SCREENING OF PHOTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF LIME ROOT EXTRACTS (Citrus Aurantifolia (Cristm.) Swingle) USING DPPH METHOD

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Abstract

Indonesia is a country that is very rich in natural ingredients, and has natural products that are very beneficial for health. One natural ingredient that are beneficial for health is lime root which is generally used as an herbal medicine. In Tumbang Kunyi , Central Kalimantan , lime root is believed for generations to treat ulcers. Based on this statement, the researcher intends to conduct further research on lime root. To analyze the content of secondary metabolites and antioxidant activity in lime root extract. This type of research is qualitative descriptive observational. Extraction by maceration method. Phytochemical screening with color reagent test and KLT. Antioxidant activity by DPPH method. Extraction of lime root as much as 400 grams with 7 liters of 96% ethanol obtained a thick extract of 14.52 grams. Phytochemical screening results with the color reagent and KLT showed that lime root contains alkaloids, flavonoids, saponins and tannins and antioxidant activity test results are obtained is 24 , 81 ppm. From this study, it can be concluded that secondary metabolites contained in lime root (Citrus aurantifolia (Cristm.) Swingle) based on phytochemical tests and KLT contain alkaloids, flavonoids, saponins and tannins. Lime root also has a very active antioxidant activity based on the obtained IC50 value of 24 , 81 ppm.

Keywords: Lime root, Antioxidant, Phytochemical

1. Introduction

Natural ingredients that are used as traditional medicine are increasing nowadays. The use of traditional medicine is considered to have fewer side effects compared to drugs obtained from synthetic compounds and the cost is more affordable. Many Indonesians live in rural areas and in some cases are difficult to reach by current clinical and medical groups. The high cost of treatment today makes most people turn to traditional recipes that come from nature(Princess, 2010).

The use of medicinal plants has long been used since ancient times until now, both in developed and developing countries. According to the World Health Organization (WHO) records, the use of biodiversity (bioprospecting) is very large, it is estimated that almost 80% of mankind, especially in developing countries such as Africa, Asia and Latin America, use traditional medicine as a complement to the primary treatment they get, and still rely on plants (extracts and bioactive ingredients) as treatment and maintenance of health(Ismail, 2015).

Indonesia is a country that is very rich in natural ingredients, and has natural products that are very beneficial for health. In a very developed country like now, and in the health sector, various medical technologies and drugs that can cure a disease also appear. But behind the advances in medical technology, there are still many people who are interested in doing home

remedies. Such as turmeric rhizome, ginger, star fruit wuluh, celery, guava, soursop, cocor duck, noni and one of the home remedies that is often used is lime.(Sambara, 2016).

Lime (Citrus aurantifolia (Cristm.) Swingle) is a polyembryonic plant grown in various countries. In Indonesia this plant is quite a lot because of its tropical climate. Lime is one of the plants that are generally used by the people of Indonesia, either as a food flavoring or empirically, to be precise, it is used as a cough medicine, shed phlegm, influenza and acne.(Lauma et al., 2014). In addition to the fruit of lime, lime leaves are also often used as medicine by the general public(Julizan, 2019). People in Tumbang Kunyi (Central Kalimantan) have traditionally used the roots of lime (Citrus aurantifolia (Cristm.) Swingle) as a home remedy to treat ulcers. Lime is used as an appetite enhancer, antipyretic and antibacterial(Parama et al., 2019). Lime contains a variety of useful chemical compounds, namely citric acid, amino acids (tryptophan and lysine), essential oils (cytal, limonene, geranylacetate, linalylacetate, felandren, kadinen, aktildehid, nonyldehid) glycosides, fats, resins, citric acid, calcium, phosphorus, iron, sulfur, vitamins B, and C. In addition, lime also contains saponins and flavonoids, especially hesperidin, naringin, tangeretin, eriocitrin, and eriocitrocid(Princess, 2010). Lime peel also contains antioxidants so it is used as an antidote to free radicals(Ulfah & Sumantri, 2014a).

