



Phytochemical and Antioxidant Activity Evaluation of Lime (*Citrus aurantifolia*) Juice Powder

(Evaluasi Fitokimia dan Aktivitas Antioksidan Serbuk Perasan Jeruk Nipis (*Citrus aurantifolia*))

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ABSTRACT

Background: Many chronic diseases that occur in society can be caused by free radicals. Excessive free radicals in the body can contribute to oxidative stress. Therefore, the role of antioxidants is needed to inhibit the effects of free radicals. Lime (*Citrus aurantifolia*) is a plant that contains several active compounds. The lime juice was reported to contain flavonoids and ascorbic acid which have several bioactivities including antioxidants. **Objectives:** This study aims to evaluate the chemical compounds and determine antioxidant activity in the lime juice powder of lime fruit collected from Ujung Pangkah, Gresik. **Methods:** Lime was squeezed and dried by a freeze-dryer to remove the water content. The phytochemical profile of lime juice powder was evaluated by thin layer chromatography (TLC) method and ascorbic acid content in lime juice powder was determined further. The antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method. Results: The results of the study showed that lime juice powder contained flavonoids, saponins, terpenoids, and steroids. The ascorbic acid content was 0.38 mg/100mg. Antioxidant activities revealed strong antioxidant activity with an IC50 value of 32.59 µg/mL, while an IC50 value of ascorbic acid showed 8.57 µg/mL. **Conclusions:** Lime juice powder has potential as an antioxidant with an IC50 value of lime juice powder being categorized to possess very strong antioxidant activity.



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INTRODUCTION

Many chronic diseases that occur in society such as premature aging, cardiovascular disease, hypertension, and diabetes can be caused by free radicals (Puspitasari *et al.*, 2020). Free radicals are molecules that contain more electrons unpaired in a separate orbital highly unstable (Gulcin, 2020). Free radicals can be formed endogenously through normal metabolic processes in the body, and formed exogenously through various pollution, such as exposure to air pollution, cigarettes, ozone, industrial chemicals (Kurniasih *et al.*, 2015; Azman *et al.*, 2019). Excessive free radicals in the body can cause various damages and contribute to oxidative stress and cause the majority of oxidative illnesses (Suriyakala *et al.*, 2022). Therefore, the role of antioxidants is needed to inhibit the effects of free radicals in the body.

Lime is a fruit that contains a lot of ascorbic acid which can act as an antioxidant. Lime or *Citrus aurantifolia* a plant from the genus *Citrus* that belongs to the family of Rutaceae contains compounds such as flavonoids, terpenoids, and ascorbic acid that have antioxidant activities (Al-Aamri *et al.*, 2018). Moreover, all parts of this plant such as leaves, peel, and seed, were reported to possess various metabolites that have antioxidants activities such as limonene in *C. aurantifolia* leaves and peel, flavonoids and steroids in lime peel, as well as flavonoids and alkaloids in lime seed (Lemes *et al.*, 2018; Ghosh *et al.*, 2020 ; Fahrurroji & Riza, 2020). There are various plants that have been reported to their antioxidant activities (Islamiati *et al.*, 2022). However, the juice of lime fruit also has metabolites that have antioxidant activity. Lime juice contains several natural antioxidant compounds, such as flavonoids (hesperidin, rutin, didymin) and limonoids (limonin), as well as ascorbic acid (Vitamin C) (Kazeem *et al.*, 2020)). Lime juice contains ascorbic acid from 21.8 g/100g to 38.9 g/100g, and seedless lime has lime juice 60-66% that contains ascorbic acid of 118.2 - 140.8 mg/100g (Shrestha *et al.*, 2012). Those metabolites may play an important role in its bioactivity such as antioxidant activity (Ghosh *et al.*, 2020). In addition, lime is also widely used by people in everyday life as an addition to drinks and food. In addition, lime plants are also widely cultivated, one of which is in the Ujung Pangkah area, Gresik. The lime garden in Ujung Pangkah, Gresik produces around 2-3 tonnes per month. However, many of these fruits are not distributed and are ultimately wasted (Antara Jatim, 2023). Therefore, in this study, a phytochemical evaluation of lime juice powder was carried out using the TLC method to determine the content of compounds in lime from Ujung Pangkah, Gresik, and their antioxidant activity. Antioxidant activity was determined using the DPPH method. The DPPH method is an in vitro method for testing antioxidant activity that is often chosen because it is a simple, easy, fast, sensitive method and only requires a small number of samples (Lung & Destiani, 2018).

