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ANALYZING ETHANOL'S ANTIOXIDANT EXTRACT OF PANDANUS LEAVES THROUGH DPPH METHOD

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ABSTRACT

Background: Aging is a natural process and no one can avoid it. The aging process is also a physiological process that will occur in all living things. This process covers all organs of the body including the skin which is one of the body tissues that directly shows the occurrence of the aging process
Aim: This research aims to analyze ethanol's antioxidant extract of pandanus leaves through DPPH method.
Method: This type of research is using experimental research designs to determine the antioxidant activity affected by time, and temperature with the DPPH method, a formulation of the effects of the ethanol extract of fragrant pandan leaves *(Pandanus amaryllifous Roxb)*.
Findings: It has active antioxidant activity and IC_{50} extract of fragrant pandan ethanol on the 5th day with a temperature of 50° C and on the 3rd day with a temperature of 35° C which is the best antioxidant activity.

KEYWORDS *ethanol; antioxidant; pandanus leave*

INTRODUCTION

Aging is a natural process and no one can avoid it. The aging process is also a physiological process that will occur in all living things. This process covers all organs of the body including the skin which is one of the body tissues that directly shows the occurrence of the aging process (Cunnningham, 2003; Yaar & Gilchrest, 2007). Various skin problems and disorders can arise in skin aging, namely dry and rough skin, sagging, wrinkles and skin folds, pigmentation patches, and skin tumors (Leijden, 1990).

In the United States, an estimated 31% of the population is 55 years of age or older by 2030 (Yaar & Gilchrest, 2007) and the number of elderly residents will double even triple during the beginning of the first quarter of the 21st century (Smith, 2001). In Indonesia according to data from the Central Statistics Agency, in the period 2000-2005, the life expectancy of the Indonesian population (men and women) was 67.8 years, in the period 2020-2025 increased to 73.6 years (Indonesian Statistics Data, 2011). This encourages increasing interest and concern for people towards efforts to slow down the aging process (Yaar & Gilchrest, 2007). The aging process of the skin has two interrelated phenomena, namely the intrinsic aging process (*chronologic aging*) and the extrinsic aging process (Leijden, 1990; Jenkins, 2000).

Antioxidants are chemical compounds that can donate one or more electrons to free radicals so that they can be muted (suhartono, et al., 2002). In addition, antioxidants are compounds that are able to eliminate, cleanse, resist the formation or combine the effects of reactive oxygen species (Lautan, 1997). Antioxidants serve as an effort to minimize the oxidation process of fats and oils, minimize the occurrence of damage processes in the food, extend the service life in the food industry, improve the stability of fats contained in food and prevent the loss of sensory and nutritional quality (Hernani and Raharjo, 2005).

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Sources of antioxidants can be synthetic antioxidants as well as natural antioxidants. Synthetic antioxidants include *Butylated hydroxytoluene (BHT), Butyl hydroxyanisole (BHA), and Tert butyl hydroquinone (TBHQ).* Natural antioxidants include tocopherols, phenols, and polyphenols. Antioxidant ingredients derived from synthetics until now are still one of the choices used by the community as food additives. However, currently, the use of synthetic antioxidants began to be limited because excessive use of antioxidants causes muscle weakness, nausea, dizziness, and loss of consciousness, while the continuous use of low doses causes bladder tumors, cancer around the stomach, and lung cancer (Cahyadi, 2006).

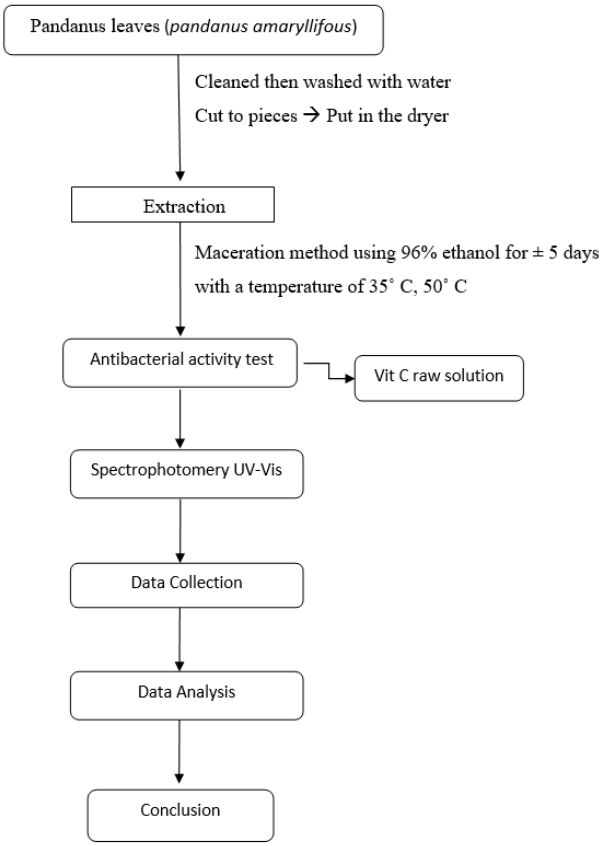
Indonesia is a tropical country that has a very abundant natural wealth. Among the types of flora that grow in Indonesia, there are many plants that have the potential as medicine and have been used for traditional medicine. Finally, the use of herbs. Indonesia began to be redeveloped in an effort to back to nature, namely efforts to extract the potential of nature to find raw materials for drugs by utilizing plants that have been known to benefit the community (Euis H. Hakim, 2007).