According to Winarti (2010), free radicals are atoms, molecules or compounds that can stand alone which have unpaired electrons, therefore they are very reactive and unstable (Giriwijoyo, 2004 in Giriwijoyo, 2004). Kesuma, 2015).

Antioxidants are compounds that can donate protons to free radicals, so that no further harmful reactions occur. Phenolic compounds or secondary metabolites found in plants are responsible for antioxidant, anticancer, antiviral and anti-inflammatory activities. Oranges can be consumed fresh or made juice which is very important as a source of antioxidants because of the content of vitamin C (ascorbic acid), flavonoids and phenolic compounds.(Julizan, 2019).

2. Method

This research was conducted with a qualitative descriptive observational type of research. Descriptive research is a research method by describing and describing something, for example existing conditions or relationships, developing opinions, ongoing processes, consequences or impacts that occur.(Linarwati et al., 2016). Observation is a technique for collecting data by paying attention and recording efficiently. Observation descriptions are used to clarify, give and manifest what is happening(Hasanah, 2017).

1. Tools and materials
   a. Tool

      The tools used in this study were aluminum foil, porcelain dish, funnel, chamber, evaporator, beaker, measuring cup, filter paper, measuring flask, oven, capillary tube, dropper pipette, volumetric pipette, KLT plate, horn spoon, UV light. , spatula, UV-Vis spectrophotometry, analytical balance, glass jar, plastic wrap, and water bath.

   b. Ingredients

      The materials used in this study were Lime Root (Citrus aurantifolia (Cristm.) Swingle), hydrochloric acid, glacial acetic acid, aquadest, butanol, DPPH (1,1-diphenyl-2-picrylhydrazil), 96% ethanol, ethyl acetate, FeCl3 1%, HCl P, HCl 2N, H2SO4 P, chloroform, liebermann-burchard, methanol, n-Hexane, Dragendroff's reagent, Mayer's reagent, Mg powder, and vitamin C.
2. Plant Determination

Determination of lime (Citrus aurantifolia (Cristm.) Swingle) was carried out at the Biology Laboratory of Lambung Mangkurat University, South Kalimantan. The parts of the plant such as roots, stems, fruits, and leaves are done by sending files in the form of plant photos. Determination is done with the aim of ascertaining the name or type of plant specifically.

3. Sample Collection

Sample preparation of lime root (Citrus aurantifolia (Cristm.) Swingle) was obtained from the Tumbang Kunyi Region, Central Kalimantan.

4. Work procedures

a. Making Simplicia Root of Lime (Citrus aurantifolia (Cristm.) Swingle)

Samples of lime root (Citrus aurantifolia (Cristm.) Swingle) were obtained from the Tumbang Kunyi Region, Central Kalimantan. The manufacture of lime root simplicia (Citrus aurantifolia) begins with the collection of raw materials, wet sorting, and washing thoroughly. Furthermore, the lime root is chopped and dried, the dried samples are sorted dry to proceed to the next stage.

b. Preparation of Lime Root (Citrus aurantifolia (Cristm.) Swingle) Ethanol Extract

Lime root simplicia (Citrus aurantifolia (Cristm.) Swingle) was put into a maceration vessel (glass jar), then extracted using 96% ethanol solvent until the simplicia was completely submerged 1 cm above the surface of the simplicia. Extraction was carried out for 3 x 24 hours, every 24 hours the liquid extract was replaced while occasionally stirring and steaming the liquid extract with the evaporator until a thick extract was obtained.

c. Phytochemical screening with color reaction

1). Alkaloid Test

The test was carried out by taking each 2 ml sample of lime root which had been extracted with ethanol solvent into 2 different test tubes. After that, the extract was added with 3 drops of concentrated hydrochloric acid and 3 drops of Dragendroff's reagent. If the solution forms an orange precipitate, it is positive that it contains alkaloids. Furthermore, the Alkaloids test using Mayer's reagent was carried out by taking as much as 2 ml of lime root samples, then adding 3 drops of concentrated hydrochloric acid and 3 drops of Mayer's reagent. If the solution forms a white precipitate, the positive sample contains alkaloids (Mustikasari & Ariyani, 2010).

