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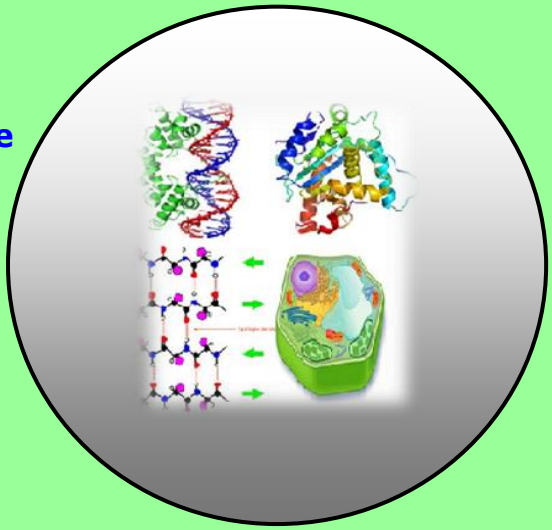

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RESEARCH PAPER

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Characteristics and Antioxidant Activities of Gaharu (*Gyrinops versteegii*) Leaves

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ABSTRACT

Oxidative stress occurs because of an imbalance of the amount of free radicals with a number of endogenous antioxidant produced by the body such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT). This imbalance can be overcome by taking antioxidants natural or synthetic antioxidants. One of them is Gaharu leaves. This study aims to determine the characteristics and antioxidant capacity of Gaharu leaves in vitro. This study was a laboratory experimental study is to analyze the characteristics and bioactivity of Gaharu leaves in the form of water content, chlorophyll content, total content of phenol/flavonoids, antioxidant capacity in the solvent n-hexane, ethyl acetate, chloroform, ethanol, methanol, and water. The results showed that the water content of the fresh leaves and dry is 60.9% and 10.8%. Chlorophyll content (a) 2771.159 ppm, chlorophyll (b) 1111.004. Total phenol content of dried leaves in water extracts showed the highest levels of phenol tolal ie 14.980 mg GAE/100 mg. Highest antioxidant capacity was also demonstrated by the water extract with IC_{50} = 3.44 mg/mL (5 minutes) and 3.03 mg/mL. Phytochemical screening showed that the chemical content of the water extract of Gaharu leaf is in the form of phenolic compounds, flavonoids, and steroids. The content of phenolic compounds/flavonoids are high enough and work in synergy to capture or neutralize free radicals cause leaf water extract of Gaharu have a high antioxidant capacity. The conclusions of this research is to extract water from the leaves Gaharu can be developed as an alternative to natural antioxidants in reducing oxidative stress.
Keywords: Gaharu Leaves, Antioxidant Capacity, Total Phenolic Content and Free Radicals.

INTRODUCTION

Maintenance and development of traditional medicine as a national heritage (ethnomedicine) continues to be enhanced and encouraged development through excavation, research, testing and development as well as drug discovery, including the cultivation of traditional medicinal plant which is medically justifiable. In this case can be formulated into five main things that must be considered, namely etnomedicine, agro-industry medicinal plants, pharmaceutical and medical technology, chemical technology and processes, guidance and supervision of the production or marketing materials and traditional medicinal products. One among these traditional medicinal plants is Gaharu (*Gyrinops versteegii*).

Etnomedicine is a legacy handed down from ancestors who had to be developed, scientifically studied and recorded/documented as possible before going extinct or lost. The Etnomedicine used as reference are: (i) Cabe Puyang, (ii) Ayur Vedas, (iii) Usada Bali, (iv) Atlas of medicinal plants Indonesia, (v) Plant Medicine Indonesia, and (vi) Useful Plants Indonesia.

Agroindustry, especially medicinal plants cultivation of medicinal plants developed to be easy to come by and are not experiencing shortages. Especially for a plant that is almost rare need for the development of tissue culture cultivation and further developed in the field. After cultivated just as much need to be further developed process technology through pharmaceutical technology and medicine through the exploration of natural resources of medicinal plants native to Indonesia through research, testing bioactivity, preparation of phytochemical and standardization of materials/botanicals that legacy hereditary used by ancestors can developed scientifically or medically. Such as cultivation, characterization, phytochemicals test, antioxidants bioactivity test, and manufacturing formulas availability of phytochemicals from plant Gaharu as traditional medicinal plants so that the benefits of the Gaharu plant can be improved (Tarigan, 2004).

