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COMPARISON OF ANTIOXIDANT ACTIVITY ETHANOL EXTRACT OF SEED AND ROOT OF NYIREH (XYLOCARPUS GRANATUM) EXTRACT WITH FRAP (FERRIC REDUCING ANTIOXIDANT POWER) METHOD

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ABSTRACT

Nyireh plant (Xylocarpus granatum) is one of the coastal species of trees that grow and live in mangrove forests known as cannon ball mangroves. The purpose of this research is to compare the antioxidant activity of Nyireh (Xylocarpus granatum) seeds and roots by determining the total phenolic content using the Folin-Ciocalteau method and determining the antioxidant activity with the Ferric Reducing Antioxidant Power (FRAP) method. Determination of phenolic content was determined with linear regression equation y = 0.0182 + 0.000712x with r = 0.9605. The total phenolic content of Nyireh seeds is 31.29 mg/g while the nyireh root is 42.98 mg/g. In the determination of antioxidant activity the results of the analysis with UV-Visible Spectrophotometry obtained the maximum wavelength of absorption at 510.5 nm and the linear regression equation of the calibration curve is y = 0.0713 + 3.255x with r = 0.9990. The results of the determination of root antioxidant activity is higher antioxidant activity value is 10.81 mmol Fe(II)/100 g, while of seeds nyireh is lower antioxidant activity value is 8.81 mmol Fe(II)/100 g.

Keywords:
Nyireh (Xylocarpus granatum), Phenolics, antioxidants, seed, root

INTRODUCTION

Indonesia is a tropical country with a large enough area that has a high diversity of coastal and marine resources and can be a source of various drugs and products that are useful both for the pharmaceutical, chemical, cosmetic, agricultural and so on. One of the coastal resources that has been widely used traditionally both as medicine and cosmetics is a plant type Xylocarpus granatum, which is a coastal plant that lives in mangrove forests (Sari, 2008). Xylocarpus granatum. have fruit, seeds, flowers, fruit skins, roots, stems and bark that can be used as a remedy for various types of diseases.

Phenolic compounds from plants have several effects, namely antioxidants, anti-inflammatory, antiproliferation, antimutagenic, antimicrobial, anti-carcinogenic, and prevention of heart disease. The antioxidant effect of phenolic compounds is due to the nature of oxidation which plays a role in neutralizing free radicals (Panovska TK et al., 2005). Excessive amounts of free radicals cause oxidative stress, which can cause oxidative damage starting from the cellular level, tissue, to the organs of the body which accelerate the aging process and the emergence of disease. Therefore, antioxidants are needed to delay or inhibit oxidation reactions by free radicals (Widyastuti, 2010). Naturally the human body has an antioxidant system to recognize free radical reactivity, which is continuously formed by the body itself. Natural antioxidants are more desirable than synthetic, because the level of security is better (Firdiyani, 2015). One method that is often used for the analysis of antioxidant activity is the FRAP method. One method that is often used for the analysis of antioxidant activity is the FRAP method. The FRAP (*Ferric Reducing Antioxidant Power*) method is a simple, fast method, the reagents used are quite simple and do not use special tools to calculate total antioxidants. The principle of this method is the reduction of ferric ions to ferrous ions (Jayanthi, 2011) .

Based on several studies that have been done and based on Increased prevalence of diseases caused by free radicals and exploited seeds and roots nyireh as traditional medicine by some of the community, so need to research on a comparison of the seeds and roots *Xylocarpus granatum* as an antioxidant.

MATERIALS AND METHOD

Tools used is beaker glass (*Pyrex*®), measuring cup (*Pyrex*®), volumetric flask *(Iwaki ®),* pipette volume (*Pyrex*®), test tube (*Pyrex*®), *rotary ovaporator* (*Heidolp*®), *UV-Vis spectrophotometer* (*Shimadzu 1800*), analytical scales (*Kenko ®*), *glass beaker (Pyrex®),* brown bottles, elements (*Pyrex*), filter paper, *blender,* micro pipettes (*Pyrex* ®).