Fragrant pandan leaves *(Pandanus amaryllifolius)* is one of the plants that contain the chemical content of alkaloids, *flavonoids*, saponins, tannins, polyphenols that serve as natural antioxidant substances. Polyphenols are phenol derivatives that have antioxidant activity. Phenolic compounds function in absorbing and neutralizing free radicals or reducing peroxide. Phenolic antioxidants as prevention of damage due to oxidation reactions. Ethanol 96% can extract the content of polyphenol compounds from pandan (Osawa, 1994).

Based on the above explanation, the author wants to explore more about fragrant pandan leaves. This study aims to find out the type of chemical compound (Phytochemicals) contained in fragrant pandan leaf extract and the antioxidant potential of ethanol extract.

METHOD

This type of research is using experimental research designs to determine the antioxidant activity affected by time, and temperature with the DPPH method, a formulation of the effects of the ethanol extract of fragrant pandan leaves *(Pandanus amaryllifous Roxb)*. The tools used in this study are electronic scales, Hitachi U-1800 Spectrophotometer, a set of glass tools, liquid extract bottles, filter paper, macerators, stirrer rods, vapor vapors, digital balance sheets, analytical balance sheets, rotary evaporators, blenders, spatulas, refrigerators, micropipets, ball fillers, stopwatches, ultrasonic cleaners, aluminum foils, drop pipettes, vials, and kuvets. The ingredients used are fragrant pandan (*Pandanus amarylifollius* Roxb.), standard gallic acid (Sigma-Aldrich), diphenylpicrilhydrazil/DPPH (Sigma-Aldrich), vitamin C, folin solution ciocalteu (Merck), Na2CO3 (technical), methanol (technical), ethanol 96%, and aquadest. The sample used in this study was a fragrant pandanus leaf extract obtained from Pasar Pagi, Sentosa Baru Street, Medan Perjuangan Subdistrict, Medan, North Sumatra.



Picture 1. The Research's Processes

RESULTS AND DISCUSSION

Phytochemical Screening Results

Table 1. Phytochemical Screening Results

Test	Result	Information
Flavonoid	+	Brownish green
Alkaloid	+	White deposits (Mayer)
	+	Yellow deposits (Dagendorf)
Tannin	+	Greenish brown deposits
Trepenoid	+	Chocolate cincin
Saponin	+	Formed foam

Source: Data Processed

From the table above it can be seen that the ethanol extract of fragrant pandan leaves contains phenols so that it can act as an antioxidant. Oxidant ability is due to the presence of phenolic groups in the molecular structure.

Characteristic Results of Fragrant Pandan Leaf Extract

Tabel 2. The Characteristic Value of Fragrant Pandan Leaf Extract with Maceration Technique with 96% Ethanol for ± 5 days

Wet Simplicia weight	3 kg
Viscous Simplicia Weight	700 gr
Condensed extract	79.85 gr
Yield	100%
Organoleptic	
• **Shape**	• Thick
• **Color**	• Brownish green
• **Construction**	• Special Pandan

Source: Data Processed

IC50 Measurement Results

IC50 measurements of fragrant pandan leaf ethanol extract were performed at a wavelength of 515 nm Division of the extract group based on length of immersion (Maceration) and heating temperature at the time of extracting. The results of IC50 measurements from each group of fragrant pandan leaf ethanol extract treatment can be seen in Table 3 below.

