Antioxidant potential and determination of total phenolic and flavonoid content of Bugis ginseng (*Talinum paniculatum* Gaertn) leaf water extract

Emelda Andi* **and Trihadi Kusuma Andi**
Faculty of Pharmacy, Universitas Muslim Indonesia, Jl. Urip Sumiharjo KM.05, Makassar, INDONESIA
*andi.emelda@umi.ac.id

Abstract
Bugis ginseng (Talinum paniculatum Gaertn.) leaves function as a traditional medicine to increase breast milk production, to increase appetite, to improve the immune system and as an antibacterial as we as medicine for ulcers. Flavonoids are phenolic compounds, one of the secondary metabolites in plants that function as antioxidants. This study aims to determine the total phenol content, total flavonoids and antioxidant activity of water extract of Bugis ginseng leaves. Quantitative determination of total phenols using the Folin-Ciocalteu method was expressed as gallic acid equivalent (GAE) per gram of extract. Total flavonoid content using the $AlCl_3$ method was expressed as Quercetin equivalent (QE) and in vitro antioxidant activity with DPPH (2, 2-diphenyl-1-picrylhydrazyl) expressed in terms of IC50 (inhibition concentration). The results showed that water extraction of ginseng leaves showed a total phenol content of 8.37 mg GAE/g extract, a total flavonoid content of 2.12 mg QE/g extract and an IC50 value of 60.27 ug/ml.

Keywords: *Talinum paniculatum* Gaertn., antioxidants, phenols, flavonoids, folin-ciocalteu, $AlCl_3$, DPPH method

Introduction

Changes in human lifestyle can cause degenerative diseases. The human body does not have excess antioxidant reserves. Therefore, if excessive radical exposure occurs, it can cause structural damage at the mitochondrial DNA level as well as functional changes in several enzymes and cellular structures that cause deviations in gene expression. Modern lifestyles associated with processed foods, exposure to various chemicals and lack of exercise play an important role in the induction of oxidative stress[18]. Concerns about the possibility of unknown side effects of synthetic antioxidants leading to natural anti-oxidants are potential alternatives to develop.

Natural antioxidants can protect the body from damage caused by reactive oxygen compounds, can inhibit the occurrence of degenerative diseases and can block lipid peroxide in food. The body actually produces endogenous antioxidant compounds such as SOD (Superoxide Dismutase), Gpx (Glutathione peroxidase) and catalase which play a role in maintaining the function of the blood vessel endothelium from free radical attacks. However, the human body has limited antioxidant reserves. In conditions of oxidative stress where the body's ability to ward off free radicals is less than the number of free radicals present, the body will need an external intake of antioxidants (exogenous antioxidants). Exogenous antioxidants can come from natural or synthetic sources[14].

Synthetic antioxidants are reported to have toxic side effects and carcinogenesis[17]. Concerns about the side effects of synthetic antioxidants lead to the use of natural anti-oxidants, becoming one of the most wanted alternatives because they are more effective and less toxic. Medicinal plants are natural sources of exogenous antioxidants. The natural antioxidants contained in plants are polyphenol compounds, carotenoids and vitamins. This antioxidant has a variety of pharmacological effects such as anti-inflammatory, anti-cancer, antibacterial, antiviral and slow aging effects[11,16]. One of the plants in Indonesia, especially in South Sulawesi, which is traditionally used by the community as medicine and food is Ginseng leaf (*Talinum paniculatum* Gaertn). This plant in South Sulawesi is known as Bugis ginseng. The parts of the plant that are commonly used are leaves and roots. Plants have been used to increase milk production, increase appetite, to increase endurance and as antibacterial[6] as well a medicine for ulcers.

Flavonoids are a class of secondary metabolites produced by plants that are included in the large group of polyphenols. These phenolic compounds are found in many plant species[1,2]. These compounds are found in all parts of plants including leaves, roots, wood, bark, pollen, nectar, flowers, fruit and seeds. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation[5,15]. The antioxidant activity of the phenolic and flavonoid components by reducing free radicals depends on the number of hydroxy groups in their molecular structure[10]. This study aims to determine the linear contribution of total phenolic content, total flavonoids and potential antioxidant activity in the aqueous extract of Bugis ginseng (*Talinum paniculatum* Gaernt) leaves.

