Food Research 7 (3) : 148 - 152 (June 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antibacterial and antioxidant activities of ethanol extract of *Artocarpus altilis* leaves

[1]Effendy, D.L., [2,*]Mahatir, M., [2]Denny, S. and [2]Nasri.

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara. Jl. Tri Dharma No.5, Medan, North Sumatera, Indonesia*
[2]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara. Jl. Tri Dharma No.5, Medan, North Sumatera, Indonesia*

Article history:
Received: 1 November 2021
Received in revised form: 5 December 2021
Accepted: 16 March 2022
Available Online: 10 June 2023

Keywords:
Antibacterial,
Antioxidant,
Artocarpus altilis,
Total phenol,
Total flavonoid

DOI:
https://doi.org/10.26656/fr.2017.7(3).834

Abstract

Nowadays, people are very concerned about the dangers of synthetic antibacterial agents and antioxidants. Their preference for using natural products of good quality with environmental friendliness continues to grow and can improve body health. In recent years, the antimicrobial and antioxidant actions of medicinal plants have received much attention. *Artocarpus altilis* or breadfruit is a versatile plant that can be used by humans. Starting from the fruit as food, the leaves are used to treat various diseases, the flowers can be used as mosquito repellent, and the stems are used as building materials. Breadfruit is one of the native plants from North Sumatra, disseminating information about the efficacy and nutritional content that is still lacking, with the diversification of technology in research on breadfruit leaves is expected to provide benefits for the community. This study aimed to determine the antibacterial and antioxidant activities, total flavonoid, and total phenolic content of *A. altilis* leaves. The extract was prepared using ethanol with the maceration method. The antibacterial activity was determined with the minimum inhibitory concentration method. The concentration of 300 mg/mL up to a concentration of 25 mg/mL can inhibit the growth of *Staphylococcus aureus*, *S. epidermidis*, *Propionibacterium acnes* and *Pseudomonas aeruginosa*. The antioxidant activity was determined with the 1,1- diphenyl-2-picrylhydrazyl (DPPH) method. Total flavonoid and total phenolic content were determined with colourimetric methods. Antioxidant activity from the DPPH assay measured as IC_{50} was 29.60±0.14 µg/mL. The extract was found to contain high levels of total phenolic (295.22±3.20 mg GAE/g) and total flavonoid (28.55±0.54 mg QE/g). The results revealed that the ethanol extract of *A. altilis* leaves has antibacterial and antioxidant potential.

1. Introduction

Natural products from medicinal plants play a considerable role in the discovery and development of new drugs (Mercy *et al*., 2013). About 25% of drugs contain compounds obtained from higher plants (Ebong, 2015). According to the World Health Organization, about 65–80% of the world's population which lives in developing countries depends essentially on plants for primary health care (Johnson and Ayoola, 2015). In recent years, the antimicrobial and antioxidant actions of medicinal plants have received much attention (Al-Rifai *et al*., 2017).

Antioxidants are defences to neutralize free radicals. An imbalance will occur if the formation of free radicals exceeds the defence system so that free radicals are unable to be detoxified. The occurrence of changes in the balance due to excess ROS or reduced antioxidants that function to neutralize ROS is a positive status of oxidative stress. Body conditions associated with oxidative stress are chronic disease, ageing, exposure to infectious toxins, inflammation, infertility, and degenerative diseases (Agarwal *et al*., 2003).

Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant (Medina *et al*., 2005; Romero *et al*., 2005). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Several phytotherapy manuals have

*Corresponding author.
Email: *mahatir.muhammad@usu.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

mentioned various medicinal plants for treating infectious diseases such as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infection (Duraipandiyan *et al*., 2006; Djeussi *et al*., 2013).

Artocarpus altilis is a plant that can be utilized by humans for a variety of purposes. Beginning with the fruit as a source of nourishment, the leaves are used to treat a range of diseases, the flowers as a mosquito repellent, and the stems as building materials. Breadfruit is a native plant of North Sumatra, and sharing knowledge on its efficacy and nutritional content, which is currently lacking, is expected to help the community with technology diversification in the breadfruit leaf study (Pradhan *et al*., 2012)

This study aimed to determine antioxidant activity using the DPPH method, total phenols, total flavonoids and antibacterial activity against *Staphylococcus aureus, S. epidermidis, Propionibacterium acnes* and *Pseudomonas aeruginosa*.

2. Materials and methods

2.1 Materials

Artocarpus altilis leaves were collected from the Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. *Artocarpus altilis* leaves were harvested in the morning.

2.2 Preparation of extraction

Artocarpus altilis leaves were dried at 45°C temperature in the drying cabinet and powdered. *Artocarpus altilis* leaves simplicia powder (500 g) was extracted using absolute ethanol by maceration method until it was submerged in a tightly closed container for three days protected from sunlight while stirring several times, then filtered to obtain the macerate. The obtained macerate was transferred to another container that was tightly closed and protected from sunlight. At the same time, the pulp was added to the filtered liquid and then macerated until a clear final macerate was obtained. The obtained macerate was collected, and the solvent was evaporated using a rotary evaporator at a temperature of not more than 40°C until a thick extract was obtained (Dalimunthe *et al*., 2018; Harahap *et al*., 2018; Hasibuan *et al*., 2020).

