

Antioxidant Properties of Beringin (*Ficus Benjamina* Linn.) Fruit

Anderson Arnold Aloanis*, Marlina Karundeng

Chemistry Department, Universitas Negeri Manado, Tondano, 95619, Indonesia

INFO ARTIKEL

Diterima 23 Desember 2021

Disetujui 28 Desember 2021

Key word:

Ficus benjamina Linn

Fosfomolybdenum

DPPH

* email:

andersonaloanis@unima.ac.id

*Telp.: +62813-4000-0093

ABSTRACT

Ficus Benjamina Linn. is a plant that can be used as a source of antioxidants. This study aims to determine antiradical activity and total antioxidant capacity of extracts and fractions of *Ficus Benjamina* Linn. fruit. Fruit of *Ficus benjamina* Linn. which has been dried is macerated with methanol. The maceration results were then evaporated to obtain a thick extract of methanol. The extract was then partitioned to obtain the n-hexane fraction, ethyl acetate fraction, and water fraction. Antiradical testing of extracts and fractions of *Ficus benjamina* Linn. fruit using 2,2-diphenyl-1-picrylhydrazyl and testing total antioxidant capacity using the phosphomolybdenum method. The results of the anti-radical and antioxidant analysis showed that the ethyl acetate fraction was the most active fraction. Ethyl acetate fraction showed IC₅₀ value of 63.55 µg / mL and the total value of antioxidant capacity was 127.05 ± 1.62 mg / gram AAE.

Introduction

Free radicals are chemical compounds that produce from homolytic disconnection of a chemical reaction [1]. Free radicals have one unpaired electron in the outer shell. These electrons make free radical molecules unstable and very reactive. Even though it is very reactive, there are several types of free radicals that last long and do not react quickly. These free radicals are grouped into three namely: Stable free radicals, permanent free radicals, and radicals [2].

Reactive oxygen species and reactive nitrogen species are the most common free radicals. The addition of electrons to oxygen and nitrogen is the initial stage in the process of forming free radicals. These electrons can come from the environment and result from chemical reactions in the human body. This means that free radicals will continue to exist in the human body. This reactive species attacks DNA to cause DNA mutations, attacks proteins causing protein modifications, and attacks lipids that cause oxidized lipids [3]. The important thing is

that our body should not achieve a condition called oxidative stress. Oxidative stress is a condition where the number of reactive species and antioxidant compounds experience imbalances. This condition can cause tissue damage even to cell death. To prevent oxidative stress from happening, we need a source of antioxidants from outside the body.

Antioxidants are compounds that have the ability to fight and inhibit the reactivity of free radicals. Antioxidants are generally divided into two, namely enzymatic antioxidants and non-enzymatic antioxidants [4]. There are enzymatic antioxidants that function as neutralizing free radicals and some function as oxidation inhibitors. Non-enzymatic antioxidants in the form of compounds grouped as flavonoids, cofactors, vitamins, phenolic, minerals, organosulfur compounds, carotenoids, and non-protein nitrogen compounds [5]. These compounds can prevent our bodies from diseases caused by free radicals such as cancer, Alzheimer, heart disease, decreased nerve function, rheumatism, and diabetes mellitus [6].

Ficus Benjamina leaves also consider as antioxidant

Methods

Materials

The materials used in this study were methanol pa (Merck), technical methanol, technical n-hexane, technical ethyl acetate, distilled water, 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich), Ammonium molybdate (99.98% trace metal base-Sigma Aldrich), Sodium Phosphate (Merck), Sulfuric Acid (Merck).

Extraction and Fractionation

Beringin (*Ficus benjamina* Linn.) fruit is taken in Minahasa Regency, North Sulawesi. Fruit taken is ripe fruit which is purplish red. The fruit is dried and not given direct contact with sunlight for 1-2 weeks. The dried fruit is smoothed to form powder. Samples weighing 500 grams which have been mashed are extracted by maceration using a technical methanol solvent which has been redistilled. The maceration process is carried out for 24 hours at room temperature. This process is repeated eight times. The filtrate from maceration was then evaporated at 40 ° C to obtain a thick methanol extract.