Material and Methods

Samples and Reagents: The main material used in this study is the leaves of Bugis ginseng obtained in Makassar, South Sulawesi, Indonesia. Other materials are quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), acetic acid, ethanol, 5%, glacial acetate acid, $AlCl_3$ 2%, HCl 25%, hexamethyltetramine (HMT) 0.5%, acetone, ethyl acetate,

**** Author for Correspondence***

Ciocalteu Folin reagent and Na_2CO_3. The instruments used are the UV-Vis Spectrophotometer Orion Aquamatt 8000, the EPOCH Microplate Spectrophotometer and Sonicator Power Soni405.

Sample and extract preparation: Ginseng leaves are dried in an oven at 40°C until they reach a moisture content of <10% and then powdered. The pollinated samples were extracted by decoctal extraction using water as a solvent. The decoct extraction results obtained were then freeze-dried to obtain dry extracts.

Quantitative Assay of Total Phenolic Content: The extracted sample was put into a 0.2 ml cuvette. Then 1 ml of Folin-Ciocalteu diluted 10 times and 0.8 ml of 7.5% sodium carbonate were added. Incubate for 30 minutes. The absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 765 nm[8].

The determination of the level of flavanoid extract from ginseng leaf water was conducted using the method of colorimetric aluminum chloride. The used extract of 0.5 g is dissolved in methanol solvent of 50 ml in a measuring pumpkin. Pick by pipette of 0.5 ml into a 50 mL pumpkin and add 20 mL of purified water. The absorption of the solution is measured at 510 nm. The standard used is quercetin with different concentrations of 2, 4, 6, 8 and 10 µg/ml[3,7,12].

Antioxidant Activity Assay using the DPPH Method: The extracts were produced at concentrations of 16.6 ppm, 33.3 ppm, 50 ppm and 66.6 ppm using methanol. 4 ml is taken from each concentration, then a solution of 1,1-diphenyl- 2-picr (DPPH) is added in 3 ml of 100 ul methanol. Incubate for 30 minutes at 37°C. The absorption is measured at 517 nm wavelengths. The controls used are BHT[4,8].

Results and Discussion

Total Phenolic and Flavanoid Test: The determination of the total phenol content by the Folin-Ciocalteu method is based on the ability of the reagent to oxidize the hydroxyl group (OH-) of phenolic compounds. Table 1 and figure 1 show the results of the determination of the total phenol content with the phenolic acid standard curve equation y = 0.1127x + 0,0005 (R^2 = 0.9988) using a raw solution of gallic acid. Based on the determinations of phenols, ginseng leaf water extract was obtained at 8.37 mgGAE/g (Table 3). The flavonoid content was determined using the colorimetric method of aluminum chloride with the raw solution of quercetin. Table 2 and figure 2 show the standard absorption and curve of the quercetin.

Table 3 shows the results of determining the level of flavonoid of ginseng leaf water extract was obtained at 2.12 mgQE/g. (Table 1). Environmental factors such as soil composition, temperature, rainfall and ultraviolet radiation can affect the concentration of phenol components including flavonoids. Flavonoids are widely distributed in plant tissues in the form of polar glycosides.