Table 3. The IC_{50} value of fragrant pandan leaf ethanol extract against DPPH free radicals

Day	Temperature	IC50
I	35˚	896,3509
III	35˚	325,3751
V	35˚	165,6976
I	50˚	902,5043
III	50˚	226,7275
V	50˚	107,3364

Source: Data Processed

From the table above can be seen the IC50 value of the ethanol extract of pandan leaves with immersion for 1 day and a warming temperature of 35 ° is 896.3509. On day 3 and the warming temperature of 35° is '325.3751. On day 5 and the temperature of 35° is 165.6976. And on day 1 and the temperature of 50°is 902.5043. On day 3 and the temperature of 50° is 226.7275. And on the 5th day and the temperature is 50°107.3364.

In this study, the sample used is a fresh sample of pandan leaves which are then cleaned, then cut into small pieces, and then dried by winding until the sample is completely dry and then extracted by maceration. Maceration was chosen because of its easy workmanship process and fairly simple equipment. In this maceration, pandan leaves are used (*Pandanus amaryllifous Roxb.*). A total of 700 grams are put in a vessel and dissolved using a 96% ethanol solvent until the simplicia is perfectly submerged. Simplicia is mixed well and the container is tightly closed then stored at room temperature.

Maceration is done while occasionally stirred to increase the extraction speed. After 5 days the resulting maceration is filtered using filter paper and the dregs are put back into the

container (accelerated as many as 3 repetitions). The extract obtained is then evaporated using rotavapor until a thick extract is obtained. The extract is then dried using a dryer cabinet to speed up and streamline time.

Quantitative testing of antioxidant activity is done using the DPPH method. This DPPH method was chosen because it is a simple, easy, fast, and sensitive method and requires only a small sample for testing the antioxidant activity of natural material compounds (Molyneux, 2004).

Based on phytochemical tests it is known that ethanol extracts and ethyl acetate fractions have almost the same compound components, namely alkaloids, flavonoids, phenolics, terpenoids, and saponins except in terpenoid compounds. Terpenoid compounds were identified in ethanol pandan leaf extract.

Ethanol extract of pandan leaves is fragrant with zero characteristics as presented in table 4.1.2. The extraction process with a 96% ethanol extract solvent followed by evaporation produces a viscous extract weighing 79.85gr and brownish-green color. Ethanol extract of fragrant pandan leaves smells special to pandan. This is because pandan contains essential oils and aromatic compounds that are volatile or volatile so pandan has a strong distinctive aroma. Pandan leaves have a distinctive aroma that is thought to come from the hereditary compound of the amino acid phenylalanine, *namely 2-acetyl-1-pyroline.* According to Dalimartha (2002), the content of pandan leaf chemical compounds includes alkaloids, saponins, flavonoids, polyphenols that serve as natural antioxidants, and dyes in extracts. While according to Margaretta et al research the extraction process is done using 96% ethanol solvent so that extracts containing phenolic compounds as natural antioxidants are obtained.

Quantitative testing of antioxidant activity is done using the DPPH method. This DPPH method was chosen because it is a simple, easy, fast, and sensitive method and requires only a small sample to test for antioxidants from natural compounds (Molyneux, 2004).

Based on table 4.3 of the study results, the IC_{50} value of pandan leaf ethanol extract with a maximum wavelength of 515nm can be seen ic50 value from pandan leaf ethanol extract with immersion for 1 day and warming temperature of 35° is 896.3509. On day 3 and the warming temperature of 35° is '325.3751. On day 5 and the temperature of 35° is 165.6976. And on day 1 and the temperature of 50°is 902.5043. On day 3 and the temperature of 50° is 226.7275. And on day 5 and the temperature of 50° is 107.3364. This shows that the ethanol extract of pandan leaves on the 5th day with a temperature of 35° 165.6976 and on the 3rd day with a temperature of 50° C is 226.7275 is the best activity.

The results of the study Margaretta et al (2011), in the study antioxidant activity of *TBHQ* has a % *scavenging activity* of 96.60% while the % *scavenging activity* obtained from a temperature of 50° C is 93.21%. *Scavenging activity of* pandan leaf extract at a temperature of 50° C which is 93.21% close to *scavenging activity* from *TBHQ* which is 96.60% means the ability of pandan leaf extract that acts as an antioxidant is quite good. This shows the results obtained in this study using a temperature of 50 ° C is not good than previous studies this is revealed because of the warming of the liver in the process of evaporation of pandan leaf extract that can cause partial damage to antioxidants in pandan leaves.

Desrosier (1998) states that the higher the temperature and the longer the drying time used to dry material, the more water evaporated from the material. In Lubis research (2008) that the

water content of pandan leaf flour at 40° C 15.70% and the water content of pandan leaf flour dried at a temperature of 50° C is 15.16%.