The ingredients used are aquadest, ortho-phenanthroline , seeds and nyireh roots (*Xylocarpus granatum*), ethanol 70 %, ethanol 96%, Folin-Ciocalteus, powder magnesium metal, Vitamin C, Dragendorf , ammonia, chloroform, concentrated HCl , glacial acetic acid, Fe (II) sulfuric Heptahydrate, $H_2 SO_4$ (sulfuric acid), $FeCl_3$ (iron (III) chloride) , acids citrate , sodium acetate trihydrate ($CH_3COONa.3H_2 O$).

This research was carried out experimentally including sampling, processing seeds of nyireh seeds and roots (*Xylocarpus granatum*), determining phenolic content, determining and comparing the antioxidant activity of seeds and nyireh roots (*Xylocarpus granatum*) using FRAP method and data analysis.

RESULTS AND DISCUSSION

Extraction was carried out by cold extraction method, namely maceration. Maceration is immersion of the sample with certain solvents or suitable solvents. The maceration method was chosen because of the simple way it works. This maceration process uses 70% ethanol solvent because ethanol contains 30% water and the sample or simplicia will be dissolved in a dry state, so that the cells in the simplicia have shriveled up and enough water is needed for the cells to expand again to facilitate the process diffusion and withdrawal of compounds (Suharti, *et al* 2017). Ethanol is a polar solvent, where solvents with high polarity levels are suitable solvents for all types of active substances (universal) because in addition to attracting polar compounds, polar solvents can also attract compounds with lower polarity levels. Ethanol is considered as a search fluid because it is more selective, non-toxic, neutral, good absorption. Ethanol can be mixed with water in any ratio, requires less heat for the concentration process, and confounding agents are limited (Sa'adah & Nurhasnawati, 2015). Each sample of seeds and root of Nyireh (*Xylocarpus granatum*) which has been mashed weighed 500 mg and maceration with 70% ethanol solvent Then maserat evaporated using a *rotary ovaporator,* then evaporated again with an oven to get a thick extract. Viscous extract obtained with the results weighed heavy seed 53.22 g while a kar 36.11 g then calculated the value of the yield that aims to determine kemaksimalan of solvent to sum up the compounds in crude drugs and to determine the amount of approximately bulbs required for the manufacture of a number of extract. The yield value of Nyireh (*Xylocarpus granatum)* seed extract is 10.64% and the root of Nyireh (*Xylocarpus granatum*) is 7.22% . According to the Extract Quality Standards According to the Indone-

sian Herbal Pharmacopoeia I edition 2008 Good Extract Extract is not less than 5.9%.

The determination of these parameters is done to provide a simple and objective initial introduction (Depkes RI, 2000). Organoleptic test samples showed differences in the color of the sample extracts, with the color of Nyireh seed extract concentrated red, while the roots were dark red.

Phytochemical screening results showed that the seeds and roots of Nyireh (Xylocarpus granatum) contained flavonoid, tannin, saponin chemical compounds, while the alkaloid, steroid and terpenoid compounds were not contained in the extract. Bioactivity of plants is strongly influenced by the content of chemical compounds contained in the material and differences in the content of chemical compounds indicate differences in pharmacological activity of plants.

The results of the gallic acid calibration curve are y = 0.0182 + 0.000712x with r = 0.9605. The coefficient value of r is almost close to 1, which means a linear relationship between the concentration of gallic acid and the absorbance produced. Phenolic levels obtained in the extract are seeds at 315.730 mg / L and roots at 433.708 mg/L.