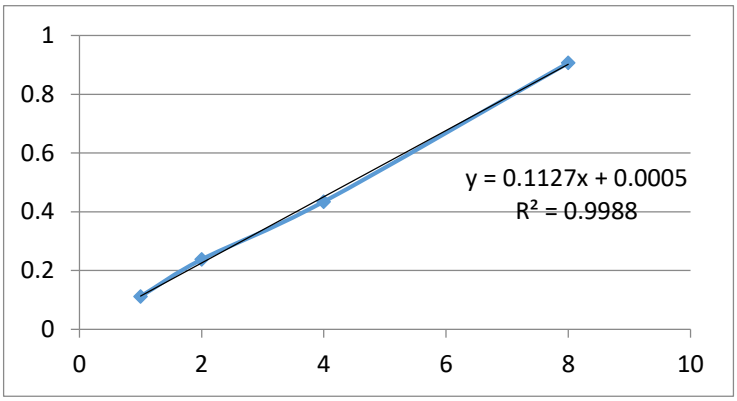

Figure 1: Calibration curve of gallic acid standard solution

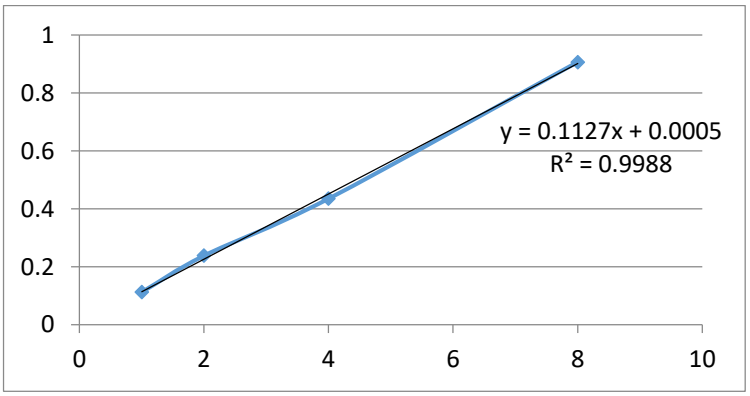

Figure 2: Calibration curve of quercetin standard solution.

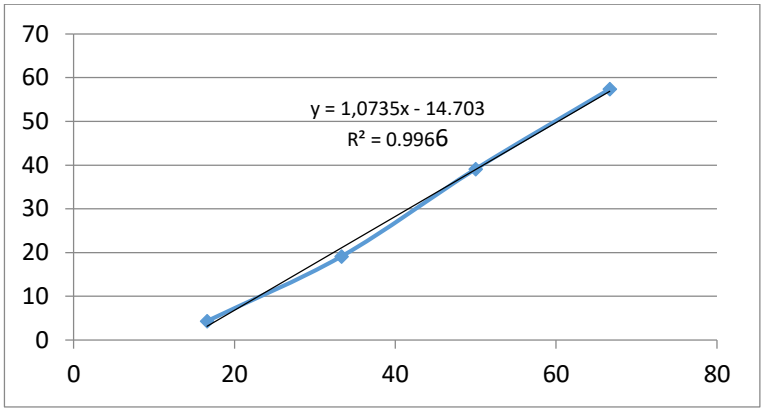

Figure 3: Relation between the extract and % inhibition

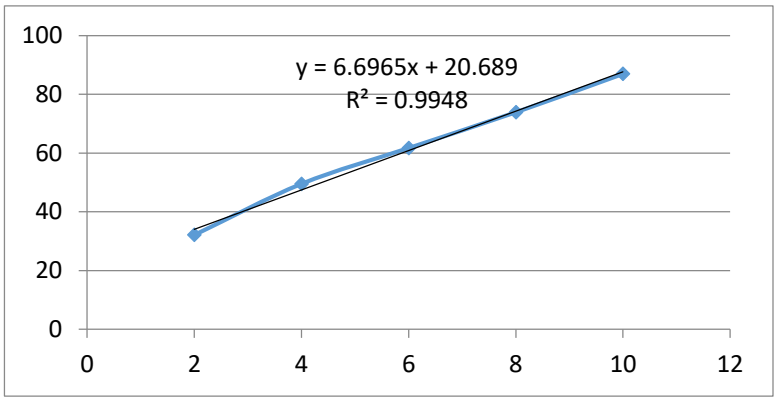

Figure 4: Relation between controlled concentration (BHT) and % inhibition

Determination of antioxidant potential: Antioxidant assay of Bugis ginseng leaf extract was made using the DPPH method**.** The results of the antioxidant potential test of ginseng leaf water extract can be seen in table 4 and figure 3.