The thick methanol extract is then fractionated by liquid-liquid extraction using n-hexane, and ethyl acetate. The filtrate and residue from the liquid-liquid extraction were evaporated to obtain the n-hexane, ethanol, and water fractions.

Determination of antiradicals

The free radicals scavenging activity of *Ficus benjamina* Linn. fruit extract and fraction was carried out according to the method by Aliyu with a slight modification [7]. The samples solutions were prepared and diluted to 10-400 µg/mL and 1.5 µg/mL, 10 µg/mL, 15 µg/mL and 20 µg/mL for the standard. Each concentration of sample and standard is made three times repetition. A solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was weighed as much as 8 mg, dissolved in 50 ml of methanol p.a to make a DPPH solution of 0.4 mM. Pipette a 500 µL DPPH solution into a test tube. All samples were incubated at 37°C for 30 minutes. Measured using a spectrophotometer

at a wavelength of 517 nm. The IC₅₀ value (extract concentration needed to reduce 50% of the initial DPPH amount) was calculated using regression analysis.

Determination of total antioxidant capacity

The total antioxidant capacity was measured by the phosphomolybdenum test using the Kasangana method with little modification [8]. Extract/fraction as much as 0.3 mL (1000 µg/mL) was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) into a test tube. The tube containing the reaction solution and the extract were incubated at 95°C for 90 minutes. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against the blank after cooling to room temperature. Distilled water (0.3 mL) at the extract site are used as blanks. The total antioxidant capacity is expressed as the number of grams equivalent to Ascorbic Acid. The calibration curve is made by Ascorbic Acid (250, 125, 62.5, 31.25, and 15,625 µg/mL).

Results and Discussion

Determination of antiradical

DPPH or 2,2-diphenyl-1-picrylhydrazyl is a free radical that is often used to test the activity of sample against free radicals. The DPPH test is considered a valid, easy, and economical method that is valid for evaluating antioxidant radical activity [9]. The DPPH method is a method of trapping purple DPPH free radicals in solution by donating electrons [10]. Absorbance value from extract and fraction of *Ficus benjamina* Linn. fruit were converted to percent inhibition through the formula:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \quad (1)$$

Where A₀ is the absorbance of 0 or absorbance DPPH and A₁ is the absorbance of *Ficus benjamina* Linn. fruit samples.

The activity of free radical scavenging can be seen from table 1. Ethyl acetate fraction has the best activity against DPPH with IC₅₀ of 63.55 µg/mL. This value indicates that ethyl acetate fraction has strong activity against free radicals. The second strongest fraction after ethyl acetate was a water fraction with an IC₅₀ value of 105.89

µg/mL. After the water fraction, the methanol extract shows a figure of 141.70 µg/mL. The water fraction and methanol extract showed moderate activity against DPPH free radicals. The n-hexane fraction is the fraction with the lowest activity with an IC₅₀ value of 293.02 µg/mL. As a standard, ascorbic acid shows IC₅₀

of 7.63 µg/mL. In previous studies, *Ficus benjamina* Linn. fruit extracted with ethanol showed greater activity compared with extracted with ethanol [11]. Differences in solvents and sample treatment can be a contributing factor.

Table 1. Antiradical activity of *Ficus benjamina* Linn. fruit

No	Sample	Linear regression equation	R ²	IC ₅₀ (µg/mL)
1	Methanol	y = 0.4318x - 11.187	0.9883	141.70
2	n-hexane	y = 0.1181x + 15.394	0.9628	293.02
3	Ethyl Acetate	y=0.6934x + 5.9314	0.9574	63.55
4	Water	y = 0.3451x + 13.457	0.9784	105.89
5	Ascorbic acid (standard)	y = 6.5102x + 0.3011	0.9939	7.63

Determination of total antioxidant capacity

Determination of total antioxidant capacity were using the phosphomolibdenum method. This method is a quantitative method for investigating the rate of reduction reactions between antioxidants, oxidants and molybdenum ligands [12]. This method measures the reduction from Mo (VI) to Mo (V). The blue colored complex of phosphate and Mo (V) shows antioxidant activity from the sample. The measurement of the total antioxidant capacity with the standard standard ascorbic acid means the value of the ability to reduce Mo (VI) from extracts / fractions of *Ficus benjamina* Linn. fruit will be equivalent to the ability to reduce ascorbic acid.

