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Functional Properties of Essential Oils Distilled from Areal Parts of Mandarin Varieties (*Citrus reticulata* Blanco.)

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Abstract Essential oil extracted from mandarin (*Citrus reticulata* Blanco.) (Rutaceae) is widely used in traditional medicine, pharmaceutical, food and cosmetic industries. However, the use of citrus leaf oil is not well established, and information is lacking. This study aimed to quantify the yield of essential oil content and evaluate the functional properties of essential oil extracted from immature and mature leaves of five different mandarin varieties (*Indu*, *Heennaran*, *Madhu*, *Juicy* and *Rahangala*) grown in Sri Lanka. The essential oil was distilled using Clevenger-type apparatus. Quantification of total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) were carried out by using the modified Floin-Ciocalteu method, colourimetric method and ferric reducing antioxidant power (FRAP) assay, respectively. The results revealed that all tested leaf essential oil of mandarin varieties contained marked amounts of essential oil content and TPC, TFC, and TAC. Significantly highest TPC (69.80 ± 5.14 mg GAE/g DW), TFC (265.08 ± 7.95 mg RE/ g DW), TAC (87.70 ± 4.02 mg TE/ g DW) and essential oil content (1.89 ± 0.19 mL/ 100 g DW) were observed in the *Indu* mandarin variety. Moreover, there were positive correlations of TAC with TPC (R^2 = 0.8822) and TFC (R^2 = 0.4004). The greater availability of bioactive compounds in *C. reticulata* leaf essential oil highlighted that the essential oil of mandarin is a potential source of valuable components. This research highlights the promising potential of utilizing the leaf essential oil of *C. reticulata* as a rich source of these beneficial components in the pharmaceutical, food and cosmetic industries.

Keywords: *Areal plant parts, Citrus reticulata, Essential oil, Functional properties, Mandarin*

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1. Introduction

Mandarin, also known as "*Naran*", is a popular fruit crop that belongs to the family Rutaceae. [1]. It is widely grown worldwide and is thought to have originated in South Eastern Asia, the Middle East, and Southern Europe. It is China's most extensively cultivated mandarin variety due to favourable weather conditions, higher cultivation extent and yield [2]. In Sri Lanka, mandarin is grown as a major fruit crop and medicinal plant in home gardens, especially in Ampara, Batticaloa, Polonnaruwa, Anuradhapura, Vauniya and Hambanthotathat are suited for almost all agroclimatic regions of the country [1]. The foliage is relatively small, green, and shiny. The flowers are produced individually or in small clusters in the leaf axils.

The fruit is round or oblate. The fruits may be seedless or contain a small number of seeds. The different varieties slightly change morphological characters [3]. The main Mandarin varieties developed in Sri Lanka suited for different geographical regions consist of "*Heennaran*", "*Rahangala*", "*Indu*", "*Juicy*", and "*Madhu*" [1]. The prominent aroma and taste make it a popular commodity with essential dietary requirements. Moreover, different plant parts of mandarin varieties are rich in phytochemicals and bioactive compounds. Phytochemicals and bioactive substances reduce the risk of various physiological and pathological abnormalities, such as cancer, cardiovascular disease, neurodegenerative diseases, and ageing, and they also act as food preservatives [4].

Essential oil is one of the citrus by-products that is appealing to many industries. The food, pharmaceutical and cosmetic sectors have extensively used essential oils due to their extensive biological activities. Bioactive substances found in mandarin fruit include phenols, flavonoids and terpenoids. The ability of bioactive compounds and antioxidants to regulate critical physiological processes in the human body is well documented. Antioxidants function as singlet oxygen formation inhibitors and free radical scavengers [5,6]. The mandarin fruits are a good source of essential oil, but production of essential oil from fruits is limited due to seasonality and availability. During the pruning of mandarin crops, more areal parts are wasted, and it can be utilized to produce essential oil. Areal parts (mature and immature leaves) of mandarin have aroma compounds similar to mandarin fruits. If essential oil similar to fruit essential oil can be extracted from various parts of the mandarin, it will help to incorporate in diversified products. So far, studies on extracting essential oil from areal parts of mandarin are scattered or lacking. Therefore, this study aims to determine the yield and functional properties of essential oil extracted from areal parts of selected mandarin varieties.