Therefore, the information from this research can be beneficial for further development of lime juice powder as an antioxidant in various preparations, especially for the use of lime fruit collected from Ujung Pangkah, Gresik.

MATERIAL AND METHODS

Materials

Lime fruit (*C. aurantifolia*) was taken from Ujung Pangkah, Gresik, East Java, Indonesia and has been verified by expert botanist researchers from Materia Medika Indonesia, Batu, East Java, with document number 074/372/102.20-A/2022. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Chemindo[®], Analytical reagent, Indonesia), Ascorbic acid (Merck[®], Germany), Aquadest (Chemindo[®], Analytical reagent, Indonesia), anisaldehyde reagent, ethyl acetate (EMSURE Merck[®], Germany), FeCl₃ reagent, methanol p.a (EMSURE Merck[®], Germany), n-hexane (EMSURE Merck[®], Germany), Liebermann-Burchard reagent, Citro Borate reagent, and Dragendroff reagent.

Methods

Sample collection

Three kg of Lime fruit was collected from the citrus cultivation garden in the Ujung Pangkah area, Gresik, East Java. Fully matured green lime was harvested three weeks after fruiting, round in shape, and has a diameter of between 4-5 cm.

Lime juice powder preparation

Lime fruit collected as much as 3 kg and was cleaned from dirt and washed with water. The juices were extracted by cutting the lime into two parts horizontally and separated from the seeds, then carefully squeezed using a citrus juicer (IdeaLife) for 15 seconds. The collected juice was filtered and then freeze-dried using freeze dryer (Buchi, L-Lyovapor[™] L-200).

Organoleptic characteristics

Lime juice powder was examined for its organoleptic characteristics in the form of color, odor, and shape.

Phytochemical screening

The phytochemical screening of lime juice powder was carried out using thin-layer chromatography (TLC). The stationary phase used was silica gel 60 F₂₅₄ (Merck). The mobile phases used were n-hexane (Merck) and ethyl acetate (Merck) (6:4) with specific detection reagents for each group of compounds, such as anisaldehyde (terpenoid), Liebermann-Burchard (saponin and steroid), citro borate (flavonoid), FeCl₃ (phenol), and dragendroff (alkaloid). Spotting observations were carried out in visible light, UV 254, and UV 366 using TLC visualizer (CAMAG[®] TLC Visualizer 2) (Fahrurroji & Riza, 2020).

Determination of ascorbic acid content

The ascorbic acid content of lime juice powder was determined by spectrophotometry UV-Vis (Hitachi UH5300) method. The standard solutions of ascorbic acid were prepared by dissolving 50 mg of ascorbic acid powder in 50 mL of distilled water, then the solution was diluted using several concentrations (4, 6, 8, 10, and 12). The absorbance of ascorbic acid solutions was measured at 277 nm. A standard curve of the ascorbic acid standard was determined by evaluating the absorbance by plotting the absorbance value against each concentration (Yunita *et al.*, 2019).

Lime juice powder 100 mg was dissolved in 10 ml of distilled water, then the solution was diluted 5 times. The absorbance of lime juice powder solutions was measured at 277 nm. The ascorbic acid content of lime juice powder (x) was obtained from the standard curve of the ascorbic acid standard ($y = bx + a$) (Yunita *et al.*, 2019).

