IDENTIFICATION OF ORGANIC COMPOUNDS FROM EXTRACT LOTUS SEEDS (*Nelumbo nucifera*)

Salmiyah[1], Ainunnisa[1], Eka Nur Afiah[1], Nurmilasari[1],
Endah Handayani[1], Firdaus[1*]

[1]Department of Chemistry FMIPA Hasanuddin University Tamalanrea Makassar 90425
*Corresponding author: firdaus_tdg@yahoo.com

Abstrak. Biji *Nelumbo nucifera* merupakan tanaman rawa yang banyak ditemukan di Indonesia. Sulawesi Selatan merupakan salah satu provinsi yang memiliki potensi rawa terbesar sehingga sering ditemukan tanaman *Nelumbo nucifera*. Uji fitokimia kandungan metabolit sekunder tumbuhan spesies ini telah dilakukan. Pengujian yang dilakukan meliputi uji alkaloid, tanin, saponin, steroid, terpenoid dan flavonoid dari ekstrak sampel metanol, n-heksan, kloroform dan etanol. Berdasarkan hasil uji yang dilakukan ekstrak sampel metanol positif mengandung senyawa terpenoid, tanin dan saponin; ekstrak kloroform positif mengandung senyawa alkaloid, terpenoid dan tanin; ekstrak etanol positif mengandung flavonoid, tanin dan saponin; sedangkan ekstrak n-heksan memberikan hasil uji negatif terhadap pengujian yang dilakukan.

Kata Kunci: Biji *Nelumbo nucifera,* uji fitokimia.

Abstract. Seeds of *Nelumbo nucifera* is many found in Indonesia. South Sulawesi is one of the most potencial provine swamp of Indonesia. The Phytochemical test of this plant contain of alkaloid, tanin, saponin, steroid, terpenoid and flavonoid from methanol exract sample, n-hexane, chloroform and ethanol. The result of this phytochemical test is positively contain of terpenoid, tanin, and saponin; chloroform extract positively contain of alkaloid, terpenoid, and tanin; ethanol extract positively contain of flavonoid, tannin, and saponnin; n-hexane extract showed the negative result of this test.

Key words : Seeds of *Nelumbo nucifera*, phytochemical test.

INTRODUCTION

Indonesia is one country that has a vast biological diversity compared to other countries in the world. Approximately 250,000 species of the world's plants are estimated to be about 30,000 species found throughout the archipelago in Indonesia (Achmad, 2006). Among the various types of plant species diversity, Indonesia has the potential to become a country capable of producing various types of medicinal herbs from traditional medicinal plants. It is reviewed based on the content of chemical compounds and bioactivity of which is owned by the plant. In all the work required a method of separation, purification, and identification of the content contained in plants that have different properties and also has a large number. Thus, the advancement of our knowledge of phytochemistry is directly related to the success of known techniques and the development of new techniques to solve a prominent new problem (Robinson, 1995).

Chemical compounds isolated from plants are widely used as medicine. In Indonesia plant species that have not been utilized as medicine one of them comes which has several common names (e.g. Indian lotus, Chinese water lily, and sacred lotus). Lotus is a large and rhizomatous aquatic herb with slender, elongated, branched, creeping stem consisting of nodal roots; leaves are membranous, peltate (60-90 cm and above), orbicular, and concave to cupshaped; petioles are long, rough with small distinct prickles; flowers are white to rosy, sweet-scented, solitary, hermaphrodite, 10-25 cm diameter; ripe
carpels are 12 mm long, ovoid and glabrous; fruits are ovoid having nut like achenes; seeds are black, hard and ovoid (Sridhar and Rajeev, 2007)

Nelumbo nucifera in Asia and Australia and *Nelumbo* lutea in America is used as an ornamental plant in the pond because of its beautiful flowers. Flowers from *Nelumbo* are used to treat diarrhea, cholera and liver disease. *Nelumbo nucifera* Gaertn. Or lotus, roots and seeds and flowers for cough medicine, famous for its very long-lasting seeds, can be up to 3000 years under suitable conditions (Astuti dan Agung, 2007). *Nelumbo nucifera* is widely cultivated in East Asia especially in China. This plant is very potential to be developed because in addition to having a high economic value, this plant also produces various types of chemical compounds (alkaloids, flavanoids, steroids and tannins) are beneficial to health (Zhu, 2015).