2). Flavonoid Test

The test was carried out by taking 2 ml of lime root samples that had been extracted with ethanol solvent into a test tube, then heated for approximately 5 minutes. After heating, add magnesium powder to the tip of a spoon and 5 drops of concentrated HCl. If the solution forms a yellow-orange to red color, it is positive that it contains flavonoids (Mustikasari & Ariyani, 2010).

3). Terpenoid Test

The test was carried out by taking as much as 2 ml of lime root samples that had been extracted with ethanol solvent into a test tube. After that the extract was added with 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. If the solution forms a red or purple color, it is positive that it contains terpenoids (Septianingsih, 2013).

4). Steroid Test

The test was carried out by taking as much as 2 ml of lime root samples that had been extracted with ethanol solvent into a test tube. After that the extract was added with 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. If the solution is green, then it is positive that it contains steroids (Septianingsih, 2013).

5). Tannin Test

The test was carried out by taking 2 ml of lime root samples that had been extracted with ethanol solvent into a test tube, then heated for approximately 5 minutes. After being heated, a few drops of 1% FeCl3 were added. If the solution forms a greenish-brown or blue-black color, it is positive that it contains tannins (Marlinda et al, 2012).

6). Saponin Test

The test was carried out by taking as much as 2 ml of lime root samples that had been extracted with ethanol solvent into a test tube. After that, add 5 ml of hot aquadest, then shake it to form a stable foam with a height of 1-3 cm for 30 seconds and after adding 2 drops of 2 N hydrochloric acid the foam does not disappear. If foam is formed, it is positive for saponins (Bintoro et al, 2017).

d. Phytochemical Screening With Kromatografi Lapis Tipis (KLT)

1). Alkaloid Test

To identify the mobile phase alkaloid compounds used are ethyl acetate, methanol and water in a ratio (12.5:1,6:1,2). A positive reaction is indicated by some alkaloids giving blue or yellow fluorescence (Hanani, 2014 in).La et al., 2020).

2). Flavonoid Test

To identify flavonoid compounds, the mobile phase used was glacial acetic acid: butanol: water (1:4:5), with ammonia vapor staining as visible. A positive reaction was indicated by the formation of a yellow-brown stain after being vaporized by ammonia on observation with visible light and blue at 366 nm, confirming the presence of flavonoid content (Marliana, 2005).Wardani & Santoso, 2017).

3). Terpenoid Test

To identify terpenoid compounds, the mobile phase used was n-hexane: ethyl acetate (6:4), after which it was put into the chamber and allowed to saturate. On the KLT plate, the extract that had been dissolved in ethanol was spotted, then put into the chamber, delusional to the limit mark, taken and allowed to dry. Then viewed under UV light 254 nm and UV 366 nm. Detection was carried out using a Liebermann Burchard sprayer, then heated for 5 minutes at 105℃. The presence of terpenoids is indicated by the formation of a blue-violet or red-violet color (Hanani, 2015 in).Fajriaty et al., 2018).

4). Steroid Test

To identify the mobile phase steroid compounds used were Chloroform: methanol (9:1), with the appearance of Lieberman-Buchard reagent stains accompanied by heating at a temperature of 105℃ for 5 minutes. A positive steroid reaction is indicated by the presence of a blue green stain (Kristanti et al., 2008 in).Wardani & Santoso, 2017).

5). Tannin Test

To identify the tannin compound, the mobile phase used was methanol: water (6:4), with 5% FeCl3 reagent as a stain. A positive reaction is indicated by the formation of a black stain (Banu and Nagarajan, 2014 in).Wardani & Santoso, 2017).