DPPH radical scavenging activity

The antioxidant activity of lime juice powder was measured by using the DPPH method. The ascorbic acid standard was used as a positive control with several concentrations of 2.5, 5, 7.5, 10, and 12.5 ppm. In labeled test vials, 1 mL of ascorbic acid standard solution was mixed with 3 mL 0,004% DPPH solution. The lime juice powder was also prepared by applying the same procedure such as the ascorbic acid standard solution. The lime juice powder was prepared with 100 mg of lime juice powder was dissolved in 100 ml of methanol p.a to obtain a concentration of 1000 ppm, then the solution was diluted to several concentrations. 1 mL of lime juice powder solutions (15, 20, 25, 30, 35, and 40 ppm) were treated with 3 ml of 0.004% DPPH solution. The mixtures were shaken and incubated in a dark place for 30 min at room temperature. The absorbances of the samples and positive control were measured at 517 nm using a spectrophotometer UV-Vis (Hitachi UH5300) (Ogundele & Bolade, 2021). All measurements were made in triplicate, and the DPPH radical concentration was calculated using the following equation :

$$\%inhibition = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

The IC₅₀ was calculated using a calibration curve in the linear range by plotting the lime juice powder concentrations vs % inhibitions (Oulebsir *et al.*, 2022).

RESULTS AND DISCUSSION

Lime juice powder preparation

The lime juice was prepared by extracting lime fruit and 3 kg of lime fruit was extracted using citrus juicer (Idealife). From this process, 830 ml or 861.38 g of lime juice was obtained.

830 ml of lime juice obtained through the extraction process, 780 ml was taken and used for freeze drying, and the results were as shown in Table 1. The total weight of lime juice before freeze-drying was 791.76 g and the total weight of lime juice after freeze-drying was 71.78 g, from these results, the total % yield of lime juice was 9.07%.

Table 1. Yield of freeze drying process of lime juice

Description	Total
Total weight of juice before freeze dry	791.76 g
Total weight of juice after freeze dry (lime juice powder)	71.78 g
Total % yield	9.07%

Organoleptic characteristics

The organoleptic examination showed that the lime juice is a viscous liquid with pale yellow color and had a distinctive lime odor, Meanwhile, lime juice powder is a yellowish-dry powder with a distinctive lime odor. The organoleptic of lime juice and lime juice powder can be seen in Figure 1.

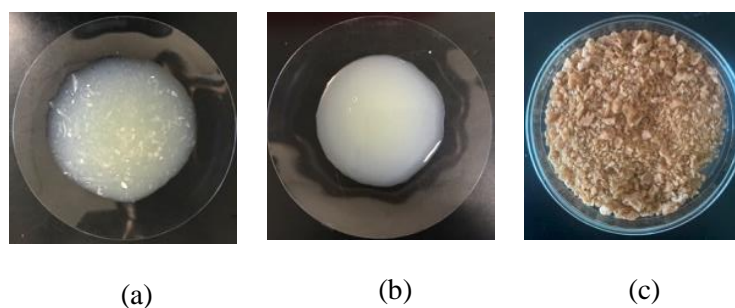


Figure 1. Macroscopic characteristics of (a) Lime juice before filtering, (b) Lime juice after filtering, (c) Freeze dry powder (lime juice powder).

Phytochemical screening

The phytochemical screening of lime juice powder was carried out using the thin layer chromatography (TLC) method, which is an identification method to determine the profile of secondary metabolites present in a sample. The results of the phytochemical screening of lime juice powder can be seen in Table 2. The choice of the mobile phase was based on the polarity of the sample and the stationary phase, where the lime juice powder was dissolved in aquadest as a polar solvent, while the TLC plate used polar silica gel 60 F₂₅₄, resulting in a strong interaction between the sample and the stationary phase. Therefore, the mobile phases were chosen n-hexane and ethyl acetate with a ratio (6:4), where N-hexane is a non-polar solvent that is stable and volatile (Hastuti *et al.*, 2018), while ethyl acetate is a semi-polar solvent which can attract polar and non-polar compounds, so that the mobile phase mixture will tend to be non-polar, which causes the compounds to separate properly.