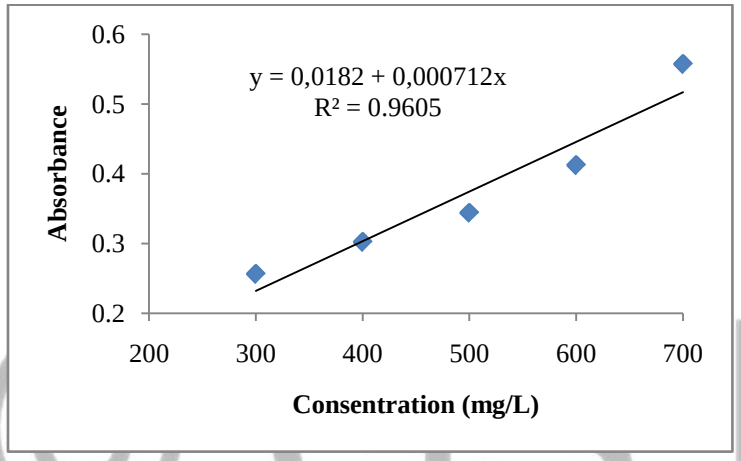

Figure 1. Gallic Acid Calibration Curve

Table 1. Total Phenolate Levels Seed and Root of Nyireh.

NO.	SAMPLE	ABSORBANCE	AVERAGE	CONC. X (mg/L)	CONTENT (mg/g)
1	Seed of Nyireh (*Xylocarpus granatum*)	0.239 0.243 0.248	0,243	315,730	31,29
2	Root of Nyireh (*Xylocarpus granatum*)	0.335 0.326 0.320	0,327	433,708	42,98

Antioxidant activity test on the determination of the maximum wavelength resulting from the measurement of a standard solution of 0.09 mmol / L with a wavelength of 400-800 nm, the maximum wavelength produced is 510.5 nm.

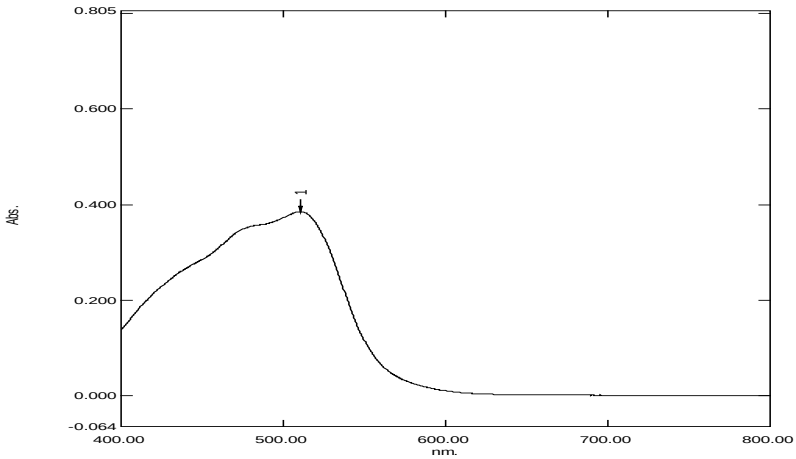

Figure 2. Maximum Wavelength Spectrum of Iron (II) Sulfate Heptahydrate with a concentration of 0.09 mmol/L

The results of the iron (II) Sulfate Heptahydrate standard curve are y = 0.0713 + 3.255x with r = 0.9990. The coefficient r value is almost close to 1 which means a linear relationship between the concentration of Iron (II) Sulfate Heptahydrate and the absorbance produced.

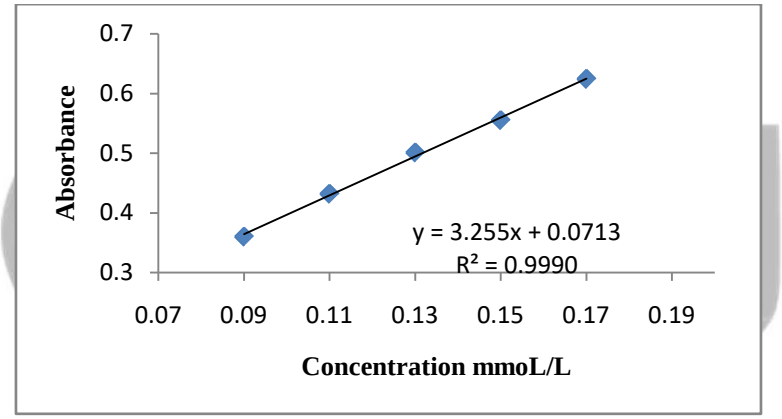

Figure 3. Calibration Curve Series of Iron (II) Sulfate Standard Concentration Series