Traditionally in China, the Gaharu plant is used as a stress reliever medication, kidney disorders, hepatitis, cirrhosis, liver and kidney swelling, material antibiotics for tuberculosis, rheumatism, cancer, malaria, and peptic ulcers. Traditionally in Tibet, the Gaharu plant can be used as a drug: anti-asthmatic, antimicrobial, the nerve stimulant, stomachache, aphrodisiac, painkillers, cancer, diarrhea, kidney, and lung tumors. The chemical content of the Gaharu plant include: norox-agarofuran, agarospirol, 3, 4-dihidroxy dihydroagarufuran, p-methoxy-benzylaceton, aquilochin, Jinkohol, jinkohol ermol, and kusunol (Mega and Swastini, 2010)

The essence of Gaharu or Eaglewood or agarwood which is the core of Gaharu, damarwangi or resin. The essence of Gaharu an aromatic substance (resin aromatic/fragrant) belonging to the sesquiterpene and has a specific chemical structure and until now there has been bias synthesized in the laboratory. This core contains many essential oils so widely used as perfumes, cosmetics, incense/incense and medicines.

Antioxidants are free radical absorbers, epidemiologically, antioxidants in foods, especially in fruits and vegetables are protective against cancer. However, the results of experimental studies on the relationship between antioxidants and carcinogenesis inconsistent. The opinion states that the antioxidants are anticancer still requires more detailed study. The fact that there has been demonstrated that consumption of a single high dose of antioxidants should be avoided (Prakash, 2001; Prangdimurti *et al*., 2006).

Antioxidants have a very important role for the human body because its function is to inhibit and neutralize oxidation reactions involving free radicals. Oxidation reactions involving free radicals often leads to some degenerative diseases such as cancer. These reactions can damage cell membranes and damage DNA composition that can cause a mutation. DNA mutations that cause uncontrolled cancer (Ardiahansy, 2007; Shafie, 2011).

In the normal or healthy, turnover and rejuvenation of cells in the body will occur as needed through cell proliferation and apoptosis (programmed cell death) under the influence of proto-oncogenes. The resistance of the process typically occurs when the antioxidant reactions in the reaction initiation or propagation.

Oxidative damage to DNA as a result of radiation, free radicals and reactive oxygen compounds that are oxidative is an important cause of cancer. The free radicals formed in the body will induce apoptosis process which causes the death of cells, including tumor cells so as to inhibit carcinogenesis. Inhibition of the antioxidant process usually occurs during reactions initiation or propagation on fat oxidation reaction or other molecules in the body by absorbing and neutralizing free radicals (Zheng and Wang, 2009).

This neutralizing done by giving the electron so that it becomes a more stable compound or termination reactions and reactions stopped radical or oxidative stress does not occur in the cell. Besides prevent or inhibit oxidative stress and tissue damage, antioxidant serves to inhibit the increased production of cytokines, such as interleukin-6 (Il-6) or Tumor Necrosis Factor (TNF-α), which is a pro-inflammatory substance or inflammation.

As an antioxidant, it is in neutralizing free radicals, can also be regarded as free radical. Free Antiradical can prevent free radical reactions are natural and free radicals in the body's metabolism. The presence of sufficient free radical that can prevent free radical reactions with fat and protein can be prevented. These reactions lead to changes in fat or protein or DNA or cell division changes as a result of oxidation reactions can not occur.