2. Materials and Methods

2.1. Sample Collection

Five mandarin varieties, namely, *Heennaran*, *Rahangala*, *Indu*, *Juicy* and *Madhu* were selected for this study. One kilogram of immature (first five leaves) and mature (fifth leaf onwards) leaf samples from each variety were collected from Fruit Crop Research and Development Institute, Horana and Government Seed and Planting Material Production Farm, Walpita Sri Lanka. Homogeneous representative samples were collected from ten trees in each variety and three replicates from each mandarin variety was used for the experiment. The mature and immature leaves were air-dried at room temperature (28 ± 2 °C) for three days.

2.2. Distillation of Essential Oil

Hydro-distillation method was used to distil essential oil from dried samples by using Clevenger arm apparatus [7]. The 100 g of dried leaf sample was immersed in 500 mL of distilled water. The distillation was carried out for 6 hr. The volume of the extracted essential oil was recorded and stored in a refrigerator (4 °C) until further analysis.

2.3. Methanolic Extraction of Essential Oil

Essential oil (0.1g) was suspended in 10 mL of 80% methanol and vortexed for 10 min. Then centrifugation at 4,000 rpm for 5 min and stored at 4 °C until further analysis [8].

2.4. Determination of Total Phenolic Content

The total phenolic content (TPC) was determined by using a modified Folin Ciocalteu method [9].

2.5. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined by a colorimetric method with slight modifications [10].

2.6. Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) was determined using ferric ion reducing antioxidant power (FRAP) assay as described by Benzie and Strain [11] with slight modifications.

2.7. Statistical Analysis

Mean values ± SD were computed for all parameters to verify their statistical significance. Using SAS statistical software (SAS Version 9.5), the General Linear Model (GLM) of ANOVA followed by the Tukey Multiple Range Test was used to compare mean values statistically.

3. Results and Discussion

3.1. The Essential Oil Yields

As shown in Table 1, the mean values of the essential oil content of the five mandarin varieties ranged between 0.56 ± 0.10 and 1.89 ± 0.19 mL / 100 g DW. Significantly highest oil yield was observed in mature leaves of variety *Indu* (1.89 ± 0.19 mL / 100 g DW) followed by immature leaves (1.39 ± 0.10 mL / 100 g DW) of the same variety. However, the reported mean value of essential oil content in mature leaves of variety *Indu* (1.89 ± 0.19 mL / 100 g DW) was slightly lower than the findings of Senarathne et al. [4] for the same variety (2.61 ± 0.10 mL / 100 g DW). Moreover, it also reported that essential oil content in mature leaves of variety *Rahangala* (1.89 ± 0.19 mL / 100 g DW) is higher than the results obtained from the current study for the same species (0.78 ± 0.10 mL / 100 g DW) [4]. There was no significant difference between the essential oil yield of immature and mature leaves in the *Heennaran*, *Madhu*, *Juicy* and *Rahangala* varieties. However, there was a significant difference in oil yield between immature and mature leaves of variety *Indu*. Thus, compared with the previous studies, variations in essential oil yield may be due to geographical conditions such as soil conditions, altitude, climate change and other conditions related to the distillation method, such as distillation time and temperature [4].

Table 1. Essential oil yield of areal parts of mandarin varieties

Plant material	Oil yield (ml/ 100 g DW)
Indu (IL)	1.39 ± 0.10 ^{b}
Indu (ML)	1.89 ± 0.19 ^{a}
Heennaran (IL)	0.78 ± 0.10 ^{cd}
Heennaran (ML)	1.00 ± 0.17 ^{c}
Madhu (IL)	0.78 ± 0.10 ^{cd}
Madhu (ML)	0.83 ± 0.00 ^{cd}
Juicy (IL)	0.83 ± 0.00 ^{cd}
Juicy (ML)	0.84 ± 0.02 ^{cd}
Rahangala (IL)	0.56 ± 0.10 ^{d}
Rahangala (ML)	0.78 ± 0.10 ^{cd}

Mean with the same letters represent non-significant differences ($p<0.05$); DW = Dry weight; IL = Immature leaves; ML = Mature leaves