6). Saponin Test

To identify the saponin compounds, the mobile phase used was chloroform: ethanol: water (10:6:1).

e. Testing of antioxidant activity using the DPPH method

1). DPPH Solution Preparation

Weighed as much as 3.9 mg of DPPH and put into a 100 ml volumetric flask, then dissolved using ethanol solvent and determined the volume up to the mark (Nugrahcni, 2007 in Widyowati et al., 2014). Next, measure the maximum wavelength of DPPH from 450-550 nm.

2). Preparation of Vitamin C Larutan Solution

Weighed as much as 10 mg of ascorbic acid and put into a 100 ml volumetric flask, then dissolved using ethanol and set the volume up to the mark. Furthermore, concentrations of 1 ppm, 2 ppm, 4 ppm and 8 ppm were made.

3). Extract Solution Preparation

Weighed as much as 10 mg of thick extract and put into a 100 ml volumetric flask, then dissolved using ethanol and set the volume to the mark. Furthermore, concentrations of 1 ppm, 2 ppm, 4 ppm and 8 ppm were made.

4). Determination of Operating Time

Determination of the operating time was carried out by reacting 4 ml of the comparison standard of vitamin C and 4 ml of DPPH solution, homogenized for 1 minute and measuring the absorbance at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 at the maximum length that has been obtained.

5). Antioxidant Activity Test With DPPH Method

To determine the antioxidant activity, each sample with various concentrations was pipetted as much as 4 ml with a volume pipette and put into a test tube, then added 4 ml of DPPH solution. Shake the mixture until homogeneous and left for 45 minutes in a dark place, then measure the absorption with UV-Vis spectrophotometry at the maximum wavelength of DPPH. The antioxidant activity of the sample by the amount of DPPH radical absorption inhibition can be determined by calculating the percentage of DPPH absorption inhibition using the formula:

$$\%\text{Inhibition} = \text{x } 100\% \frac{\text{Abs.blanko} - \text{Abs.sampel}}{\text{Abs.blanko}}$$

Information:

abs. Blank = DPPH Absorbance

abs. Sample = Absorbance Test Sample

3. Results

1. Simplicity Making

Lime root (Citrus aurantifolia (Cristm.) Swingle) was made simplicia by making powder using a wood pollinator and obtained the results of simplicia dry powder as much as 400 grams.

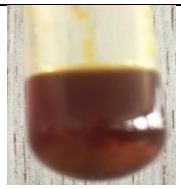
2. Extract Making

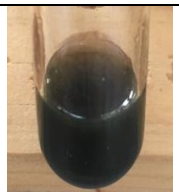
The results of the extraction of lime root (Citrus aurantifolia (Cristm.) Swingle) using 96% ethanol as a solvent obtained 7 L of liquid extract and after being thickened, a thick extract of 14.52 grams was obtained.

3. Identification of Phytochemical Compounds by Color Reaction

To identify the phytochemical compounds of lime root (Citrus aurantifolia (Cristm.) Swingle) which was carried out using the color reagent test, the following results were obtained:

Table 1. Color Reagent Test Results

No.	Phytochemical Compounds	Reactor	Results	Information
1	Alkaloid	HCL P + Dragendroff		(+) produces an orange precipitate
		HCL P + Mayer		(+) produces a white precipitate
2	Flavonoid	Mg Powder + HCL P		(+) red color
3	Terpenoid	HCl P + H2SO4 P		(-) does not produce a red or purple color
4	Steroid	HCl P + H2SO4 P		(-) does not produce green color
5	Saponin	Aquadest + HCL 2 N		(+) produces foam

6	Tannin	FeCl3 1%		(+) black color

4. Identification of Phytochemical Compounds by KLT

To identify phytochemical compounds in lime root (Citrus aurantifolia (Cristm.) Swingle) which was carried out by Kromatografi Lapis Tipis (KLT), the following results were obtained:

Table 2. Kromatografi Lapis Tipis Test Results

No.	Phytochemical Compounds	Results			Information
		Visible light	254	366	
1	Alkaloid				(+)
2	Flavonoid				(+)
3	Terpenoid				(-)
4	Steroid				(-)
5	Saponin				(+)

6	Tannin				(+)

5. Testing of antioxidant activity using the DPPH method

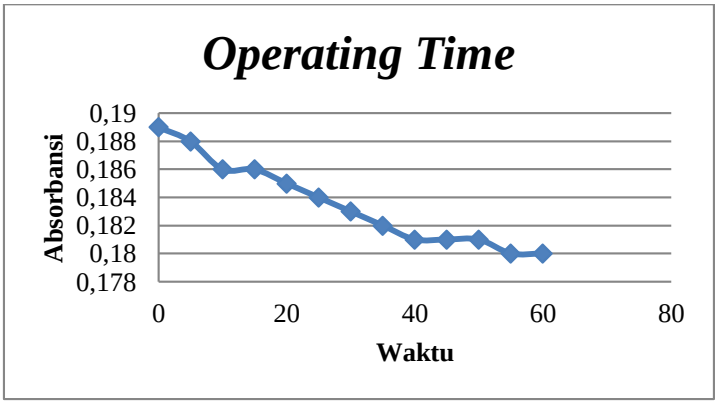


Figure 1. Operating Time Graph

Table 3. Results of Antioxidant Activity

Sample	Concentration (ppm)	Average Absorbance	% Inhibition	Linear regression equation	IC50 value
Vitamin C	1	0.434	9.95	y = 8.5285x + 3.643R² = 0.9726	5.43
	2	0.391	18.87		
	4	0.268	44.39		
	8	0.148	69.29		
Lime root extract	1	0.477	1.03	y = 2.0246x - 0.2348R² = 0.8029	24.81
	2	0.474	1.65		
	4	0.422	12.44		
	8	0.413	14.31		