Table 2. Phytochemical screening results on lime juice powder

Test	Spotting reagent	Result (+/-)	Description
Flavonoid	Citro borate	+	Visible blue smudges before being sprayed with a spotting reagent
Saponin	Liebermann-Burchard	+	Faded brown spots are visible after sprayed with a spotting reagent at 366 nm
Terpenoid	Anisaldehyde	+	Visible purplish spots in visible light
Steroid	Liebermann-Burchard	+	A blue spot is visible after sprayed with a spotting reagent at 366 nm.
Alkaloid	Dragendroff	-	No orange spots visible
Fenol	FeCl ₃	+	Visible blue spot at 366 nm

Based on Figure 2, it can be seen that lime juice powder contains flavonoid compounds. This can be seen from the results of testing where the flavonoid compounds are found in the white lamp (400-800 nm) there were no spots, and in UV 366 there was a blue stain before spraying with citro borate, indicating that the sample contained isoflavones and flavonols compounds (Harborne, 1998). The screening for saponin compound which can be seen in Figure 2, in the white lamp (400-800 nm) after spraying with Liebermann-Burchard, there are faded brown and purplish spots. At UV 366 after spraying with Liebermann-Burchard a faded brown fluorescence appears. From these results, it can be concluded that lime juice powder contains saponins (Fahrurroji & Riza, 2020).

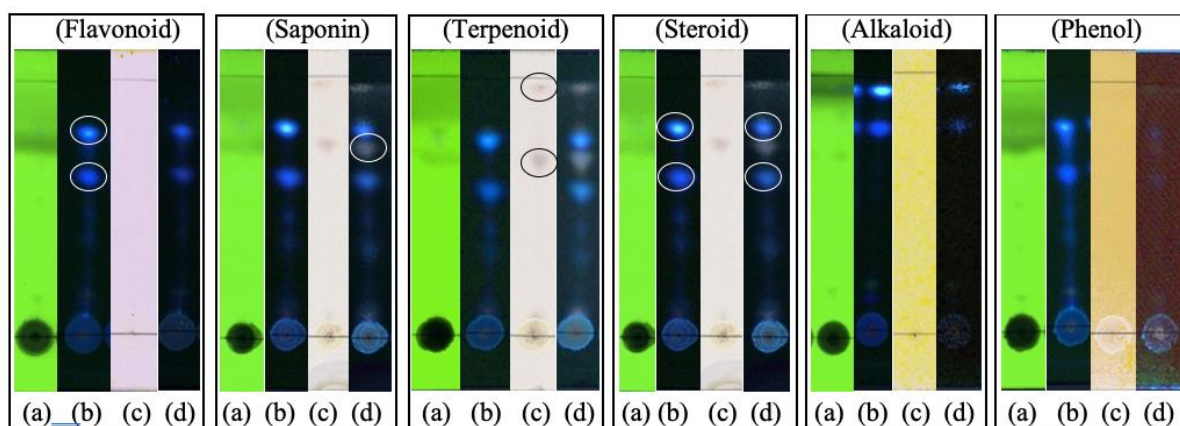


Figure 2. TLC profile of screening compound of lime juice powder. Silica gel 60 F254 served as the stationary phase. N-hexane: ethyl acetate (6:4) was used as mobile phase: (a) UV 254 before spraying; (c) UV 366 before spraying; (d) White lamp (400-800 nm) after spraying and heated at 105°C for 5 minutes; (e) UV 366 after spraying and heated at 105°C for 5 minutes.

The results of the terpenoid compounds screening in Figure 2 showed purplish spots in the white lamp (400-800 nm), and in UV 366 light there is blue fluorescence after spraying with anisaldehyde and heated at 105°C for 5 minutes. The appearance of purple, blue, gray, red, or green spots after spraying with anisaldehyde indicates that sample contains terpenoid compounds (Fahrurroji & Riza, 2020). Based on Figure 2, it can be seen that after spraying with the Liebermann-Burchard in UV 366, it showed blue

fluorescence. From these results, it can be concluded that lime juice powder contains a steroid compound (Fahrurroji & Riza, 2020). The result of screening of alkaloid compounds can be seen in Figure 2, that in UV 366 before and after spraying with dragendroff showing blue fluorescence, but the sample indicate contain alkaloid compounds if after spraying with dragendroff, orange-brown or red-orange spots appeared (Fahrurroji & Riza, 2020). Based on Figure 2, it can be seen that in the screening of the phenolic compounds before and after spraying FeCl_3 there was no color change in the white lamp (400-800 nm) and UV 254. In UV 366, it showed blue fluorescence, but the sample indicate contains a phenolic compound if after spraying with FeCl_3 showed strong spot of green, red, brown, purple, and blue color. While the samples showed faded blue spots, it was concluded that the sample not contained phenolic compounds (Fahrurroji & Riza, 2020). So, from the description, it can be concluded that the lime juice powder sample contained flavonoids, saponins, terpenoids, and steroids.