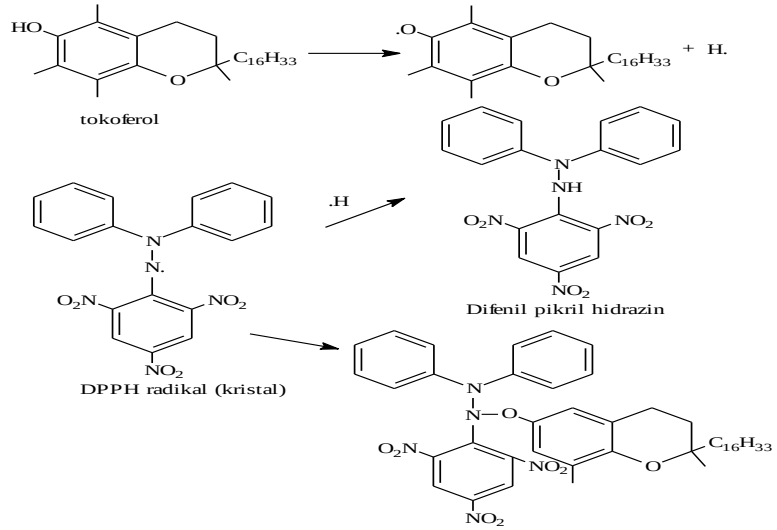
There are two sources of free radicals, the radical source of exogenous and endogenous radicals. Exogenous radical sources usually come from outside the body such as air pollutants, radiation, carcinogenic chemicals, cigarette smoke, bacteria, viruses, and the effects of the drug (anesthetic drugs and pesticides). Radical endogenous sources, namely the free radicals that are the result of normal metabolic within the human body, such as food oxidation process, the oxidation process xanthine, and excessive exercise (Shafie, 2011).

Without realizing in our body are constantly formed free radicals through the events of normal cell metabolism, inflammation, malnutrition, and result in response to influences from outside the body, such as environmental pollution, ultraviolet rays, and cigarette smoke. Consequences caused by the polluted environment, diet, and lifestyle, it stimulates the growth of free radicals that can damage our bodies (Mega and Swastini, 2010). Results of research in the field of nutrition at the cellular level, proving that antioxidants can protect body tissues from the negative effects of free radicals.

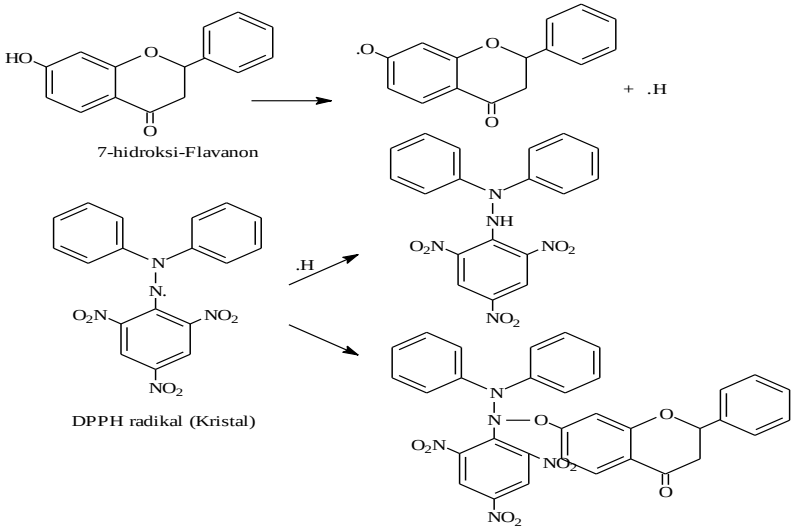
Our bodies need a vital substance, which is an antioxidant that may help protect the body from free radical attack to reduce the negative impact of these compounds. However, this depends on the lifestyle and diet we should correct. Adequate consumption of antioxidants can reduce the occurrence of various diseases, such as cancer, cardiovascular, cataracts, digestive problems, as well as other degenerative diseases (Ardiansyah, 2007).

Antioxidants include phenolic acids, flavonoids, β-carotene, vitamin E (tocopherol), vitamin C, uric acid, bilirubin, and albumin (Shafie, 2011). Minerals, such as manganese, zinc, copper, and selenium (Se), also acts as an antioxidant. Among these antioxidant substances suspected of aqueous extracts of leaves of aloes, such as phenolic compounds and flavonoids.

