Identification of Saponins and Flavonoids in Lime (Citrus aurantifolia) Peel Extract

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Abstract

Lime (*Citrus aurantifolia*) peel has an important role in human health. Components contained in lime (*Citrus aurantifolia*) peel can be utilized in medical field. Lime peel is the site of accumulation of flavonoid and saponin compounds. Both compounds are useful for human health. The purpose of this study was to identify saponin and flavonoid compounds in lime (*Citrus aurantifolia*) peel. This study was a descriptive study. Foam test on lime (*Citrus aurantifolia*) peel extract revealed saponin compound, while extract test of lime (*Citrus aurantifolia*) peel using UV-Vis spectrophotometer revealed the presence of flavonoid compound with a level of 1.12%.

Keywords : Saponin, Flavonoid, Lime (*Citrus aurantifolia*) peel extract.

1. Introduction

Lime (*Citrus aurantifolia*) is one type of citrus (Citrus) which contains chemical compounds that are beneficial for health, including citric acid, amino acids (tryptophan, lysine) essential oils (citral, limonene, phellandrene, lemon campher, cadinene, glycosides, calcium, phosphorus, fats, iron, sulphur, vitamin B1 and C. Lime also contains saponin and flavonoid compounds, namely hesperetin (hesperetin 7-rutinoside), tangeretin, naringin, eriocitrin and eriocitrocid (Adindaputri et al., 2013).

Lime (*Citrus aurantifolia*) peel has an important role to human health. Components contained in lime (*Citrus aurantifolia*) peel can be used to lower cholesterol levels in blood. Lime peel contains flavonoid compounds, namely the naringin, hesperidin, naringenin, hesperitin, routine, nobiletin and tangeretin (Pratiwi et al., 2010)Flavonoids are the largest group of polyphenol compounds that can act as antioxidants and also as antibacterials. The actions of flavonoid are denaturing bacterial cell proteins and damaging bacterial cells (Adina et al., 2014; Pelczar and Chan, 2015).

Flavonoids are secondary plant metabolites and derivatives of 2-phenyl-benzyl-γ-pyrone which can be found in almost all plants. More than 9,000 compounds from this group have been studied. The group's biosynthetic pathway begins with the condensation of one Co-koumaroyl-CoA molecule with three malonil-CoA molecules to produce chalcone, which is catalyzed by chalcone synthase (Mierziak et al., 2014). The properties of flavonoids depend on class structure, degree of hydroxylation, substitution and other conjugations (Kumar, 2014). The end result of this substance is in the form of polyphenolic compounds as antioxidants that have potential in health. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by binding to free radicals or by chelating metal ions (Gurnani et al., 2016). The metal chelation is very important in preventing the destruction of biomolecules (Gurnani et al., 2016)

Saponin, one of the secondary metabolites of plants, is a glycoside composed of sugars that bind to the aglycon. Aglycon (sapogenin) has a structure consisting of a triterpenoid or steroid chain and non-polar. The saponin structure causes saponins to be like soaps or detergents, so saponins are referred to as natural surfactants (Fahrunida and Pratiwi, 2009) Saponins have effective anti-inflammatory properties to cure edema in mice and have anti-inflammatory activity (Fahrunida and Pratiwi, 2009).

Based on information, lime peel is a site of accumulation of flavonoids and saponins. In order to utilize lime peel as a source of flavonoids and saponins, this study aims to identify the content of flavonoids and saponins in lime peel.

2. Methodology

The tools used in this study include blender, glass jar, aluminium foil, filtr paper, rotary evaporator, digital scales, measuring glass, and UV-Vis Spectrofotometer. Materials used include lime peel, 96% ethanol, hexamethylenetetramine solution, acetone, 25% HCl, ethyl acetat, water, glacial-methanol acetic acid, and 2N HCl.

To perform the research procedure through several methods ie first method (1) Sampling (2) Sample preparation (3) Samples extraction (4) Total Flavonoid Identification. First method (1) Sampling. Lime peel was taken and collected from the garbage of bakso, soto and sate eateries in Surabaya area. Second Method (2) Sample Preparation. Lime peel was cleaned and dried in a shaded room (30º-35ºC), then mashed with a blender until powder is formed. The powder was put in a clean and covered container (Tumane et al., 2014).