Determination of ascorbic acid content

Determination of ascorbic acid content in lime powder was carried out using the UV-Vis spectrophotometry method, this is because the ascorbic acid structure contains chromophore and auxochrome groups which can be read by UV spectrophotometry (Puspitasari *et al.*, 2020). The maximum wavelength of ascorbic acid standard solution obtained is 277 nm (Figure 3).

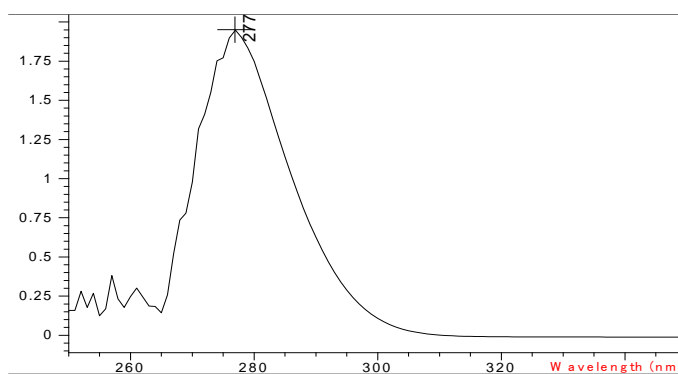


Figure 3. Maximum wavelength of ascorbic acid

The standard curve of ascorbic acid was determined by evaluating the absorbance of series concentrations (4, 6, 8, 10, 12). The linear regression equation of the ascorbic acid standard curve was obtained (Figure 4).

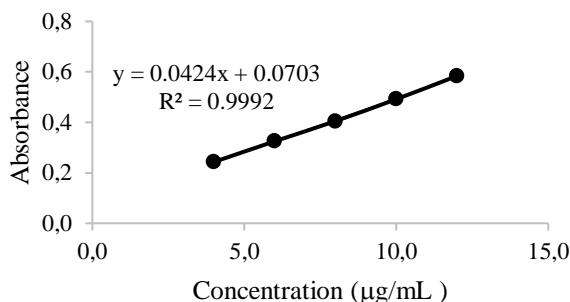


Figure 4. Ascorbic acid linearity and regression equation

The absorbance measurement of the lime juice powder was carried out by UV-vis spectrophotometry at 277 nm, and the absorbance measurement results and the determination of ascorbic acid content in the lime juice powder were obtained, which can be seen in Table 3.

Table 3. Determination of ascorbic acid content in lime juice powder

Replication	Ascorbic acid content (mg/100mg)	ascorbic acid content (%)± SD
1	0.38	0.38 ± 0,001
2	0.38	
3	0.38	

Based on Table 3, it can be seen that the ascorbic acid content in lime powder is 0.38 mg/100mg. whereas in a research conducted by Shrestha *et al.*, (2012), lime juice was found to contain ascorbic acid of 118.2 - 140.8 mg/100g, and the results of ascorbic acid content were from Ogundele & Bolade's research (2021) of 21.8 g/100g to 38.9 g/100g. The ascorbic acid content among samples is different from the references because of the different areas of the lime fruit used. The lime fruit used in this study was harvested from the Citrus Cultivation Garden in the Ujung Pangkah area, Gresik.

DPPH radical scavenging activity

The DPPH is a free radical that is often used to measure the antioxidant activity of extracts of natural materials or several compounds and is stable at room temperature (Prasonto *et al.*, 2017). The result of DPPH radical scavenging activity showed that lime juice powder can scavenge the free radical, with an IC₅₀ value of 32.587 µg/mL, while ascorbic acid had an IC₅₀ value of 8.567 µg/mL (Figure 5).