Antioxidant testing control (BHT): The results of the BHT antioxidant activity test can be seen in table 5. Antioxidant activity testing with the DPPH method is conducted based on the antioxidant's ability to inhibit free radicals by donating hydrogen atoms to DPPH. The reaction of DPPH with antioxidants will neutralize free radicals from DPPH and form reduced DPPH: DPPH +AH DPPH-H + A. Table 4 and figure 3 show that Bugis ginseng leaf extract has an IC50 of 60.27%. The results of this research are much better than the IC50 of soursop leaf ethanol extract of 1.512 ug/ml[10]. Table 5 and Figure 4 show a comparison of % inhibition for the comparison used (BHT) with IC 50 as 4.38%.

The DPPH method is used in testing antioxidant activity because it has an easy and fast procedure for evaluating the radical scavenging activity of non-enzymatic antioxidants. DPPH radical is a stable radical and has a maximum absorption at a wavelength of 517 nm. The principle is that there is electron transfer and hydrogen atom transfer between the antioxidant and the DPPH radical, so that DPPH (Diphenyl Picryl Hydrazyl) will be reduced to DPPH-H (diphenyl picryl hydrazine) and the color changes from purple to yellow[5,8,10,13].

The phenol and flavonoid compounds have a linear contribution to antioxidant activity, so the higher are the levels, the better are the antioxidants. In addition to flavonoids, other phenolic components such as tannins are known to have antioxidant activity. Phenolic components (flavonoids and tannins), alkaloids, terpenoids and organic sulfur components act as natural antioxidants. Antioxidant activity is not always correlated with phenol or flavonoid levels.

Table 1
Concentrations and absorbances of gallic acid standards

Concentrate (x)	Absorbance (y)
1	0.112
2	0.238
4	0.435
8	0.907

Table 2
Concentration and absorption of quercetin standard

Concentrate (x)	Absorbance (y)
2	0.054
4	0.208
6	0.336
8	0.51
10	0.65

Table 3
Total phenolic and flavonoid of Bugis ginseng *(Talinum paniculatum)* leave water extract

Sample	Measurement	Average absorption	Average rate
Talinum paniculatum leaves water extract	Total phenolic	0.095	8.37 mgGAE/g
	Total Flavonoid	0.066	2.12 mgQE/g

Table 4
Value of % inhibition of ginseng leaves water extract

Extract	Concentration	Absorbance	% inhibition
I	16.6	0.11	4.347826087
II	33.3	0.093	19.13043478
III	50	0.07	39.13043478
IV	66.66	0.049	57.39130435

Table 5
Value of % inhibition Control (BHT)

BHT	Concentration	Absorbance	% inhibition
I	2	0.078	32.17391304
II	4	0.058	49.56521739
III	6	0.044	61.73913043
IV	8	0.03	73.91304348
V	10	0.015	86.95652174

This may be due to several factors such as differences in active components in plants, synergistic effects or antagonistic effects between the active ingredients contained, the conditions of the research and the methods used affecting antioxidant activity on plants[9].

Conclusion

Ginseng leaf water extract has a moderate phenol content of 8.37 mg GAE/g extract while its flavonoid content is 2.12 mgQE/G extract. The IC50 value of the extract by the DPPH method is 60 µg/ml with antioxidant potential with a strong category.

Acknowledgement

We would like to thank the Faculty of Pharmacy, Universitas Muslim Indonesia for funding this research.

