 3 to 4 hours, the liquid obtained was left to cool to room temperature. Because essential oil is less dense than water, it floated naturally and rested on top of the hydrosol. The essential oil was carefully separated from the hydrosol using a separation funnel. The oil was transferred to the pre-labelled, dark glass storage vessel. The hydrosol was preserved for eventual use in cosmetic applications⁽¹⁵⁾.

Storage

Essential oil extracted from *Citrus reticulata* was placed in a firmly sealed glass bottle and stored in a cool, dark environment to avoid light exposure as well as oxidative degradation.

Quantification of mandarin essential oil yield

The essential oil was extracted from peels of mandarin orange and transferred into a vial. Subsequently, the percentage yield was determined by calculating the ratio of mass of the extracted mandarin orange essential oil to the initial mass of the mandarin orange peels used in this study. The following formula was used to compute the

$$\text{Yield (\%)} = \frac{\text{Mass of extracted mandarin orange essential oil (g)}}{\text{Initial mass of mandarin orange peel (g)}} \times 100 \quad (1)$$

Characterization of bioactive compound in mandarin orange essential oil

The analysis of mandarin essential oil was carried out using a gas chromatograph (Agilent 7890B) configured with a mass spectrometric detector and a capillary column HP-5MS (60 m × 0.32 mm × 0.50 μm film thickness). Helium was utilised as the carrier gas at a controlled flow rate of 1.2 mL/min. A 1 μL diluted sample was manually injected using a splitless injection method. The oven initial temperature was set to 50°C and subsequently increased at a rate of 4°C per minute until reaching 280°C. The detector and injector were maintained at temperature of 250°C. The mass spectrometer functioned in electron impact ionization (EI) mode at an ionization energy of 70 eV. The ion source and quadrupole were maintained at temperatures of 230°C and 180°C, respectively. Volatile compounds were characterized based on their mass spectral fragmentation patterns and identified by comparing them with reference spectra from the WILEY/NIST mass spectral database.⁽¹⁶⁾

Mandarin oil roll-on aromatherapy formulation

The required quantity of ingredients was weighed as listed in (**Table 1**). The addition of menthol and camphor to mortar and ground. An Erlenmeyer flask with a glass stopper was filled with the components, and they were

swirled until they dissolved. Coconut oil was added to the Erlenmeyer flask and stirred until homogenous mixture was obtained, then the mixture was transferred into a 10ml roll-on bottle.

Evaluation of antibacterial activity

The antibacterial activity of essential oil formulation was evaluated against *Lactobacillus acidophilus*, *S. mutans*, and *S. aureus*. The bacterial strains were emulsified in nutrient agar and inoculated. Discs infused with the extract (10 µg/mL) were placed on the cultured plates and incubated for 48 hours at 37°C(98.6°F). The antibacterial activity of the formulation (M1) was assessed by measuring the zone of inhibition (ZOI) around the discs. The results confirmed the antibacterial properties of essential oil formulation against the tested bacterial strains^{(17),(18)}.

ASSESSMENT OF THE PHYSICAL CHARACTERISTICS OF CITRUS RETICULATA ESSENTIAL OIL ROLL-ON

Evaluation of organoleptic parameters

The preparation physical characteristics, such as its shape, color, and smell, were examined in order to conduct the tests⁽¹⁹⁾.

Measurement of pH

The pH of the essential oil formulation was measured using pH meter (Mettler Toledo Seven Compact S220). Prior to each measurement, the device was calibrated using buffer solutions at pH 4, 7, and 10. A formulation sample(50mL) was collected in a beaker, and a pH probe was dipped into the solution. The reading obtained was recorded⁽²⁰⁾.

Assessment of density

A 10 mL volumetric pycnometer was used to determine the density. Before use, it was thoroughly cleaned and dried. The empty pycnometer was weighed, before the sample was added. To prevent air bubbles, the pycnometer was carefully sealed and weighed again⁽²¹⁾.