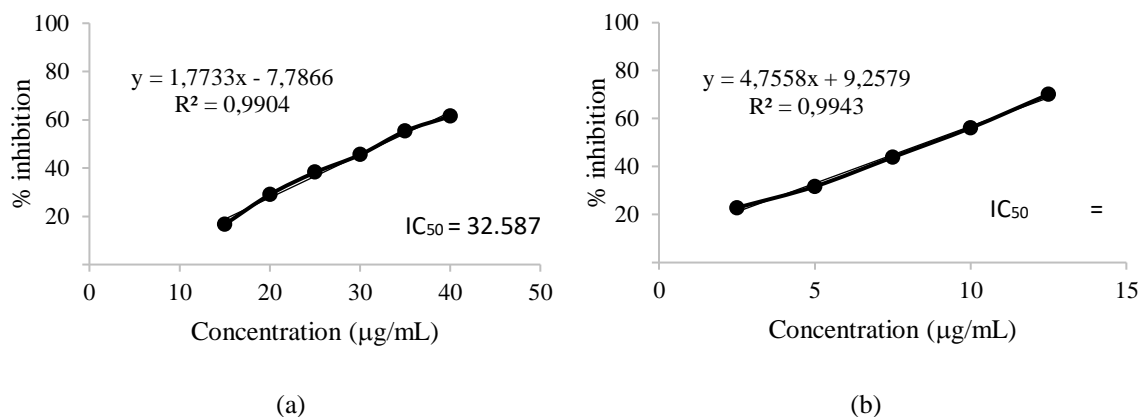


Figure 5. The IC₅₀ value for radical scavenging activity of : (a) lime (*C. aurantifolia*) juice powder; (b) ascorbic acid.

The free radical scavenging reaction by ascorbic acid can be seen in Figure 6. When the DPPH solution is mixed with an antioxidant molecule that can donate a hydrogen atom, DPPH would oxidize and be decolorized with the loss of the deep violet color in the DPPH solution to a pale yellow solution (Basma *et al.*, 2011).

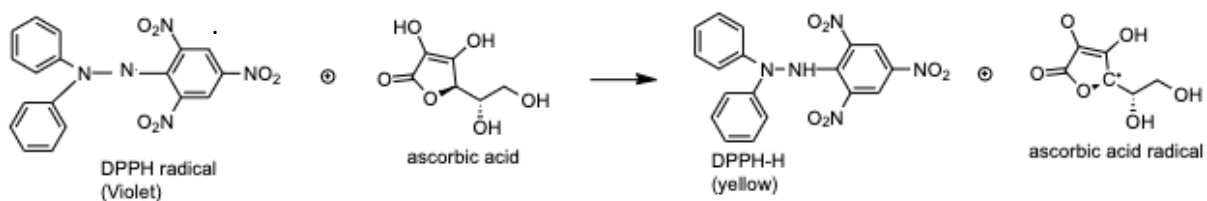


Figure 6. The reaction between DPPH radical and ascorbic acid

The parameter used to measure antioxidant activity is the inhibition concentration (IC₅₀), which is the concentration of antioxidants needed to inhibit 50% of the free radical oxidation process. The lower IC₅₀ value makes the higher substance or material has antioxidant properties (Boligon, 2014). The value of IC₅₀ less than 50 µg/mL was the category to possess very strong antioxidant activity, 50-100 µg/mL was the strong category, 100-250 µg/mL was the moderate category, 250-500 µg/mL was the weak antioxidant activity, and more than 500 µg/mL was inactive antioxidant activity (Lung & Destiani, 2018).

CONCLUSION

The lime juice from lime fruit (*C. aurantifolia*) was taken from Ujung Pangkah, Gresik, East Java, Indonesia contains flavonoids, saponins, terpenoids, and steroids compounds, it also has ascorbic acid content concentration of 0.38 mg/100mg. The IC₅₀ value of the ascorbic acid was 8.57 µg/mL, while the

IC₅₀ value of the lime juice powder was 32.59 µg/mL. According to the IC₅₀ value of lime juice powder had a category as very strong antioxidant.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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