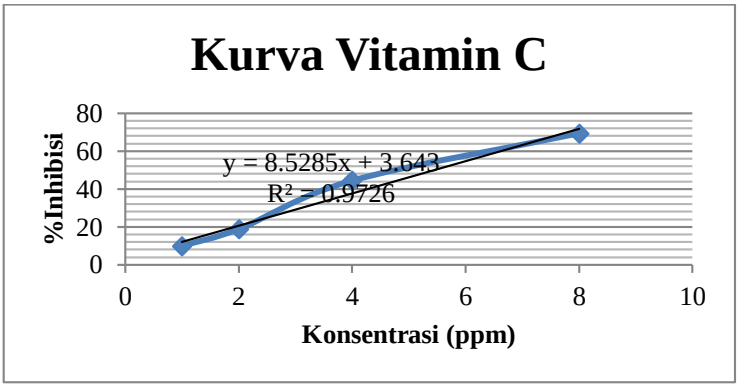


Figure 2. Graph of Vitamin C

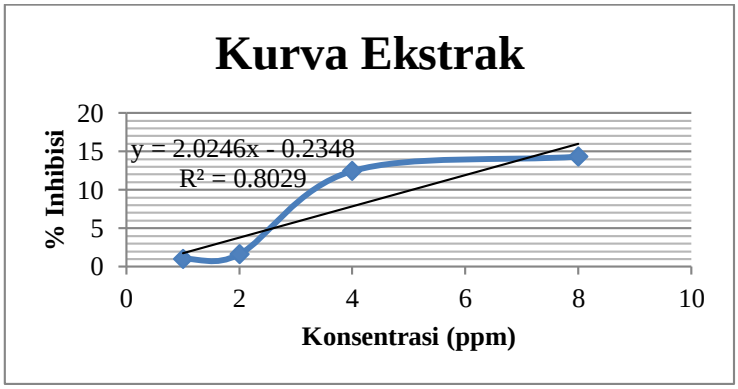


Figure 3. Graph of Lime Extract

4. Discussion

From the research that has been carried out, lime root simplicia was dried and the yield of 400 grams of dried simplicia was obtained, and after drying, lime root was macerated using 96% ethanol solvent and obtained maceration results as much as 7 L. After the maceration process, lime root extract was thickened. using a rotary evaporator and obtained a thick extract of 14.52 grams. After obtaining the thick extract, a phytochemical screening identification test was carried out with a color reagent. The phytochemical compounds tested were alkaloids, flavonoids, terpenoids, steroids, saponins and tannins.

In the alkaloid test, the addition of HCl positive results were obtained by the formation of a precipitate from the replacement of the ligand. The nitrogen atom having a lone pair of electrons in the alkaloids replaces the iodo ion in Dragendorff and Mayer . reagents(ES, 2014).

The flavonoid test showed positive results with a change in color to red. The addition of magnesium powder and hydrochloric acid to the flavonoid test will cause the reduction of the existing flavonoid compounds, causing a red color reaction which is a feature of the presence of flavonoids (Robinson, 1995).ES, 2014). Flavonoids are a class of polyphenolic compounds which are said to have properties as free radical scavengers, inhibitors of hydrolysis and oxidative enzymes, and work as anti-inflammatory (Pourmourad et al, 2006). According to Robinson (1995), flavonoids function to regulate growth, photosynthesis, antimicrobial and antiviral. Flavonoids are useful for protecting cell structures, increasing the effectiveness of vitamin C, anti-inflammatory, preventing bone loss and as antibiotics (Haris, 2011; Robinson, 1995 inIkalinus et al., 2015).

The steroid and terpenoid tests showed negative results because there was no color change in the ethanolic extract of lime root. If the results are positive, the color changes to green for steroid compounds and becomes red or purple for terpenoid compounds (Septianingsih, 2013).

The saponin test showed a positive result due to the presence of foam. This condition forms foam, in this test the sample contains saponin compounds, 2N HCl is added which aims to increase the polarity so that the hydrophilic group will bind more stable and the foam formed becomes stable.(ES, 2014).

The tannin test showed a positive result because of the change in color to greenish black. In this test it produces a greenish black color which means the sample contains tannin compounds (Sangi et al., 2013; Artini et al., 2013 inES, 2014). Tannins are polyphenolic compounds that are widely distributed in plants. The nature of tannins as astringents can be used as antidiarrheal, bleeding and preventing inflammation (Hanani, 2014).

The next test was carried out by testing the identification of phytochemical screening of lime root extract using thin layer chromatography. The KLT test observes the color directly and observes the color of the spots on the KLT plate under UV 254 and 366 lamps. The principle of KLT is the distribution of compounds between the stationary phase in the form of a solid placed on a glass or plastic plate and the mobile phase in the form of a liquid, which moves over the stationary phase.

In the alkaloid test with ethyl acetate: methanol: water (12.5:1,6:1,2) as mobile phase, the results were positive with the appearance of stains after spraying with Dragendrof reagent. The results of KLT before spraying with reagents visually look faintly yellow, at UV254 it looks black and UV366 looks blue fluorescence. After being sprayed with Dragendorph reagent, the visible light appears clear yellow and at UV 254 it is black, while at UV 366 it looks blue. Based on the literature, some alkaloids give a blue or yellow fluorescence, for example, strychnine, purine and brusine alkaloids (Hanani, 2014).