Assessment of viscosity

A Brookfield viscometer was used to perform the viscosity test. A formulation sample of 15 mL was placed in the beaker, and a spindle (type A3) was carefully positioned and completely submerged in the liquid. Rotation speed of the spindle was adjusted to 250 rpm, furthermore viscosity was measured directly on the viscometer⁽²²⁾.

Testing for clarity

The clarity determination of the *Citrus reticulata* roll-on was performed by visually examining the sample within a vial against a dark backdrop. The formulation was observed for any visible particles or sediment. It was considered homogeneous if no solid particles were observed⁽²³⁾.

Accelerated Stability studies

The roll-on aromatherapy formulation was subjected to an accelerated stability study employing the thermal cycling method, in which the product was maintained at 41°F (5°C) for 720 min and then at 95°F (35°C) for next 720 min to evaluate its stability under temperature fluctuations, repeating the whole process six times, while the control sample was stored at a constant temperature of 25°C for comparison purposes⁽²⁴⁾.

Aroma intensity

The roll-on formulation was applied to the test paper and the preparation remained for nine hours. The assessment of the formulation was done every hour on a score basis from 1 to 4, where (1 meant extremely strong, 2-very weak, 3-weak, and 4-very weak.) The formulated roll on given in the (Fig.2)⁽²⁵⁾.

RESULTS AND DISCUSSION

Quantification of mandarin essential oil yield

The amount of essential oil extracted from peels of *Citrus reticulata* was 0.54% by steam distillation for one hour at 100°C. Moreover, some studies suggested that the duration of extraction might affect the ability of extraction and the amount of oil harvested.

Characterization of bioactive compounds in mandarin orange essential oil

The composition of bioactive compounds in essential oil was identified through GC-MS analysis, as indicated in the chromatogram. This essential oil contains a total of 18 compounds (Table 2), wherein limonene is the most abundant, constituting 28.58% of its total composition. limonene is considered the predominantly abundant in mandarin orange essential oil. Other components include monoterpenes, sesquiterpenes, alkanes, and aldehydes. The bioactive compounds are given in Table 2.

Evaluation of antibacterial activity

The Mandarin Orange (*Citrus reticulata*) essential oil was evaluated for its antibacterial activity against *Lactobacillus acidophilus*, *S. mutans* and *S. aureus* and the zone of inhibition was mentioned in the table 5.

ASSESSMENT OF THE PHYSICAL CHARACTERISTICS OF *CITRUS RETICULATA* ESSENTIAL OIL FORMULATION

Evaluation of organoleptic parameters

The organoleptic evaluation results of the essential oil formulation (roll-on) are summarized in Table 3. The analysis revealed that the roll-on aromatherapy had a transparent, yellow liquid appearance with a mild to strong mandarin orange peel scent. The color variation in the roll-on formulation is influenced by differences in the essential oil content extracted from mandarin orange peels. The intense yellow coloration of the formulation results from a higher concentration of essential oil.

Measurement of pH

The pH of the mandarin orange peel oil roll-on formulation was measured at 0, 7, 14, and 21 days. The recorded values, are shown in Table 4. The pH readings of the formulation M1, recorded with a pH meter, ranged between 5.33 and 5.24. The comparator product displayed a pH range of 5.07 to 5.93. During storage, the pH of the roll-on aromatherapy preparation increased but remained within the ideal pH range of 4-6. Topical preparations should maintain a normal skin pH, as an excessively alkaline pH can cause scaly skin, whereas an overly acidic pH may lead to skin irritation.

Assessment of density

The density of M1 formulation is 0.932 g/ml; whereas for the comparator product, it is 0.905 g/ml. The density depends on the concentration of mandarin orange peel oil in the formulation.