Third Method (3) Sample Extraction. A total of 60 g of lime peel in a glass jar was soaked (macerated) with 96% ethanol of 225 ml, then covered with aluminum foil and left for 5 days with occasional stirring to be evenly distributed. After 5 days, it was filtered using filter paper, produced filtrate 1 and residue 1. Residue 1 was added with 75 ml of 96% ethanol solution and covered with aluminum foil, left for 2 days with occasional stirring. After 2 days, the sample was filtered using filter paper to produce filtrate 2 and residue 2. Filtrate 1 and 2 were mixed together, and evaporated using a rotary evaporator at 80°C. Subsequently, the solvent was evaporated until the ethanol solvent was removed, leaving a pure, viscous extract. The extract was weighed and stored in a glass jar before being used for testing (Mpila et al., 2012).

Fourth Method (4) Total Flavonoid Level Identification. (a) Sample Preparation. A total of 1 g of the sample was fed into a round bottom flask and added 1.0 ml of 0.5% (b/v) hexamethylenetetramine solution, plus 20 mL acetone and 2.0 mL HCl 25% (w/v), and refluxed for 2 hours since boiling. The mixture was filtered using cotton into a 100 mL measuring flask. The cotton was rinsed with acetone, added acetone to 100 mL, and then homogeneously shaken. The filtrate was inserted 20.0 mL into a separating funnel, plus 20 mL of water, plus 15 mL of ethyl acetate, shaken for 10 min, allowed to separate and the ethyl acetate phase was taken. It was continued with 3 times, extraction each with 10 mL of ethyl acetate. The ethyl acetate phase was combined and washed 2 times each with 50 mL of water. The extraction results were fed into a 50 mL measuring flask, added with ethyl acetate to the sign line, and homogeneously shaken. (b) Flavonoids Level Determination. As much as 10.0 mL of ethyl acetate fraction was introduced into a 25 mL flask, added with 1 ml of AlCl3 solution, added with glacial-methanol acetic acid to the volume marks. The blank treatment was the same as the determination procedure. (c) Saponin Compound Screening. A total of 0.3 g of the sample was added with 10 mL of water and shaken strongly for 30 seconds. Froth test result positively contains saponins if stable froth is produced for more than 30 minutes with froth height of 1-10 cm above the surface and if one drop of 2N HCl is added the froth will not disappear.

3. Results And Disussion

Testing of flavonoid content in lime (*Citrus aurantifolia*) peel was quantitatively done by UV-Vis spectrophotometry method. Testing of saponin content in lime (*Citrus aurantifolia*) peel was done qualitatively with froth test.

Based on the results of flavonoids test quantitatively, lime (*Citrus aurantifolia*) peel samples contained Flavonoid compounds with a level of 1.12% as shown on table 1. Based on the results of flavonoids test quantitatively, lime (*Citrus aurantifolia*) peel samples contained Flavonoid compounds with a level of 1.12% as shown on table 1.

Table 1. Results of flavonoid level test on lime (*Citrus aurantifolia*) peel using spectrophomotery UV-Vis method

Samples (g)	Absorbance	Flavonoid (%)
1.0180	0.909090	1.12
1.0082	0.909620	1.13
	Mean	**1.12**
	SD	0.00814
	% RSD	0.73
	Uncertainty	0.02

Table 1 shows the mean value of flavonoid compounds of 1.12%. The absorbance of the test solution was measured at maximum wavelength (± 425 nm) and then as shown on figure 1.

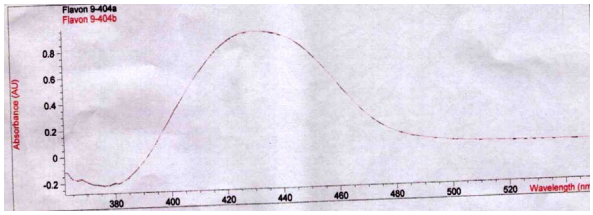

Figure 1. UV-Vis spectrum of flavonoid test level on lime (*Citrus aurantifolia*) peel.

Testing of saponin content in lime peel was qualitatively done by froth test. The qualitative froth test showed that the sample contained saponin with the formation of stable froth with a height of 1-10 cm above the surface and the froth was able to last more than 30 minutes and did not disappear after added with 1 drop of 2N HCl as shown on figure 2.