The results showed that the antioxidant activity of methanol extract plumule lotus and also lotus flower together has a powerful reduction and activity of free radical capture (Wu *et al*., 2003). In addition, extracts from some parts of the lotus plant have shown potential as anti-ischaemia, antioxidant, anticancer, antiviral, antiobesity, lipolytic, hypocholesterolemic, hypoglycemic, antipyretic, hepatoprotective, antidiarrhea, antifungal, antibacterial, anti-inflammatory and also have diuretic activity (Mukherjee *et al*., 2009). Identification of *Nelumbo nucifera* seeds contains flovonoids,

tannins, saponins and lignins from ethanol extract (Baehaki, et.al., 2015) . In a study to identify secondary metabolite compounds on *Nelumbo nucifera* seeds using methanol, n-hexane, chloroform and ethanol extracts.

RESEARCH METHODS

Research Tools

The tools used in this research are the glass tools commonly used in laboratory, separating funnel, analytical balance, freeze dryer and rotary evaporator.

Materials Research

The materials used in this study were Nelumbo nucifera seeds, methanol p.a, aquadest, n-hexane p.a, chloroform p.a, ethanol p.a, filter paper whatmann 42, filter paper, KLT plate, Wagner reagent, acetal anhydride, concentrated H_2SO_4, NH_4OH , 10% NaOH, Mg, $FeCl_3$, dilute HCl, 95% ethanol.

Time and Place of Research

This research was conducted in March-June 2017 at Integrated Biotechnology Laboratory of Faculty of Animal Husbandry, Hasanuddin University, Organic Chemistry Laboratory and Biochemistry Laboratory of Faculty of Mathematics and Natural Sciences, Hasanuddin University.

Research procedure

Sample Preparation

Nelumbo nucifera's seeds is collect from Pangkep, Daya, Antang and Gowa region about 1,2 kg and then rmove the water containment using freeze dryer so that the seeds can be drying until about 700 gram. Next we crushing th seeds. The smaller size of the sample the bigger its surface and the interaction of the solvent in extraction will get bigger until the extraction process become more effective (Voight, 1995). Then we macerary using methanol solvent 1:1 during 1 week, next we do partition nhexane, chloroform and ethanol. The principle of partition is based on the distribution of solutes wit certain comparation between two solvent which not mix up to each other (Khopkar, 2003).

Phytochemical Test

Alkaloid

Into the test tube were inserted each sample as much as 1 mL after it was added Wagner reagent. Observed sediment formed.

Steroid

To the test tube were inserted each sample as much as 1 mL and added acetate anhirida and concentrated H_2SO_4. Observed changes that occur.

Terpenoid

To the test tube were inserted each sample as much as 1 mL and added chloroform and concentrated H_2SO_4. Observed changes that occur.

Flavonoid 1

Into the test tube were inserted each sample as much as 1 mL and added NH_4OH. Observed changes that occur.

Flavonoid 2

Into the test tube were inserted each sample as much as 1 mL and added 10 % NaOH. Observed changes that occur.

Flavonoid 3

Into the test tube were inserted each sample as much as 1 mL. Added Mg, dilute HCl and 95% ethanol. Observed changes that occur.

Tanin

Into the test tube were inserted each sample as much as 1 mL and added concentrated $FeCl_3$ dan concentrated H_2SO_4. Observed changes that occur.

Saponin

Into the test tube were inserted each sample as much as 1 mL and added aquades. Shake up to form foam. Observed changes that occur.

RESULTS AND DISCUSSION

Alkaloid

Sample that has been added with Wagner reactor will form brown precipitate. This shows positive result of the presence of alkaloid in chloroform sample.

Steroid

The sample was added acetate anhiridate and concentrated H_2SO_4 result obtained showed no color change of solution. It can be concluded that the sample shows a negative test. Positive test that shows the presence of steroid is when there is changes in colour to green or blue. This is based on the Liebermann-Buchard reaction that states when steroid is reacted with anhydride acetate acid and a drop of concentrated sulphuric acid will result in a solution with green or blue colour (Robinson, 1995).