Assessment of viscosity

A Brookfield viscometer was used to perform the viscosity test. The result for formulation M1 and the products that were used as a comparison were 25.32 cP. To analyse the flow properties of liquid formulations, viscosity tests were performed. The viscosity of a formulation is crucial for its ability to spread and adhere effectively to the skin. Proper adhesion and spreadability help ensure even distribution of the active ingredient, allowing for consistent delivery to the skin surface and maximizing its therapeutic benefits.

Test for clarity

The clarity evaluation demonstrated that both formulation M1 and the comparator product exhibited transparency, without any observable sediment or particulate matter. This assessment aims to identify the presence of undissolved components within the roll-on aromatherapy preparation. Throughout this research, every formulation displayed superior transparency, with the absence of sedimentation, suspended particles, or coarse aggregates. A formulation is classified as clear when it appears completely free of visible particles under strong transmitted light.

Accelerated stability Assessment

The findings from the accelerated stability study performed through the thermal cycling method are summarized in Table 6. The roll-on aromatherapy formulations underwent stability testing, comparing their characteristics before and after storage. Throughout six storage cycles, no sensory alterations were observed in formulation M1 or the comparator product. As no changes were detected before or during the stability assessment, the formulations are considered stable across different temperature conditions.

Aroma intensity

The evaluation of aroma persistence revealed that the M1 formulation of mandarin orange peel oil in roll-on aromatherapy exhibited an extended-lasting fragrance. (Table 7). While various studies have examined the use of citrus oils in aromatherapy, this research is unique in that mandarin orange peel essential oil had not previously been developed into a roll-on formulation. The findings demonstrated that the roll-on aromatherapy containing 10% mandarin orange peel essential oil (M1) was the most effective formulation. It was composed of menthol, camphor, lemongrass oil, and virgin coconut oil.

CONCLUSION

Chromatogram data revealed that mandarin oil contained 18 distinct components, with limonene being the most abundant at 28.58%. Formulation M1 exhibited the highest aroma intensity, lasting nearly five hours and producing a notably strong scent. Its antibacterial properties were tested against *Lactobacillus acidophilus*, *S. mutants*, *S. aureus*. Additionally, the physical assessment of the roll-on aromatherapy formulation met the necessary quality standards.

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Table 1: Composition of formulated aromatherapy roll on

S.no	Composition	Quantity
1	Mandarin Orange peel Essential	0.3ml
2	Menthol	0.1g
3	Camphor	0.1g
4	Coconut oil	0.2ml
5	Lemongrass oil	0.2ml

Table 2: Bioactive compounds in *Citrus reticulata* essential oil.

S.NO	Elution time	Bioactive Compounds	Area (%)
1.	7.687	α -Pinene	2.89
2.	8.653	Sabinene	5.50
3.	8.734	Pinene	10.80
4.	8.758	Myrcene	3.35
5.	9.297	Octanal	1.27
6.	9.622	δ -Carene	2.48
7.	10.034	Limonene	28.58
8.	10.183	β -cis-Ocimene	1.46
9.	10.468	Terpinene	2.19
10	10.732	1-Octanol	1.60
11	11.054	1,8-p-Menthadiene	2.54
12	11.265	β -Linalool	3.05
13	12.271	(+)-Citronellal	8.61
14	14.430	Isoeugenol	3.65
15	14.854	1-Terpinenol	10.94
16	16.107	α -Terpineol	7.15
17	13.493	Citronellol	3.58
18	22.081	α - arsenene	0.36
			100.00

Table 3: Evaluation of organoleptic parameters in *Citrus reticulata* roll-on formulation

S. No	FORMULATION	ORGANOLEPTIC PARAMETERS		
		SHAPE	SMELL	COLOR
1	M1	Liquid	Citrus reticulata aroma	Clear

Table 4: pH evaluation of *Citrus reticulata* roll-on aromatherapy preparation

S.NO	Day	M1	Comparators product
1.	0	5.33 \pm 0.023	5.07 \pm 0.08
2.	7	5.24 \pm 0.000	5.93 \pm 0.032
3.	14	5.24 \pm 0.000	5.56 \pm 0.242
4.	21	5.24 \pm 0.000	5.82 \pm 0.052