Figure 2. Saponin froth formation

The basis of froth test reaction is the nature of the saponin compound which dissolves easily in water and causes foam when shaken. The function of water is as a solvent, while 2N HCl functions as a reagent.

Extraction is a process of separating a substance based on its solubility difference against two different non-soluble liquids. The extraction in this research was the extraction of maceration. Maceration is one method of separation of compounds by immersion using organic solvents at room temperature. The extraction process by maceration method is very advantageous in the isolation of natural material compounds, because it is cheap and easy to do. By immersion, plant samples have cell walls and membranes breakdown due to pressure differences between inside and outside the cell, so that the secondary metabolites present in the cytoplasm will dissolve in the solvent. The solvent

that flows into the cell can cause protoplasmic swelling and the cell content will dissolve in accordance with its solubility. The solvent used in this study was 96% ethanol p.a. This solvent was chosen because the flavonoid compound present in lime peel was a polar compound, so it must be dissolved with a polar solvent (Wardani et al., 2017)

Flavonoids are good reducing agents, inhibiting many oxidation reactions, both enzymes and non enzymes. Flavonoids are the largest group of phenol compounds (Gafur et al., 2012) The mechanism of action of flavonoids is as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. The mechanism works by denaturing the proteins of bacterial cells and destroying cell membranes irreparably (Loizzo et al., 2012).

Saponin is a high molecular weight compound glycoside produced primarily by plants. Based on their chemical structure, saponins are grouped into three main classes, the steroid class, the alkaloid steroid class and the triphonoid class. Typical properties of saponins, among others, are bitter and foaming in water. Triterpenoids have an antibacterial mechanism by reacting with the porin (transmembrane protein) in the outer membrane of the bacterial cell wall, forming a strong polymer bond resulting in the destruction of the porine. Damage to the porine, which is the entrance and exit way of the compound, will reduce the permeability of bacterial cell membranes. This makes the bacterial cells lack of nutrients, so the bacteria will have growth inhibition and even death (Abdassah, 2009).

Research by (Choi et al., 2007) showed that the content of lime (*Citrus aurantifolia*) peel is a compound with a flavanon framework, naringin, hesperidin, nobiletin and tengeretin. Flavonoids are one of the active compounds in plants that can be exploited as antibacterial. Flavonoid mechanisms, such as quercetin, act to inhibit bacterial growth by inhibiting DNA gyrase. Saphoraflavone G and (-) - epigallocatechin gallate have been reported to inhibit bacterial cytoplasmic membrane function, whereas lichocalcones A and C can inhibit energy metabolism.

Flavonoids are one of the compounds that have the ability as an antibacterial. Research conducted by (Mpila et al., 2012) on antibacterial activity of *jarak cina* leaf ethanol extract stated that *jarak cina* leaf ethanol extract can inhibit the growth of Staphylococcus aureus bacteria with 8% concentration and Escherichia coli bacteria with concentration of 5%. Leaves of *jarak cina* and *jarak pagar* contain the same chemical compounds, the flavonoids, saponins and tannins (Nuria et al., 2009). The mechanism of action of flavonoids as antibacterial is by forming complex compounds with extracellular and dissolved proteins, which can damage the bacterial cell membrane and followed by the discharge of intracellular compounds (Nuria et al., 2009). Saponin is one of the secondary metabolites. Various studies have found that saponins can have the effect of antitussives and expectorants (Hylocereus and Weber, 2017). These effects can help cure cough. Inflammatory saponins have also been shown to be effective in curing oedema (inflammatory responses) in mice and have anti-inflammatory activity (Wulandari and Idiawati, 2013). The ability of saponins makes saponin as an important secondary metabolite in the medical field.

According to (Katzung, 2004) saponin is a compound that has a strong surface tension and can act as an antimicrobial by disturbing the stability of bacterial cell membrane that causes cell lysis. This is because saponins are soluble semipolar compounds in lipids and water, so they will be concentrated in the microbial cell membrane. The ability of saponin as the active compound can be developed as a natural antimicrobial drug. Lime peel can be utilized as a source of saponins that can be developed into commercial natural antimicrobials.

4. Conclusion

Froth test on lime (*Citrus aurantifolia*) peel extract shows the presence of saponin compounds. Lime (*Citrus aurantifolia*) peel extract test of with UV-Vis spectrophotometer method showed the presence of flavonoid compound in a level of 1.12%.

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