Testing for anti-free radical compounds from natural materials/synthesis can be performed using a chemical reaction with DPPH (diphenyl pikril hidrazil) as a stable free radical compound to see the reduction in the maximum wavelength UV-Vis spectrophotometer. Damping red purple color (absorbance at 517 nm ± 20 nm) is associated with the ability as an anti-free radicals (free radical Scavanger). As for the reaction of DPPH with anti-free radical compounds can be seen in the following example:



See the above reaction, the flavonoids in the leaves of Gaharu allegedly suffered reduction reaction process is almost the same, as shown in the following reaction:



Analysis of antioxidants can be done by measuring the antioxidant capacity of a compound to see the IC_{50}, for example, phenolic compounds such as flavonoids contained in extracts of leaf water Gaharu.

Several flavonoid compounds isolated from medicinal plants has been shown to have activity as free radical, such as pinostrobin and pinocembrin (in rimpang temu kunci), flavanones (on leaves Sudamala), and flavanones (on the noni fruit).
Functional groups allegedly involved in the reaction between compounds are free radical -OH group and a double bond (>C = C<).

METERIAL AND METHODS

Gaharu leaves plant material obtained from the area of Tabanan Bali, distilled water, n-hexane, ethyl acetate, chloroform, ethanol, methanol, reagent Leiberman-Bourchard, Wilsatter reagents, reagent NaOH 10%, reagent Meyer, $FeCl_3$. Chemicals used in this study pro analysis degree of EMERCK.
A set of tools respirators, filter paper, Buchner funnels, erlenmeyer, glass beaker, liquid extract container, rod stirrer, rotary epavourator, test tubes, pipette, porcelain cup, oven and analytical balance.

Making the water extract of leaves Gaharu

A total of 1000 grams of Gaharu leaf powder included in 2 liters of hot water (60 °C) for about 2 hours. While stirring, let stand for 24 hours, then strain the extraction. Concentrated liquid extract obtained disposable freeze dryer to obtain a thick extract. Viscous extract is then stored in -20 °C for further research. The same is done for the preparation of the extract n-hexane, chloroform, ethyl acetate, ethanol and methanol. Results obtained in the form of condensed extract n-hexane, chloroform, ethyl acetate, ethanol, methanol, and water

Determination of Water Content

Gaharu leaves were already clean cut into small pieces, then put in a porcelain cup that had been weighed beforehand. Enter the porcelain dish into the oven in time of approximately 30 minutes, then removed, then cooled, and weighed. This treatment is done to obtain a constant weight. The water content is calculated from a constant weight obtained reduced weight of fresh leaf leaves Gaharu. The same is treated at Gaharu leaves that have been dried in advance.

Total Phenol Content Analysis

Take as much as 0.1 grams of powdered samples of leaves and extracted with 5 mL of ethyl acetate, ethanol, methanol, and water. Then homogenized and centrifuged at 3000 rpm for ± 15 minutes, until the resulting two layers, then filtered and the filtrate obtained. The filtrate obtained is inserted in a 5 mL flask. Filtran much as 0.4 mL pipette and placed in a test tube, then added 0.4 mL reagent Folin-Clocalteu, divortex until homogeneous, and leave it on for six minutes, then add 4.2 Ml Na_2CO_3 solution. Let stand for 1.5 hours at room temperature before reading at λmax = 760 nm. Standard curve made with gallic acid dissolved in methanol 85% with various concentrations of 10-100 mgl^{-1}. Levels of total phenols using linear regression equation of gallic acid y = ax + b. Data calculation results expressed in units of gallic acid equivalent (GAE)/100 grams (Wolfe and Liu, 2007).

Antioxidant Capacity Analysis

Antioxidant capacity analysis begins with the manufacture of standard solution of gallic acid, then made a calibration curve between concentration of gallic acid in various concentrations (0-100 mg/L). Treatment of samples was done by weighing 0.1 gram sample in each sport, then diluted with methanol 99.9% to a volume of 5 mL flask, then in vortek to homogenize the solution, then the solution was centrifuged 3000 ppm for 15 minutes.