3.2. Bioactive Compounds

As shown in Table 2, the Total phenolic content (TPC) of five *C. reticulata* varieties ranged from 26.94 ± 2.00 to

69.80 ± 5.14 mg GAE/ g DW. Significantly high TPCs were observed in immature leaves of variety *Indu* immature essential oil extract (69.80 ± 5.14 mg GAE/ g DW), indu mature essential oil extract (66.63 ± 2.38 mg GAE/ g DW), Rahangala immature essential oil extract (62.62 ± 5.69 mg GAE/ g DW) and Rahangala mature essential oil extract (60.32 ± 2.68 mg GAE/ g DW). Significantly low TPCs were observed in heennaran immature essential oil extract (26.94 ± 2.00 mg GAE/ g DW), heennaran mature essential oil extract (29.68 ± 1.78 mg GAE/ g DW) and juicy mature essential oil extract (35.95 ± 4.55 mg GAE/ g DW). The descending order of TPC of five *C. reticulata* varieties was as follows; *Indu* > *Rahangala* > *Madhu* > *Juicy* > *Heennaran*. However, there was no significant difference observed in the TPC of *Indu*, *Heennaran*, *Madhu* and *Rahangala* varieties among immature and mature leaves essential oil extracts. But there was a significant difference between juicy immature and mature leaves essential oil extracts for TPC. High TPC (46.75 ± 4.48 mg GAE/ g DW) was reported in immature essential oil extract of juicy when compared with mature essential oil extract (35.95 ± 4.55 mg GAE/ g DW). TFC of essential oil of five *C. reticulata* varieties ranged from 115.92 ± 2.20 to 265.08 ± 7.95 mg RE/ g DW. Significantly high TFCs were reported in indu mature essential oil extract (265.08 ± 7.95 mg RE/ g DW) and indu immature essential oil extract (250.64 ± 7.56 mg RE/ g DW). However, significantly low TFCs were reported in the essential oil of the variety Madhu for both immature (128.14 ± 3.94 mg RE/ g DW) and mature (115.92 ± 2.20 mg RE/ g DW) leaves. However, there was no significant difference observed in *Indu*, *Heennaran*, *Madhu* and *Rahangala* varieties between immature and mature leaves essential oil extracts. There was a significant difference between juicy immature (176.47 ± 22.25 mg RE/ g DW) and Juicy mature (135.08 ± 8.46 mg RE/ g DW) essential oil extracts. Both high TPC and TFC were recorded in immature essential oil extracts when compared with mature essential oil extracts. Results obtained from this study agreed with the findings of Dharmadasa et al [6], who investigated higher phenol and flavonoid content in immature leaf oil extract of *Pimenta dioca* compared with its mature leaf oil extract.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of areal parts of mandarin varieties

Plant material	TPC (mg GAE/g DW)	TFC (mg RE /g DW)
Indu (IL)	69.80 ± 5.14 ^{a}	250.64 ± 7.56 ^{a}
Indu (ML)	66.63 ± 2.38 ^{a}	265.08 ± 7.95 ^{a}
Heennaran (IL)	26.94 ± 2.00 ^{d}	154.81 ± 2.5 ^{bcd}
Heennaran (ML)	29.68 ± 1.78 ^{cd}	166.19 ± 6.74 ^{b}
Madhu (IL)	46.83 ± 2.90 ^{b}	128.14 ± 3.94 ^{de}
Madhu (ML)	39.92 ± 2.84 ^{bc}	115.92 ± 2.20 ^{e}
Juicy (IL)	46.75 ± 4.48 ^{b}	176.47 ± 22.25 ^{b}
Juicy (ML)	35.95 ± 4.55 ^{cd}	135.08 ± 8.46 ^{cde}
Rahangala (IL)	62.62 ± 5.69 ^{a}	163.69 ± 9.37 ^{bc}
Rahangala (ML)	60.32 ± 2.68 ^{a}	164.81 ± 14.17 ^{bc}

Mean denoted by the same letters in a column represent non-significant differences ($p<0.05$); GAE = Gallic acid equivalent; RE = Rutin equivalent; DW = Dry weight; IL = Immature leaves; ML = Mature leaves