In figure 1, the total antioxidant capacity of the extract and fraction of *Ficus benjamina* Linn. fruit is shown. Ethyl acetate fraction showed a value of 127.05 ± 1.62 mg/gram of Ascorbic Acid Equivalent. This means that in the antioxidant ability of each gram of ethyl acetate fraction is equivalent to 127 mg of ascorbic acid. This value is quite high compared to the methanol extract fraction which only shows the number 30.43 ± 1.16 mg / gram AAE, the n-hexane fraction 24.06 ± 7.05 mg / gram AAE and the water fraction 60.27 ± 2.41mg / gram AAE.

Ethyl acetate fraction shows the best antiradical and antioxidant activity. This is because many phenolic compounds and flavonoids are semipolar and soluble in ethyl acetate.

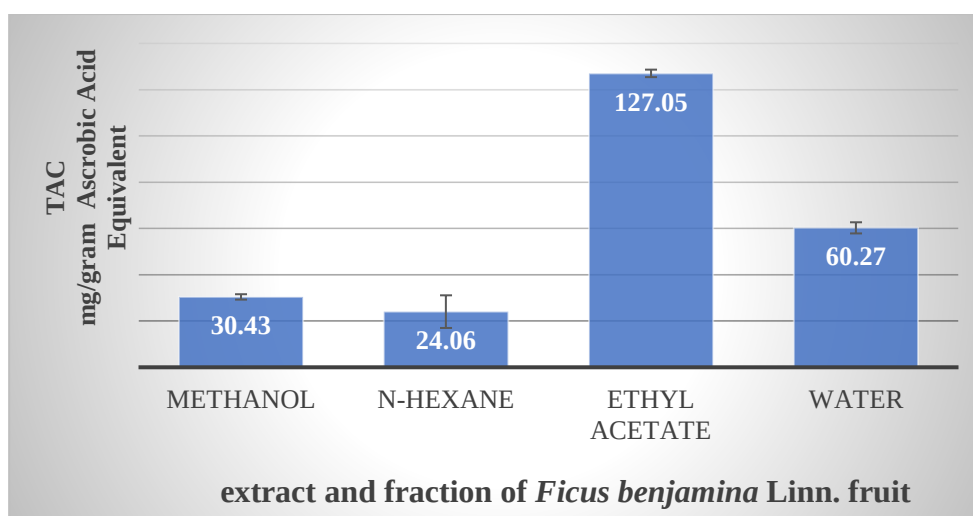


Figure 1. Total antioxidant capacity of *Ficus benjamina* Linn. Fruit

Other ficus genus have been studied for their antiradical and antioxidant abilities[13–23]. In fact, from several fruits, antioxidant activities such as flavonoids, phenolics, and alkaloids were isolated. Based on chemotaxonomy, this supports data on the

ability to reduce phosphate-(MoVI) complexes and the ability to scavenge DPPH free radicals from *Ficus benjamina* Linn. fruit. This ability is also supported by leaves, stem roots, and hanging roots of *Ficus benjamina* Linn. which shows antioxidant activity [24–28].