The antioxidant test is carried out using the FRAP (*Ferric Reducing Antioxidant Power*) method . Antioxidants are reducing agents that can reduce Fe3 + to Fe2 +. This reduced Fe2 + is complexed with an *ortho*-phenanthroline complex. Complexing iron using *ortho*- phenanthroline will produce a solution that is red orange, because of the formation of [Fe (C12H8N2) 3] 2+ complex . The orange-red color of the resulting complex is stable in the pH range of 2-9. Therefore, the advantage of the FRAP method is that research can be carried out in the range of acidic or basic pH (Yefrida *et al* ., 2014) . The absorbance of these complex compounds is measured with a *UV-Vis spectrophotometer* . The amount of complex compounds formed will be proportional to the total antioxidant content in the material (Yefrida *et al.*, 2015) . the use of fenantrolin as a complexing ligand is more profitable because the price is cheaper, simpler analysis process and the use of fewer reagents (Aleksandra *et al.*, 2013).

The results of the analysis of antioxidant activity showed that the total root antioxidant content was higher than Nyireh seeds (*Xylocarpus granatum*) with total root antioxidant content of 10.80 mmol Fe (II)/ 100g, while the nyireh root (*Xylocarpus granatum*) was 8.81 mmol Fe (Fe) II) / 100g. then if Secondly sample compared to the total antioxidant content of ascorbic acid amounted to 15.87 mmol of Fe (II) / 100g seeds and roots contain antioxidants lower. This is because the sample used in the research is still in extract form while ascorbic acid as a comparison is a pure compound .

Table 2. Antioxidant activity of Seed and Root of Nyireh.

NO.	SAMPLE	ABSORBANCE	AVERAGE	CONC. X (mmol/L)	CONTENT (mmol Fe(II)/100g)
1	Seed of Nyireh (*Xylocarpus granatum*)	0.368 0.354 0.36	0.3606	0.0888	8.81
2	Root of Nyireh (*Xylocarpus granatum*)	0.351 0.471 0.448	0.423	0.1080	10.80

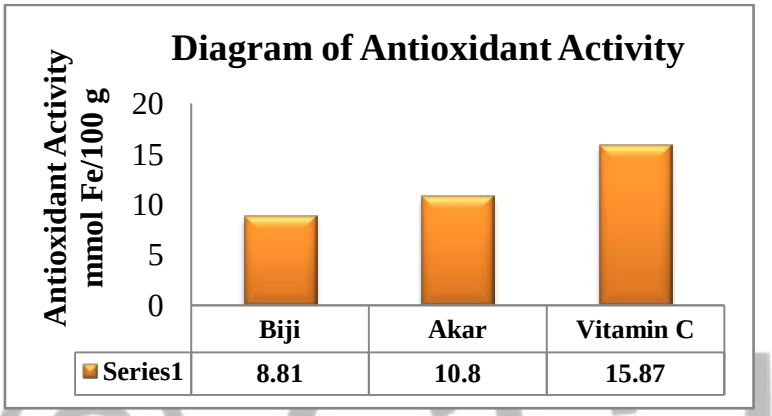

Figure 4. Diagram of Antioxidant Activity

Mastuti's research (2015) states that phenol compounds contribute significantly to antioxidant activity. The antioxidant capacity of phenol compounds is mainly due to the edox properties which enable phenol compounds to act as reducing agents and hydrogen donors. In this study, the measurement of antioxidant capacity by the FRAP method has a positive correlation with total phenols, which indicates that the higher the total phenol contained in the sample , the higher the antioxidant capacity.

Conclusion

The research results show that the nyireh root has a higher phenolic compound content than the Nyireh seed. Nyireh root has higher antioxidant activity than N- yireh seeds . However, both samples have low antioxidant activity when compared with ascorbic acid. The higher the total phenolic level , the higher the antioxidant activity .

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