Reactions that occur between steroid with acetic acid anhydride is the acetylation reaction of the -OH group on steroids. As an example of a compound 5α-Kolestan-3β, 6β-diol which undergoes acetylation in the -OH group at C3, resulting in a compound of 5α-Kolestan-3β, 6β-ol (Ahmad, 1986), The reaction can be seen in Figure 1.

Terpenoid

In this test, the methanol extracted sample and chloroform is added with chloroform and concentrated sulphuric acid. Observed result shows formation of reddish brown colour on the surface. This shows in both sample contains terpenoid compound (Odeoga, 2005).

Formation of reddish bron colour on the surface of the solution is due to the addition of chlorosulohonate acid or Brieskorn and Briner reagent that is used to differentiate specifically between reddish triterpenoid and brown steroid (Gerlach in Robinson, 1995) that reddish brown colour is shown on the sulface.

Flavonoid 1

In this test, sample extract is added with dilute ammonia. A change in the colour of the solution to yellow is observed. This happens because

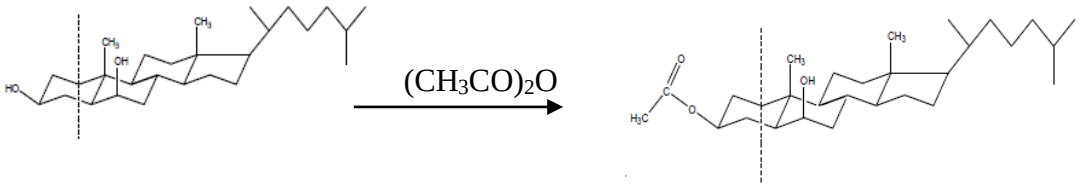
flavonoid is included as phenolic compound. When phenol is reacted with base, it will formcolour that is due to the conjugation system from aromatic chain (Markham, 1988).

Flavonoid 2

In this test the ethanol extract sample was reacted with 10% NaOH. The results showed a change in yellow color then positively contain flavonoids.

Flavonoid 3

In this test the sample added Mg, dilute HCl and 95% ethanol. Flavonoids that are reduced by Mg and HCl can give red, yellow, or orange (Baud, 2014).



a b

Figure 1. a. Compound 5α-Kolestan-3β, 6β-diol, b. Compound 3β-asetoksi-5α-Kolestan-6β-ol

Tanin

Tannin test results from methanol extract, chloroform and ethanol samples with 0,1 % $FeCl_3$ and H_2SO_4 reagents showed positive test that is the color of the solution to be blackish green. This happens because of the reduction reaction.

Tannins are polyphenols classes of compounds, polyphenols can reduce ferri to ferro (Budini, 1980). It is also a classic way of detecting phenol compounds, by adding 1% ferri chloride solution in water or ethanol to the solution of the samples giving rise to green, red, purple, blue or black (Harborne, 1987).

Saponin

In this test, methanol and ethanol extracted sample is added with aquadest, shaken until foam is formed and put aside for 10 seconds. If the foam is still present, it shows positive result of the sample containing saponin.

Table 1. Phytochemical test results

Secondary Metabolite's	Sample			
	Methanol	**N-Hexane**	**Chloroform**	**Ethanol**
Alkaloid	-	-	+	-
Steroid	-	-	-	-
Terpenoid	+	-	+	-
Flavonoid 1	-	-	-	+
Flavonoid 2	-	-	-	+
Flavonoid 3	-	-	-	-
Tannin	+	-	+	+
Saponin	+	-	-	+

CONCLUSION

There are a view kind of secondary metabolite that contain, in *Nelumbo nucifera*'s seeds with lots of extract on phytochemicals teet which is on extract positive of methanol that contains secondary metabolite compound which is terpenoid, tannin and saponnin; chloroform positive extract which contain secondary metabolite compound such as alkaloid, terpenoid, and tannin; ethanol positive extract which contain compound such us flavonoid, tannin and saponnin; and n-hexane negative conyain secondary metabolite compound on the test.

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