Standard and sample solution (water extract, ethyl acetate, chloroform, ethanol, and methanol leaves Gaharu) in 0.5 mL pipette and added 3.5 mL of 0.1 mM DPPH in methanol in the reaction tube, then divortex. The solution was then incubated at 250C for 30 minutes, so DPPH no time to react with H atoms were given or donated by antioxidants sample, and then measuring the absorbance at 517 nm λ max. Antioxidant capacity was calculated using linear regression equation y = ax + b (Wolfe and Liu, 2007).

Phytochemical screening of aqueous extract of leaves Gaharu

Extract the most active as an antioxidant (antioxidant capacity with IC_{50} least), then analyzed chemical content with the reagent Meyer (alkaloid), reagent Wilstatter, NaOH 10%, and $FeCl_3$ (phenolic compounds and flavonoids), as well as the reagent Leibermand-Bourchard (terpenoids and steroids).

RESULTS

Gaharu leaf characteristics in measuring water content and the content of total phenols. The results of the analysis of the water content of the fresh leaves of Gaharu obtained for 60.9%, while the moisture content of dried leaves Gaharu ready to be used as a tea, water levels as much as 10.5%. Results of the analysis of the antioxidant capacity of the water extract of the leaves of Gaharu *in vitro* in various solvents and the content of total phenols (flavonoids) are presented in Table 1.

Table 1. Antioxidant Capacity and Total Phenol Content of Gaharu Leaf Extract.

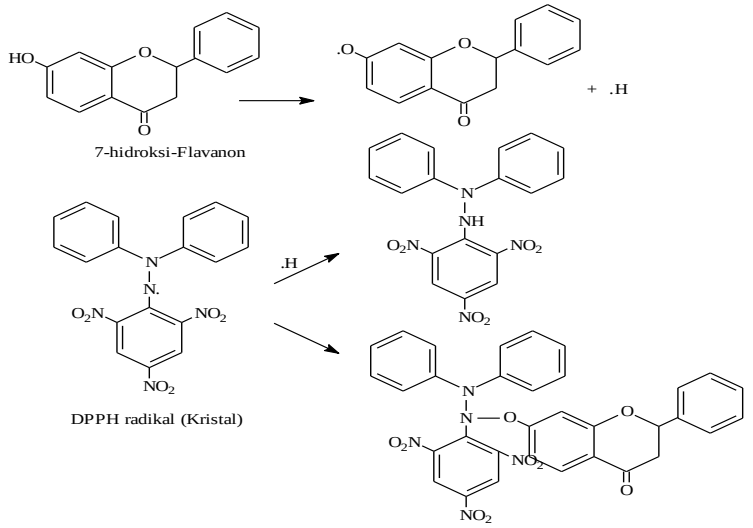
No.	Extract	Antioxidant capacity (IC_{50} = mg/mL)		Total phenolic compounds (flavonoids) (mg GAE/100 gr)
		5 menit	60 menit	
1	Water	3.44	3.03	14980
2	Methanol	16.55	24.04	6070
3	Ethanol	23.45	24.04	1508
4	ethyl acetate	19,20	19,70	442

The analysis showed that the four types of solvents used all showed inhibition of the antioxidant capacity value (IC_{50}). Water extract has antioxidant capacity with the smallest concentration (IC_{50} = 3.44 mg / mL in 5 minutes and IC_{50} = 3.03 mg / mL at 60 minutes) and has the greatest levels of total phenols. These results indicate that the water extract of the Gaharu leaves can be used as an alternative natural antioxidants and subsequently analyzed chemical content. Water extract, the leaves of Gaharu obtained = 200 mg and dark brown. Phytochemical screening Wilstater used reagents, reagent Bate Smith, NaOH 10% to test the presence of flavonoids. $FeCl_3$ solution to test for the presence of phenolic compounds, analysis by Meyer reagent to test for alkaloids, while Leiberman Burchard reagent is used to test for steroids. The results obtained in this phytochemical test showed that, leaf aqueous extract of Gaharu contains phenolic compounds, flavonoids, and steroids.