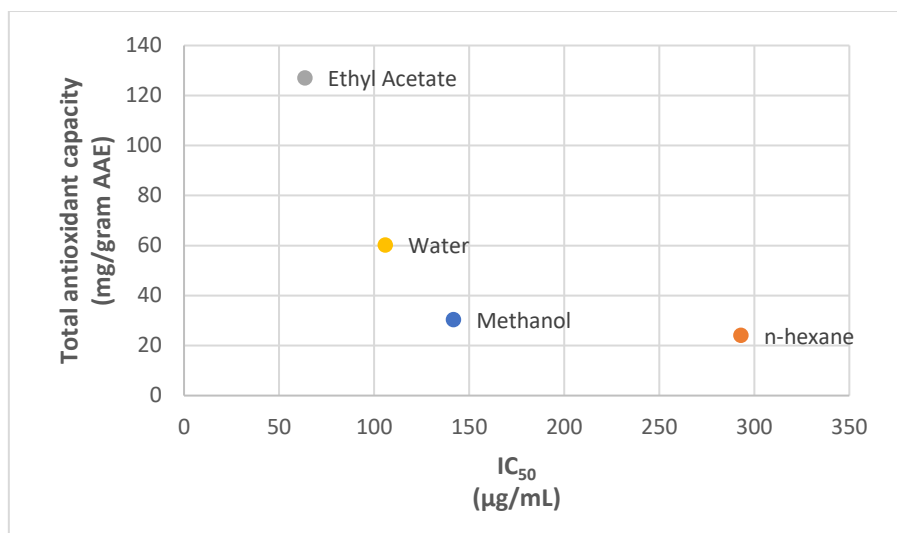


Figure 2. Relationship between antiradical activity and antioxidant of *Ficus benjamina* Linn. fruit

The total antioxidant capacity of the extract and the fraction of the *Ficus benjamina* Linn. has a relationship (figure 2). Ethyl acetate fraction which has a large total antioxidant capacity also has strong antioxidant abilities. The ability of the fraction of water in trapping antioxidants that are classified as moderate also has a total antioxidant capacity that is not too large as well. The n-hexane fraction with an IC₅₀ value that is in the weak range is also indicated by the moderate amount of antioxidant capacity. The total antioxidant capacity of the n-hexane fraction is close to the total value of the antioxidant capacity of water fraction, and methanol extract. This is possible because total antioxidant capacity analyzes antioxidants that are fat-soluble and water-soluble [29]. When compared with ethyl acetate fraction, the ability of methanol extract as antiradical and antioxidants is very low. This happens because the amount of n-hexane fraction yields is greater than the yield of ethyl acetate fraction partitioned from thick ethanol extract. The n-hexane fraction covered the antiradical and antioxidant abilities of ethyl acetate fractions in methanol extract.

Acknowledgement

The authors acknowledge the contribution of Direktorat Jenderal Penguatan Riset dan Pengembangan (Ditjen Risbang) Kementerian Riset Teknologi dan Pendidikan Tinggi for financing this research.

Conclusion

Ficus benjamina Linn. fruit has antiradical and antioxidant activity. Ethyl acetate fraction is the highest ability fraction with values of 63.55 µg / mL and 127.05 ± 1.62 mg / gram AAE while the n-hexane fraction is the smallest fraction of activity with a value of 293.02 µg / mL and 24.06 ± 7.05 mg / gram AAE.

References

1. Bhattacharya, M.; Chakraborty, S. Free Radicals and Naturally Occuring Antioxidants. **2015**, 3, 1–7.
2. Sarma, A.D.; Mallick, A.R.; Ghosh, A.K. Free Radicals and Their Role in Different Clinical Conditions: An Overview. *International Journal of Pharma Sciences and Research (JPSR)* **2010**, 1, 8.
3. Lone, A.A.; Ganai, S.A.; Ahanger, R.A.; Bhat, H.A.; Bhat, T.A.; Wani, I.A. Free

