Citric acid compounds of tangerines peel extract (Citrus reticulata) as potential materials teeth whitening

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Abstract. Peel of citrus fruit (Citrus reticulata) has a variety of possible chemical compounds that may serve as a potential whitening teeth. This research is conducted on a laboratory scale; therefore, it needs to be developed on an application scale. A quasi-experimental was employed in this study. Citric acid extraction was carried out on the type of Sweet Orange (Citrus Aurantium L), Tangerine (Citrus Reticulata Blanco or Citrus Nobilis), Pomelo (Citrus Maxima Merr, Citrus grandis Osbeck), and Lemon (Citrus Limon Linn). Citric acid's ability test as teeth whitener was performed on premolar teeth with concentrations of 2.5%, 5%, and 10%. The experiments were replicated in 3 times, and teeth whiteness level was measured using Shade Guide VITA Classical. The result of this research showed that citric acid in every kind of orange peel with various concentration has different abilities on whitening teeth. The highest colour level obtained from Tangerine peel's citric acid concentration of 5%.

Orange peel extract has the best teeth whitening abilities tested by the method of Gass Chromatography to know the active ingredients.

1. Introduction

Citrus is one of the horticultural plants which is produced in high quantity at Indonesian agriculture industry [1]. There are many ingredients inside the citrus fruit that beneficial to the body, according to Purdue University Horticulture and Landscape Architecture, in every 100 g of citrus contains 40 mg of calcium which beneficial to bones and teeth. However, with the exact amount, orange peel contained 161 mg calcium. Orange peel (Citrus reticulata) contain many compounds like Tangeraxanthin, Tangeritin, Terpinen-4-ol, Terpinolene, Tetradecanal, Threonine, Thymol, Thyme- methyl-ether, Tryptophan, Tyrosine, Cis-3-hexenol, Cis-carveol, Citric-acid, Citronellal, Citronelic-acid, Citronellyl-acetate, Cystine, Decanal, Decanoic-acid, Decanol, Nobiletin [2].

The whitening ability of citric acid extract was previously reported. The citric acid compound in citrus was known its ability in whitening the discoloured teeth, because it has OH group on the chemical structure [3]. The current research was aimed to formulate the composition of whitening compounds from the citric acid extract.
2. Methods

2.1. Materials
Sweet Orange \((Citrus auratium L)\), Tangerine \((Citrus reticulata Blanco or Citrus nobilis)\), Pomelo \((Citrus maxima Merr, Citrus grandis Osbeck)\), and Lemon \((Citrus Limon Linn)\). Citric acid's ability to test as teeth whitener was performed on premolar teeth with concentrations of 2.5\%, 5\%, and 10\%.

2.2. Procedure

2.2.1. Extraction of Orange Peels and Powder Preparation. Orange peels were separated from the fruit, and it was cut into little pieces. The orange peels were dried using the oven with 50 °C.

2.2.2. The dried orange peels were then ground into a fine powder using blender machine.

2.2.3. Teeth Colour Observation. Teeth colour was observed after discolouration process using orange peel extract. The citric acid was subjected for teeth soaking. Then, the Shade Guide Vita was applied as the guidance for teeth observation [4].

2.2.4. Teeth Discoloration. The discoloration of teeth was performed using 36 premolar teeth which were polished using nail polish on root part until CEJ part in 3 times. These teeth were then inserted to discoloration fluid that made of 10 g robusta coffee and 180 ml water (boil until 100°C). The solution was then changed every 24 hours during one week [5][6].

2.2.5. Orange Peels Citric Acid. The orange peel's powder of each 2 g, was mixed with 75 ml of water and 25 ml methanol. Then, it was stirred using a magnetic stirrer for 30 minutes with 7th speed, and it was kept in a volumetric flask. Next, the distillation phase for extracting orange peel citric acid was performed. The distillation step is to isolated citric acid compound from orange peel's powder. Sodium hydroxide (NaOH) is used to separate the citric acid, and the orange peel's powder distilled four times [7]. Then, after these processes, the distillation results were subjected to the GC-MS analysis.

2.2.6. Treatment Stage. The discoloured teeth were then employed into each flask with orange peel extract. The mixture was then placed in the incubator with 50 °C heating for 25 s, after that, it was then lowering the heat to 37 °C for 30 min. The process will produce white colour to the teeth.[6]

2.2.7. GC-MS Analysis. Gas chromatography analysis was performed by employing the samples that have been in the extraction was analysed by the method of gas chromatography FPD (flame photometer Detector) Model Shimadzu GC-FID 2014. GC tool is equipped with AFC (Advanced Flow Controller). The temperature of the injector on the GC-MS was in maintenance at a temperature of 260 °C and pressure of the gas flow of Helium Ultra High Pure (UHP) was at 100 kPa and was utilised as a carrier Gas Chromatography – Mass Spectrometry (GC-MS).