DISCUSSION

Based on the results obtained, the water extract of leaves of Gaharu (*Gyrinops versteegii*) turned out to contain secondary metabolites, namely flavonoids, steroids, and phenolic compounds.

The compounds of these secondary metabolites are expected to have activity as free radical, because the functional groups present in the compound as the OH group in the breakdown heterolitiknya, will produce a radical O (O) and H radicals (H.). These radicals which will react with DPPH radical, so as to dampen the wavelength of the DPPH. The reaction is thought to occur in this reaction is:



The content of phenolic compounds and flavonoids is what is believed to be active as an antioxidant. Flavonoids may provide antioxidant effect by preventing the formation of ROS, ROS direct capture, protect the lipophilic antioxidants, and stimulates an increase in enzymatic antioxidants, such as superoxide dismutase (SOD), GPx and catalase. Phenolic compounds and flavonoids could be expected to reduce levels of malondialdehyde (MDA) and 8-hydroxy-deoksiguanosin, so as to prevent further DNA damage.

Flavonoids may inhibit damage to DNA from HO* reaction with nitrogen bases of DNA. Flavonoids can serve as an inducer that activates Nrf2 in the cytoplasm, thereby dissociating and translocation to the nucleus. Associated Nrf2 in the nucleus on the promoter of a gene called Antioxidant Response Element (ARE), thus triggering the expression of genes encoding antioxidant to produce SOD (Goodsell, 2007).

CONCLUSION

Based on the analysis and discussion, it can be concluded that: (i) the water content of the fresh leaves of Gaharu was 60.9% and the leaf extract of dried Gaharu is 10.5%; (ii) extract water of Gaharu leaf has antioxidant capacity (IC_{50} = 3.44 ppm and 3.03 ppm), so it can be said to have the greatest antioxidant activity; (iii) extract water of Gaharu leaf has a total phenol content of most, namely 14.980 mg GAE/100 g, and contains phenolic compounds, flavonoids, and steroids.

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REFERENCES

Ardiahansy, (2007). Antioksidan dan Peranannya bagi Kesehatan. http://www.beritaiptek.com, diakses pada tanggal 8 January 2009.

Goodsell, D. (2007). Molecule of the Month: Superoxide Dismutase. http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/ pdb94_1.htm, diakses tanggal 27 Oktober 2012.

Mega, I.M. and D. A. Swastini (2010). Screening Fitokimia dan Aktivitas Antiradikal bebas Ekstrak Metanol Batang Gaharu (*Gyrinops versteegii)*. J. Kimia 4 (2). Jurusan Kimia FMIPA. Universitas Udayana. Denpasar.

Prakash, A. (2001). "*Antioxidant Activity*". Medallion Laboratories: Analithycal Progress, 19 (2): 1-4.

Prangdimurti, E., D. Muchtadi, M. Astawan dan and F. R. Zakaria (2006). Aktivitas Antioksidan Ekstrak Daun Suji *(Pleomele angustifolia* N.E. Brown). *Jurnal Teknologi dan Industri Pangan* XVII (2): 79-86.

Shafie, (2011). Hubungan Radikal Bebas dan Antioksidan Terhadap Penyakit Periodontal. Skripsi, Fakultas Kedokteran Gigi Universitas Sumatra Utara, Medan. diakses tanggal 27 October 2012.

Tarigan, (2004). Profil Budidaya Gaharu. Departemen Kehutanan, Pusat Bina Penyuluhan Kehutanan Jakarta.

Wolfe, R.H. and K.L. Liu (2007). Cellular Aantioxidant Activity (CAA) Assay for Aassessing Aantioxidants, foods, and dietary supplements. *J. Agric. Food Chem*. 55 (22): 8896-8907.

Zheng, W. and Wang, S.Y. (2009). Antioxidant Activity and Phenolic Compounds in Selected *Herbs. J.Agric.Food Chem.,* 49 (11): 5165-70, ACS Publications, Washington D.C.

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