- Radicals and Antioxidants: Myths, Facts and Mysteries. *AJPAC* **2013**, *7*, 91–113, doi:10.5897/AJPAC12.074.
4. Shebis, Y.; Iluz, D.; Kinel-Tahan, Y.; Dubinsky, Z.; Yehoshua, Y. Natural Antioxidants: Function and Sources. **2013**, *2013*, doi:10.4236/fns.2013.46083.
 5. Caroch, M.; Ferreira, I.C.F.R. A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Screening and Analysis Methodologies and Future Perspectives. *Food and Chemical Toxicology* **2013**, *51*, 15–25, doi:10.1016/j.fct.2012.09.021.
 6. Zhu, L.; Chen, J.; Tan, J.; Liu, X.; Wang, B. Flavonoids from Agrimonia Pilosa Ledeb: Free Radical Scavenging and DNA Oxidative Damage Protection Activities and Analysis of Bioactivity-Structure Relationship Based on Molecular and Electronic Structures. *Molecules* **2017**, *22*, 195, doi:10.3390/molecules22030195.
 7. Aliyu, A.; Ibrahim, M.A.; Musa, A.; Musa, A.; Kiplimo, J.; Oyewale, A.O. Free Radical Scavenging and Total Antioxidant Capacity of Root Extracts of Anchomanes Difformis ENGL. (ARACEAE). *Acta poloniae pharmaceutica* **2013**, *70*, 115–121.
 8. Kasangana, P.B.; Haddad, P.S.; Stevanovic, T. Study of Polyphenol Content and Antioxidant Capacity of Myrianthus Arboreus (Cecropiaceae) Root Bark Extracts. *Antioxidants* **2015**, *4*, 410–426, doi:10.3390/antiox4020410.
 9. Kedare, S.B.; Singh, R.P. Genesis and Development of DPPH Method of Antioxidant Assay. *J Food Sci Technol* **2011**, *48*, 412–422, doi:10.1007/s13197-011-0251-1.
 10. Garcia, E.J.; Oldoni, T.L.C.; Alencar, S.M. de; Reis, A.; Loguercio, A.D.; Grande, R.H.M. Antioxidant Activity by DPPH Assay of Potential Solutions to Be Applied on Bleached Teeth. *Braz. Dent. J.* **2012**, *23*, 22–27, doi:10.1590/S0103-64402012000100004.
 11. Karundeng, M.; Aloanis, A.A. Analisis Pemerangkapan radikal bebas ekstrak etanol buah beringin (Ficus benjamina Linn.). *Fullerene Journal of Chemistry* **2018**, *3*, 37–39, doi:10.37033/fjc.v3i2.36.
 12. Phatak, R.; Hendre, A. Total Antioxidant Capacity (TAC) of Fresh Leaves of Kalanchoe Pinnata. *Journal of Pharmacognosy and Phytochemistry* **2013**, *2*, 32–35.
 13. Arimado, J.; Santiago, L. The Antioxidant and Hepatoprotective Activities of the Ethyl Acetate Leaf Extract of Ficus Pseudopalma Blanco. **2015**, *7*, 1179–1189.
 14. Çalışkan, O.; Aytekin Polat, A. Phytochemical and Antioxidant Properties of Selected Fig (Ficus Carica L.) Accessions from the Eastern Mediterranean Region of Turkey. *Scientia Horticulturae* **2011**, *128*, 473–478, doi:10.1016/j.scienta.2011.02.023.
 15. Misbah, H.; Aziz, A.A.; Aminudin, N. Antidiabetic and Antioxidant Properties of Ficus Deltoidea Fruit Extracts and Fractions. *BMC Complement Altern Med* **2013**, *13*, 118, doi:10.1186/1472-6882-13-118.
 16. Tawfik, M.S.; Alhejy, M. Antioxidants in Fig (Ficus Carica L.) and Their Effects in the Prevention of Atherosclerosis in Hamsters. *Journal of Food and Nutrition Sciences* **2014**, *2*, 138, doi:10.11648/j.jfns.20140204.17.
 17. Solomon, A.; Golubowicz, S.; Yablowicz, Z.; Grossman, S.; Bergman, M.; Gottlieb, H.E.; Altman, A.; Kerem, Z.; Flaishman, M.A. Antioxidant Activities and Anthocyanin Content of Fresh Fruits of Common Fig (Ficus Carica L.). *J. Agric. Food Chem.* **2006**, *54*, 7717–7723, doi:10.1021/jf060497h.
 18. Tanwar, B.; Andallu, B.; Modgil, R. Influence of Processing on Phytochemical Characteristics and in Vitro Antioxidant Activity of Ficus Carica L. (Fig) Products. *International Journal of food fermentation Technology* **2015**, *5*, 223–230, doi:10.5958/2277-9396.2016.00020.9.
 19. Joseph, B.; Raj, S.J. Pharmacognostic and Phytochemical Properties of Ficus Carica Linn –An Overview. **2011**, *3*, 8–12.
 20. Harzallah, A.; Bhouri, A.M.; Amri, Z.; Soltana, H.; Hammami, M. Phytochemical Content and Antioxidant Activity of Different Fruit Parts Juices of Three Figs (Ficus Carica L.) Varieties Grown in Tunisia. *Industrial Crops and Products* **2016**, *83*, 255–267, doi:10.1016/j.indcrop.2015.12.043.
 21. Viuda-Martos, M.; Barber, X.; Pérez-Álvarez, J.A.; Fernández-López, J. Assessment of Chemical, Physico-Chemical, Techno-Functional and Antioxidant Properties of Fig (Ficus Carica L.) Powder Co-Products. *Industrial Crops and Products* **2015**, *69*, 472–479, doi:10.1016/j.indcrop.2015.03.005.
 22. Vallejo, F.; Marín, J.G.; Tomás-Barberán, F.A. Phenolic Compound Content of Fresh and Dried Figs (Ficus Carica L.). *Food*