3.3. Total Antioxidant Capacity

The total antioxidant capacity (TAC) of leaf essential oil extracted from five *C. reticulata* varieties ranged from 6.17 ± 0.53 to 87.70 ± 4.02 mg TE/ g DW (Figure 1). Significantly the highest TAC was reported in *Indu* immature essential oil extract (87.70 ± 4.02 mg TE/ g DW) whereas, significantly low TACs were observed in *heennaran* immature essential oil extract (6.17 ± 0.53 mg TE/g DW) and *heennaran* mature essential oil extract (7.48 ± 0.25 mg TE/ g DW). The ascending order of TAC of five *C. reticulata* varieties was as follows; *Heennaran* < *Juicy* < *Madhu* < *Rahangala* < *Indu*. However, there was no significant difference observed among the results of TAC in *Madhu*, *Juicy* and *Heennaran* varieties between immature and mature essential oil. Moreover, there was a significant difference in *Indu* and *Rahangala* varieties between immature and mature leaf essential oil extracts. In immature essential oil extracts of *Indu* and *Rahangala*, both varieties reported high TAC (87.70 ± 4.02 mg TE/ g DW and 78.84 ± 6.68 mg TE/ g DW, respectively). The result revealed that higher TAC is concentrated around the immature plant parts near to the bud region and decreases with the maturity. Observed high TAC of immature leaf oil extracts compared with mature leaf oil extracts are in agreement with previous studies [12].

The above results also indicated that the immature leaves of *C. reticulata* had more content of all bioactive compounds than the mature leaves. Furthermore, the immature leaves recorded higher antioxidant activity than the mature leaves. This may be due to the presence of high content of secondary metabolites during immature stages act as a barrier to protect the plant from pathological damage during the growth and development of the plant. When the plant is able to survive in the environment, the content of secondary metabolites produced within the plant is lower than at the immature stage [13] (Oulebsir *et al.*, 2022).

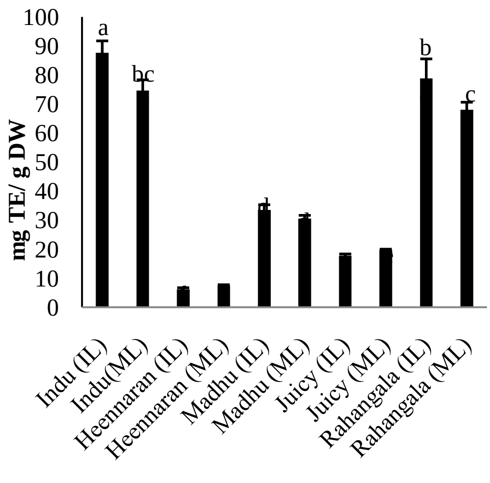

Figure 1. Total antioxidant capacity (TAC) of leaf essential oil of mandarin varieties

Mean with the same letters represent non-significant differences ($p<0.05$); TE = Trolox equivalents; DW = Dry weight; IL = Immature leaves; ML = Mature leaves

3.4. Correlation of TAC with TPC and TFC

The study found that there is a strong positive correlation between TAC and TPC, with an R^2 value of

0.88. Additionally, there was a slightly poorer but still positive relationship between TAC and TFC, with an R^2 value of 0.40. These results are consistent with previous findings by Senarathne et al. [6] who observed similar positive correlations between TAC and TPC ($R^2 = 0.724$, $p < 0.05$) and TFC ($R^2 = 0.0078$, $p < 0.05$) in methanol extracts of 28 citrus varieties. These positive correlations suggest that phenolic compounds play a significant role in the antioxidant capacity of selected mandarin varieties, as these compounds serve as electron donors in free radical reactions [13].

4. Conclusions

Present study found that all tested varieties of *C. reticulata* contain elevated levels of essential oil, bioactive compounds, and antioxidant capacity. Among them, the *Indu* mandarin variety has been identified as having the highest concentration of these valuable components. This research highlights the promising potential of utilizing the leaf essential oil of *C. reticulata* as a rich source of these beneficial components. Furthermore, it has been suggested that mandarin leaves, which are often discarded, could be transformed into a valuable source of essential oil containing bioactive compounds. The discovery could potentially revolutionize various industries, including food and beverage, flavor and fragrance, traditional medicine, as well as the pharmaceutical and nutraceutical sectors.

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