Previous research related to the DPPH method states that the optimal time for a perfect reaction to take place is 30 minutes, but the incubation time for various samples can vary according to the characteristics of antioxidant activity that the sample has.

Testing antioxidant activity with the DPPH method aims to find out the amount of concentration that can dampen 50% of DPPH free radicals expressed in IC_{50} values and concentrations, the greater the IC_{50} value, the concentration needed to cause a 50% damping effect of DPPH is getting smaller.

Based on the test results it is known that the ethanol extract of pandan leaves and their fractions has a higher ability to catch radicals than commercial vitamin E, but lower than BHT. The higher radical capture rate is indicated by the increasing sloping of the BHT curve, followed by the curve of ethanol extract and the lowest vitamin E. This correlates with rsay test results showing that vitamin E has an ethyl acetate value of 24.15%, while ethanol extract is 69.96%, hexan fraction is 54.81%, ethyl acetate fraction is 54.60%, and BHT 84.80%. (Nakiboglu et al., 2007).

The results of Jenni et al (2012), in the study antioxidant activity of *TBHQ,* has a % *scavenging activity* of 93.56% while the % *scavenging activity* obtained from a temperature of 50 ° C is 89.20%. *Scavenging activity of* pandan leaf extract at a temperature of 50 ° C which is 89.20% close to *scavenging activity* from *TBHQ* which is 93.56% means the ability of pandan leaf extract that acts as an antioxidant is quite good. This shows the results obtained in this study using a temperature of 50 ° C is not good than previous studies this is revealed because of the warming of the liver in the process of evaporation of pandan leaf extract that can cause partial damage to antioxidants in pandan leaves.

CONCLUSION

Based on the results of research that has been done, it can be concluded that the extract of fragrant pandan leaves *(Pandanus amaryllifous Roxb.)*. It has active antioxidant activity and IC_{50} extract of fragrant pandan ethanol on the 5th day with a temperature of 50° C and on the 3rd day with a temperature of 35° C which is the best antioxidant activity. Furthermore, it is highly recommended to conduct research that discusses the effects of Pandan Wangi extract, its benefits, and its antioxidant activity.

REFERENCES

Barhe, T.A. and Tchouya, G.R.,2014, Comparative Study of the Anti-oxidant Activity of the Total Polyphenols Extracted from *Hibiscus Sabdariffa L., Glycine max L. Merr*. Yellow Tea and Red Wine through Reaction with DPPH Free Radical, *Arabian Journal of Chemistry, 9, 1-8*.

Direktorat Pengawasan Obat Tradisional. (2000). *Parameter Standar Umum Ekstrak Tumbuhan Obat Cetakan Pertama*. Jakarta: Departemen Kesehatan Republik Indonesia.

Ditjen POM. (1986). Sediaan Galenik. Jakarta: Depertemen Kesehatan RI. Halaman. 10-11.

Jun, M.H.Y., Fong, X., Wan, C.S., Yang, C.T., Ho. 2003. *Camparison of Antioxidant Activities of Isoflavones From Kudzu Root (Peurarua labata O)*. Journal Food Science Institute of Technologist. 68:2117-2122.

Halliwell, B., 2012, Free Radicals and *of Antioxidant Activities of Isoflavones From Kudzu Root (Peurarua labata O)*. Journal Food Science Institute of Technologist. 68:2117-2122.

Halliwell, B., 2012, Free Radicals and Antioxidant: Updating a Personal View. *Nutrition Review*, 70, 256-265.

Handa, S.S., Khanuja, S.P.S., Longo G., Rakes D.D. (2008). Extraction Technologies for Medicinal and Aromatic Plants. Trieste: *International Centre for Sciences and High Technology*, 21-25.

Lautan, J. 1997. *Radikal Bebas Pada Eritrosit dan Leukosit.* Cermin Dunia Kedokteran. (116), 49-52

Liochev, S.I., 2013, Reactive Oxygen Species and the Free Radical Theory of *Aging, Free Radical Biology and Medicine, 60, 1-4.*

Molyneux, P. 2004. *The Use Of The Stable Free Radicals Diphenylpicryldrazyl (DPPH) For Estimating Antioxidant Activity.* Songklanakarin Journal of Science Technology, 26 (2), 211-219

Ncube, N.S., Afolayan, A.J., Okoh, A.l (2008). Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends. *African Journal of Biotechnology*, 7 (2), 1797-1806.