References

1. Alara O.R., Abdurahman N.H. and Ukaegbu C.I., Extraction of phenolic compounds: A review, *Current Res. Food Sci*., **4**, 200-214 **(2021)**

2. Aneklaphakij C., Saigo T., Watanabe M., Naake T., Fernie A.R., Bunsupa S., Satiipatipan V. and Tohge T., Diversity of Chemical Structures and Biosynthesis of Polyphenols in Nut-Bearing Species, *Front. Plant Sci.*, **12**, 1-15 **(2021)**

3. Azizah R.N., Emelda A., Asmaliani I., Ahmad I. and Fawwaz M., Total Phenolic, Flavonoids and Carotenoids Content and Anti-Obesity Activity of Purslane Herb (*Portulaca oleracea* L.) Ethanol Extract, *Pharm. J.*, **14(1)**, 8-13 **(2022)**

4. Baliyan S., Mukherjee R., Priyadarshini A., Vibhuti A., Gupta A., Pandey R.P. and Chung C.M., Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa, *Molecules*, **27(1326)**, 1-19 **(2022)**

5. Banjarnahor S. and Artanti N., Antioxidant properties of flavonoids, *Med. J. Ind.*, **23(4)**, 239-244 **(2014)**

6. Emelda A., Auliawati Arman, Taufik M. and Kurniawaty R., Immunomodulator Activity and Antirheumatoid Arthritis Extract of Ethyl Acetate Ginseng Bugis (*Talinum Paniculatum* (jacq.) Gaertn), *J. Glob. Phar. Tech.*, **12(02)**, 246-251 **(2020)**

7. Emelda A., High Levels of Flavonoids and HPLC Profile from Purified Extract of Cocoa Bean from West Sulawesi Indonesia, *Int. J. Chem Tech Res.*, **6(4)**, 2363-2367 **(2014)**

8. Emelda A., Polyphenol total content, IC50 and antioxidant activities of ethanol extract from some cocoa (Theobroma cacao) beans in South Sulawesi Indonesia, *J. Chem. and Pharm. Res.*, **7(4)**, 1211-1214 **(2015)**

9. Gengaihi S., Ella F., Emad M., Shalaby E. and Doha H., Food processing & technology antioxidant activity of phenolic compounds from different grape wastes, *J. Food Proc & Tech.*, **5(2)**, 1-5 **(2014)**

10. Gonzales M., Villena G.K. and Kitazono A.A., Evaluation of the antioxidant activities of aqueous extracts from seven wild plants from the Andes using an in vivo yeast assay, *Results in Chem.*, **3**, 1-7 **(2021)**

11. Hoang H.T., Moon J.Y. and Lee Y.C., Natural Antioxidants from Plant Extracts in Skincare Cosmetics, *Cosmetics*, **8(106)**, 1-24 **(2021)**

12. Kapil S., Sharma V. and Kumar T., Characterization of Bacillus tequilensis A1C1: A Novel Bio-reservoir of D-serine, *Res. J. Biotech*., **18(2)**, 8-14 **(2023)**

13. Liang N. and Kitts D.D., Antioxidant property of coffee component: assessment of methods that define mechanisms of action, *Molecules*, **19**, 19180-19208 **(2014)**

14. Lourenco S.C., Martin M.M. and Alves V.D., Antioxidants of Natural Plants Origins: From Sources to Food Industry Applications, *Molecules*, **24(22)**, 4132 **(2019)**

15. Treml J. and Smejkal K., Flavonoids as potent scavengers of hydroxyl radicals, *Comp. Rev. Food Sci. Food Saf.*, **15**, 720-738 **(2016)**

16. Xu D., Li Y., Meng X., Zhou T., Zhou Y., Zheng J. and Li H., Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources, *Int. J. Mol. Sci.*, **18(96)**, 1-32 **(2017)**

17. Xu X., Liu A., Ares I., Larranaga M.R.M., Martinez M., Anadon A. and Martinex M.A., Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action, *Food Chem.*, **353(1)**, 129488**(2021)**

18. Zucca P., Varoni E.M., Dini L., Panzarini E., Rajkovic J., Fokou P.V.T., Azzini E., Peluso I., Mishra A.P., Nigam M., Rayess Y.E., Beyrouthy M.E., Polito L., Iriti M., Martins N., Martorell M., Docea A.O., Setzer W.N., Calina D., Cho W.C. and Rad J.S., Lifestyle, Oxidative Stress and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases, *Front. Physiol.*, **11**, 1-21 **(2020)**.

(Received 25th October 2023, accepted 04th January 2024)