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Phytochemical screening and antioxidant activity of seeds extract of water plant (*Nymphaea stellata* and *Nelumbo nucifera)*

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ABSTRACT

The purpose of this research was to screening of phytochemical and antioxidant from seeds extract of water plant (Nymphaea stellata and Nelumbo nucifera). A powdered sample of the seeds of Nymphaea stellata and Nelumbo nucifera were extracted with ethanol to produce a crude extract. Phytochemical result of extracts confirmed the distribution of tannins, saponnins, and flavonoids. The antioxidant activities (IC50) of the extract of Nymphaea stellata and Nelumbo nucifera were 43.21 ppm and 139.84 ppm, respectively.

Keywords: Phytochemical screening, Antioxidant, *Nymphaea stellata*

INTRODUCTION

Nelumbo nucifera, now placed in the mono-generic family Nymphaeaceae, has numerous common names (e.g. Indian lotus, Chinese water lily and sacred lotus) and synonyms (*Nelumbium nelumbo, N. speciosa, N. speciosum and Nymphaea nelumbo* [1]. All parts of *N. nucifera* have many medicinal uses. The leaf, rhizome, seed and flower are traditionally used for the treatment of pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, small pox, dysentery, cough, haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia, fever, cholera, hepatopathy and hyperdipsia [2]. Different classes of phytoconstituents has been isolated from various parts of *N. nucifera*. The most important classes include alkaloids, steroids, triterpenoids, flavonoids, glycosides and polyphenols [3–9].

The major secondary metabolites present in the seeds of *N. nucifera* are alkaloids [6]. Seeds also contain saponins and carbohydrates [10]. ^{13}C-NMR and insource pyrolysis–mass spectrometry analysis showed that the fruit wall and seed coat of *N. nucifera* are composed of a complex of polysaccharides, based primarily on galactose and mannose units and insoluble tannins [11].

Nymphaea stellata referred to as blue water lily. *N. stellata* is a water plant that grows wild or cultivated. This plant grows in Borneo, the Philippines, Sri Lanka, Myanmar, Afghanistan, Pakistan, Bangladesh, Nepal, Cambodia, Malaysia, Laos, Thailand, Vietnam, New Guinea, Indochina, Taiwan and Indonesia. Distribution also been reported in Africa and Australia [12]. (Raja et al, 2010) In Indonesia, the plants of the genus *Nymphaea* is called the lotus.

In this research, we reported that phytochemical screening and antioxidant activity of the solvents' Seed extract of water plant (*Nymphaea stellata* and *Nelumbo nucifera)* were presented.

EXPERIMENTAL SECTION

Plant Materials
The seeds of *Nymphaea stellata and Nelumbo nucifera* were freshly collected at an uncultivated land in Kayu Agung, South Sumatera, Indonesia.

Preparation of Seed Samples
The seeds of *Nymphaea stellata* and *Nelumbo nucifera* were plucked from their stems and collected separately. The seeds were dried under-shade for seven days and ground into powder using clean pestle and mortar. The powdered sample was stored in a closed container and kept in the dark at room temperature until it was required for use [13].

Extraction of Seed Materials
About 2 kg of Seeds of *Nymphaea stellata* and *Nelumbo nucifera* blended rough with the addition of 96% ethanol. Samples were blended and then added 1.5 liters of ethanol and macerated for 2 hours at a speed of 230 rpm. Macerated allowed to stand for 24 h before followed by filtration. Maceration process is carried back to the filtration residue three times repetition with the same amount of ethanol, which is 2.5 liters. The whole macerated collected evaporated by using a rotary evaporator at 40 ° C and a pressure of 175 mbar. Evaporation process is carried out for three days to obtain a thick extract and the yield is calculated. Extracts are stored in dark glass bottles, sealed at a temperature of ± 4 ° C.

Yield of extraction
Yield is the ratio between the initial weight and the weight of the extract material resulting from the extraction process. Yield calculations done to measure the effectiveness of the solvent to extract the bioactive components.

Phytochemical Screening of Seed Materials
The phytochemical analyses of the fractions were conducted by subjecting the fractions to different standard confirmatory tests. This is to determine the presence of certain phytochemical classes.