2.2.8. Statistical Analysis. The data was analysed and interpreted using IBM SPSS 20 software. In this study, the analysis was conducted using two tests, there was a difference test of various types and concentrations of orange peel's extract to the ability to whiten teeth using One Way ANOVA, and the second test was Paired T-test that used to analyse level change between discoloured teeth and teeth after treatment.
3. Results and Discussion

3.1. Colour Changing After Testing

Colour measurement tests were applying the Shade Guide VITA Classical that have 16 category colours. The colour for shade guide starts from most white, bright, to most dark. The colour that has sorted as their order, and the order score is: B1=1, C1=2, A1=3, B2=4, D2=5, C2=6, B3=7, A2=8, B4=9, C3=10, A3=11, D3=12, D4=13, C4=14, A4=15, A35=16, and these the result of change discolored teeth that had soaked on extract acid citric orange peel in 30 min, with 4 kinds of orange peel and in various concentration.

Table 1. Result of ability test citric acid orange peel as teeth whitening at various concentrations

| Citrus  | Concentration | Discoloured | Score | Dental Score after Treatment Mean |
|---------|---------------|-------------|-------|----------------------------------|
|         |               |             |       | I | II | III |
| Sweet   | 2.5% 5% 10%   | C4 14       | 6 4 2 | 6 6 6 |
| Tangerine| 2.5% 5% 10%  | C4 14       | 4 2 2 | 7 4.33 |
| Pomelo  | 2.5% 5% 10%  | C4 14       | 10 7 7 | 10.6 |
| Lemon   | 2.5% 5% 10%  | C4 14       | 10 7 7 | 7 9 |

The data shown in the Table 1 indicates that citric acid on the tangerine peel with 5% concentration give the best result.

3.2. Data Analysis Result

From One Way Anova test, it is obtained the score Sig 0.000 (<0.05) which shows the different effect that significant between variation kinds and concentration orange peel (Table 2). On the other side, paired t-test also show sig 0.000 (<0.005) and prove that significant transformation between discoloured teeth and soaked teeth with orange peel extract (Table 3).

Table 2. Result of One Way ANOVA parametric statistical test

| Source   | df | Mean Square | F   | Sig. |
|----------|----|-------------|-----|------|
| Intergroup| 11 | 26.816      | 11.492 | 0.000 |

Table 3. Result of Paired T-test parametric statistical test

| Mean | T   | df | Sig. |
|------|-----|----|------|
| 7.972| 15.105 | 35 | 0.000 |
3.3. GC-MS Test Result

GC-MS tests carried out on samples that have the best ability to whiten teeth. Orange peel extract that has distilled to isolate the citric acid tested using GC-MS method for seeing active compound of citric acid as Table 4.

| Peak no. | Retention time | Area % | Name                                      |
|---------|----------------|--------|-------------------------------------------|
| 1       | 43.771         | 21.46  | methyl dihydro malvate                   |
| 2       | 47.782         | 20.17  | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester |
| 3       | 51.298         | 33.38  | 9-Octadecenal, (Z)                       |
| 4       | 67.940         | 16.69  | Cholest-5-en-3-ol, (3.beta.)-carbonochloridate |
| 5       | 67.969         | 8.30   | Androstan-3-one, 17-hydroxy-1,17-dimethyl-, (1.alpha.,5.alpha.,17.beta.) |

The table above telling us that orange peel's citric acid have several active compounds, like one that peaks no 2, there are Hexadecanoic acid 1-(hydroxymethyl)-1,2-ethanediyl ester that has the cluster as Figure 1.

![Figure 1](image1.png)

From the chart above, Hexadecanoic acid has Hydroxide (OH) group that known can whiten teeth. Also Hexadecanoic acid has potent as anti-bacterial and anti-fungi. Then, as shown Figure 2 it has active compound Cholest-5-en-3-ol or cholesterol that can be as an emulsion stabiliser on toothpaste [8], while as shown Figure 3, there are active compound Androstan which have OH group and can work as anti-inflammation on gum [9].

![Figure 2](image2.png)
4. Conclusion
Based on research which has been done, we get the result that citric acid containing on orange peel can whiten the teeth. Furthermore, these orange peel's citric acid have the active compound that functions as anti-bacterial, anti-fungi, emulsion stabiliser and anti-inflammation on gum. To obtain citric acid on an orange peel that can work as teeth whitener can be done with extraction. After testing on discoloured teeth, we have the result that tangerine peel with 5% concentration gives the best result on whitening teeth than other orange peels and concentrations.

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