- Chemistry* **2012**, *130*, 485–492, doi:10.1016/j.foodchem.2011.07.032.
23. Wojdyło, A.; Nowicka, P.; Carbonell-Barrachina, Á.A.; Hernández, F. Phenolic Compounds, Antioxidant and Antidiabetic Activity of Different Cultivars of *Ficus Carica* L. Fruits. *Journal of Functional Foods* **2016**, *25*, 421–432, doi:10.1016/j.jff.2016.06.015.
 24. Almahy, H.; Rahmani, M.; Sukari, M.; Ali, A. The Chemical Constituents of *Ficus Benjamina* Linn. and Their Biological Activities. *Pertanika J. Sci. Technol.* **2003**, *11*, 73–81.
 25. Novelli, S.; Lorena, C.; Antonella, C. Identification of Alkaloid's Profile in *Ficus Benjamina* L. Extracts with Higher Antioxidant Power. *American Journal of Plant Sciences* **2014**, *05*, 4029, doi:10.4236/ajps.2014.526421.
 26. Sirisha, N.; Sreenivasulu, M.; Sangeeta, K.; Chetty, C.M. Antioxidant Properties of *Ficus* Species - a Review. *International Journal of PharmTech Research* **2010**, *2*, 2174–2182.
 27. Jain, A.; Ojha, V.; Kumar, G.; Karthik, L.; Rao, K.V.B. Phytochemical Composition and Antioxidant Activity of Methanolic Extract of *Ficus Benjamina* (Moraceae) Leaves. *Research Journal of Pharmacy and Technology* **2013**, *6*, 1184–1189.
 28. Imran, M.; Rasool, N.; Rizwan, K.; Zubair, M.; Riaz, M.; Zia-Ul-Haq, M.; Rana, U.A.; Nafady, A.; Jaafar, H.Z. Chemical Composition and Biological Studies of *Ficus Benjamina*. *Chemistry Central Journal* **2014**, *8*, 12, doi:10.1186/1752-153X-8-12.
 29. Aliyu, A.; Ibrahim, M.A.; Hassan, I.; Musa, A.; AY, L.; JA, O.; M, U.; IE, A.; Oyewale, A.O.; JO, A. Free Radical Scavenging and Total Antioxidant Capacity of Methanol Extract of *Ethulia Conyzoides* Growing in Nigeria. *Roumanian biotechnological letters* **2012**, *17*, 7458–7465.