Test for Saponins
Each fraction (0.5g) was shaken with water in a test tube. Frothing which persists on warning confirmed the presence of saponins [14].

Test for Tannins
Each fraction (0.5g) was stirred with 10ml of water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate indicated the presence of tannins [15].

Test for Flavonoids
A portion of each fraction was heated with 10ml of ethylacetate over a steam bath for 3 min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoid [16].

Antioxidant Activity
DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured based on methods described in Hanani *et al*. [17]. DPPH solution concentration used was 1 mM. The solution used in fresh condition and protected from light. A total of 4.5 ml of test solution included in a test tube is then reacted with 0.5 ml of DPPH solution. Test tube is covered with aluminum foil and incubated at 37°C for 30 minutes then the absorbance was measured using a UV-Vis spectrophotometer at length wave 517 nm.

The antioxidant activity of each sample was expressed in percentage inhibition of free radicals which is calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{blanko absorbance} - \text{sample absorbance}}{\text{blanko absorbance}} \times 100\%$$

Concentration and barriers to extract the value of each plotted on the x axis and y. Obtained equation in the form [y = b(x) + a] is used to find the value of IC (Inhibitory concentration) with a stated value of y is 50 and the value of x as IC50. IC50 values is the concentration of the sample solution is required to reduce DPPH by 50%. This test is performed three replications. The data obtained analyzed descriptively.

RESULTS AND DISCUSSION

Yield of Extraction

Yield of extraction of *Nymphaea stellata* and *Nelumbo nucifera* were 12.6% and 13.6%, respectively. Fig. 3 showed the of extraction of water plant seed (*Nymphaea stellata* and *Nelumbo nucifera*)

Table 1. Yield of extraction of water plant seed

Sample	Yield of extraction (%)
Nymphaea stellata	12.6
Nelumbo nucifera	13.1

The phytochemical screening

The phytochemical screening indicated the presence of some secondary metabolites in the plant fractions that account for the activities of the plant. Phytochemical result of extracts confirmed the distribution of tannins, saponnins, and flavonoids. Tannins that were reported to have anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects were present in all the fractions. Plants that possess tannins are used to treat non-specific diarrhea, inflammations of mouth and throat and slightly injured skins [18 - 19]. Flavonoids are also important for human health. Like vitamins, these compounds are not produced endogenously by the body and must be supplied either through the diet or nutritional supplements [20]. These flavonoids display a remarkable array of biochemical and pharmacological actions *viz*., antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities [21-24]. Phytochemical compound of seeds of water plant (*Nymphaea stellata* and *Nelumbo nucifera*) depicted in Table. 2.

Table 2. Phytochemical compound of seed of water plant

Sample	Tannin	Saponnin	Flavonoid
Nymphaea stellata	+	+	+
Nelumbo nucifera	+	+	+
(+) indicates present while (-) indicates absent			

Antioxidant Activity

Antioxidant mechanisms include radical-scavenging (both hydrogen-donating capability and free radical quenching activity, inhibition of lipid peroxidation, metal ion chelation, or acombination of these properties [25]. Antioxidant activities might protect biological systems against damage related to oxidative stress in human disease conditions. These antioxidant peptides might also be employed in preventing oxidation reactions (such as lipid peroxidation) that leads to deterioration of foods and foodstuffs [26]. Antioxidant activities of seeds of water plant depicted in Table 3.

Table 3. Antioxidant Activity of seed of water plant

Sample	Linier Line	IC 50 Value (ppm)	Antioxidant[1]
Nymphaea stellata	y= 0.053x + 47.71	43.21	Very Strong
Nelumbo nucifera	y= 0.063x + 41.19	139.84	Medium

[1]Molyneux [27].

Scavenging radical activity of *Nymphaea stellata* seeds has higher was compared with *Nelumbo nucifera* seeds. DPPH is a stable free radical that shows maximal absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance, such as an antioxidant, the radical is scavenged. The color is changed from purple to yellow and the absorbance is reduced [28]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability [29].

CONCLUSION

Phytochemical result of extracts confirmed the distribution of tannins, saponnins, and flavonoids. The antioxidant activities (IC50) of the extract of *Nymphaea stellata* and *Nelumbo nucifera* were 43.21 ppm and 139.84 ppm, respectively.

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