Table 5: Antibacterial activity of formulation M1

Test organisms	Zone of inhibition (avg \pm STD)	
	Standard	M1
<i>S. mutans</i>	35.5 \pm 2.1mm	12 \pm 2.5mm
<i>S. aureus</i>	17.5 \pm 1.5mm	8 \pm 1.2 mm
<i>Lactobacillus acidophilus</i>	25.5 \pm 4.5mm	9 \pm 1.4 mm

Table 6: Accelerated stability tests for formulation M1

S.NO	Formulation	Parameter	Accelerated stability test	
			Before	After
	M1	Form	Liquid	Liquid
		Aroma	<i>Citrus reticulata</i> aroma	<i>Citrus reticulata</i> aroma
		Colour	Clear	Clear
2.	Comparator product	Form	Liquid	Liquid
		Aroma	Characteristic aroma	Characteristic aroma
		Colour	Clear	Clear

Table 7: Qualitative evaluation of aroma intensity in *Citrus reticulata* roll-on:

S.NO	Formulation	Time (hrs)								
		1	2	3	4	5	6	7	8	9
1.	M1	+	+	+	+	–	–	–	–	–
2.	Comparator product	+	+	+	+	+	–	–	–	–

Strong - +

Weak - -

**Fig 1: Mandarin orange peel essential oil extraction.****Fig 2: M1 formulated aromatherapy roll on.**

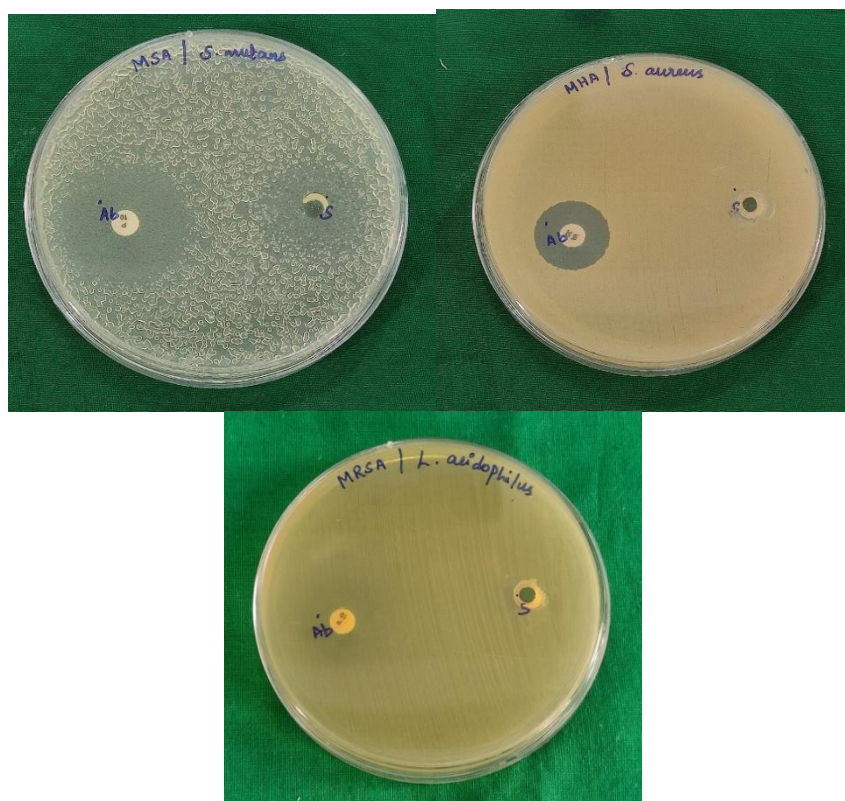


Figure 3: Anti-bacterial activity of formulation M1.

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