In the flavonoid test with acetic acid: butanol: water (1:4:5) mobile phase, the results were positive with the appearance of stains after being vaporized with ammonia. The results of KLT before spraying with reagents visually look faint yellow in color, at UV254 it looks black and UV366 looks blue fluorescence. After being evaporated with ammonia in visible light it appears yellow brown and at UV 254 it is black, while at UV 366 it looks blue. This shows that lime root extract contains flavonoids in accordance with Markham's (1998) statement that flavonoid compounds will produce a blue color when viewed in UV 366 light (Nafisah et al., 2014; Miharja et al., 2001; Harlis, 2010; Karim et al. , 2015 inWinariyanthi, 2017).

In the terpenoid test, no blue-violet or red-violet stains were formed, this indicates that the lime root extract does not contain terpenoid compounds (Hanani, 2015 in Fajriaty et al., 2018).

In the steroid test there was no blue green stain formed, this indicates that the lime root extract does not contain steroid compounds (Kristanti et al., 2008 in Wardani & Santoso, 2017).

In the saponin test with chloroform: ethanol: water (10:6:1), the mobile phase was positive with the appearance of stains after spraying with Lieberman Burchard. The results of KLT before spraying with reagents visually look faint yellow in color, at UV254 it looks black and UV366 looks blue. After spraying with Lieberman Burchard the visible light appears green. This is in accordance with the statement of Rikomah & Elmitra, that the presence of saponins is indicated by the formation of green spots. (Rikomah & Elmitra, 2017)

In the tannin test with methanol: water as mobile phase (6:4), the results were positive with the appearance of stains after spraying with 5% FeCl3. The results of KLT before spraying with

reagents visually look faint yellow in color, at UV254 it looks black and UV366 looks blue. After spraying with Lieberman Burchard the visible light appears black. This is in accordance with the statement of Banu & Elmitra (2014), that the presence of tannin compounds is characterized by the formation of black spots (Banu and Nagarajan, 2014 inWardani & Santoso, 2017).

The next test is the antioxidant activity test on lime root extract using the DPPH method. The first thing to do is to determine the maximum wavelength of DPPH, the absorbance value is 518 nm, so that the sample and positive control tests are carried out at 518 nm. Determination of the operating time shows that the absorbance value is stable at 45 minutes, then the antioxidant activity test is carried out at 45 minutes by measuring absorbance, the absorbance value reading is stable at 0.181, this reading is done at three digits behind the comma because the fourth digit is a pseudo number, so its existence can be ignored(Suharyanto & Prima, 2020). The antioxidant activity test of lime root extract was carried out by measuring the value of the inhibitory activity against DPPH free radicals using the UV-Vis spectrophotometric method. The principle of this method is the interaction of antioxidants with DPPH either by electron transfer or hydrogen radicals in DPPH will neutralize the free radical character of DPPH, if all the electrons in the DPPH free radicals become paired, the color of the solution changes from dark purple to bright yellow.(Jami'ah et al., 2018). The greater the DPPH radical scavenging activity, the smaller the remaining DPPH concentration, so that the resulting absorbance value decreases.(Ulfah & Sumantri, 2014b). The IC50 value shows that the value for lime root extract is 24.81 ppm while the IC50 value for vitamin C is 5.43 ppm which can be categorized as having very active/strong antioxidant activity. In a previous study with the title Lime peel ethanolic extract has antioxidant activity using the DPPH method. The IC50 value of the ethanolic extract of lime peel was 54,458 g/ml and that of vitamin C was 4,768 g/ml.(Ulfah & Sumantri, 2014a). and In previous studies also obtained the IC50 value of lime leaf extract worth 93.41 ppm (Airat et al., 2015). It can be concluded that the root part of the lime plant has a very strong antioxidant value, while the fruit peel and leaves have a strong antioxidant activity value.

5. Conclusion

From this research, it can be concluded that secondary metabolites found in lime root (Citrus aurantifolia (Cristm.) Swingle) based on phytochemical tests contain alkaloids, flavonoids, saponins and tannins. Lime root also has very active antioxidant activity based on the IC50 value obtained at 24.81 ppm.

Declaration of Interest Statement

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