2.3 Free radical scavenging activity test

The free radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) method. 0.2 mM solution of DPPH• in methanol was prepared and 100µl of this solution was added to various concentrations. After 60 mins, absorbance was measured at 516 nm. Quercetin was used as the reference material. All the tests were performed in triplicate and the percentage of inhibition was calculated by comparing the absorbance values of the control and test samples (Nazliniwaty *et al*., 2021; Urip *et al*., 2021).

2.4 Determination of total phenol concentration

The total phenol concentration (TPC) of the sample was determined using a folin reagent. Briefly, 100 µL of EAF (500 µg/mL) was mixed with 7.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent (1:10 v/v) and mixed with vortex for 1 minute. After mixing, 1.5 mL of 20% aqueous sodium bicarbonate was added, and the mixture was allowed to stand for 90 mins with intermittent shaking. The absorbance was measured at 775 nm using a spectrophotometer. Total phenolic concentration is expressed as gallic acid equivalent in mg per gram of extract. The methanol solution was used as a blank. All assays were carried out in triplicate (Rosidah *et al*., 2008).

2.5 Determination of total flavonoid concentration

The amounts of total flavonoids in the extracts were measured spectrophotometrically as previously reported. Briefly, 2 mL of extract in methanol was mixed with 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M), and 2.80 mL of distilled water. After incubation for 40 min, absorbance was measured at 432 nm using a spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentration is expressed as quercetin equivalents in mg per gram of extract. All assays were carried out in triplicate (Jamuna *et al*., 2012).

2.6 Determination of minimum inhibitory concentration

Antibacterial activities of extracts were examined by agar well plate diffusion assay method, minimum inhibitory concentration (MIC). The examination was carried out based on the previous procedure that used Mueller Hinton Agar (MHA) as test media and pure bacteria culture (*S. aureus, S. epidermidis, P. acne, P. aeruginosa*) with several treatment modifications. Negative blanks used a mixture of dimethylsulfoxide (DMSO, Merck®) (Boateng and Diunase, 2015).

3. Results and discussion

3.1 Antioxidant activity of ethanol extract Artocarpus altilis with DPPH, total phenolic and total flavonoid method

Based on the results obtained, the antioxidant activity test using the DPPH method showed that the

https://doi.org/10.26656/fr.2017.7(3).834

ethanol extract of *A. altilis* leaves had strong and moderate activity in the DPPH method. In determining the antioxidant activity using the DPPH method, the IC_{50} parameter determines the sample concentration required to capture 50% of the DPPH radicals. Table 1 shows the results of antioxidant testing using the DPPH method with an IC_{50} value of ethanol extract of *A. altilis* leaves, which is 29.60±0.14 µg/mL, where the smaller the IC_{50} value, the stronger the antioxidant activity (Molyneux, 2004). The level of antioxidant power is very strong (IC_{50} <50 µg/mL), strong (IC_{50} 50 - 100 µg/mL), moderate (IC_{50} 101 - 150 µg/mL) and weak (IC_{50} > 150 µg/mL) (Blois, 2003).

The total flavonoids and total phenol from the ethanol extract of *A. altilis* leaves were 295.22±3.20 mg GAE/g extract and 28.55±0.54 mg QE/g extract, respectively. The antioxidant activity obtained was influenced by the total phenol and total flavonoid content. Phenol and flavonoid compounds have a linear contribution to antioxidant activity, so the higher the levels, the better the antioxidants (Ghasemzadeh and Ghasemzadeh, 2011). High total phenolic content has an important role as an antioxidant. Besides flavonoids, other phenolic components such as tannins are known to have antioxidant activity (Amarowicz, 2007). In addition, other secondary metabolites such as alkaloids and terpenoids also contribute as antioxidant, phenolic components (flavonoids and tannins), alkaloids, terpenoids, and organic sulfur components act as natural antioxidants (Al-Jaber *et al*., 2011).

The DPPH method was chosen for testing this antioxidant activity because it has an easy and fast procedure to evaluate the radical scavenging activity of non-enzymatic antioxidants. The DPPH radical is a stable radical and has a maximum absorption at a wavelength of 517 nm. The principle of the test is the transfer of electrons and the transfer of hydrogen atoms between antioxidants and a colour change from purple to yellow occurs (Liang and Kitts, 2014).

The measurement of total phenol was used by the Folin-Ciocalteu method which is based on the reducing power of the phenolic hydroxyl group using gallic acid standard. Gallic acid was chosen because it is a pure and stable substance. All phenolic compounds including simple phenols can react with Folin-Ciocalteu reagent although they are not effective radical scavengers. The presence of an aromatic nucleus in phenolic compounds can reduce phosphomolybdate phosphotungstate to molybdenum tungsten. Phenolic compounds only react with the Folin-Ciocalteu reagent under alkaline conditions to cause the dissociation of protons in phenolic compounds into phenolic ions. Measurement of total flavonoids is used with the AlCl3 principle which will form a complex because it has a C-4 keto group and then a C-3 or C-5 hydroxyl group that is next door so that there is a shift in wavelength towards the visible (visible) which is visible from the yellow colour in the solution. In the measurement of total flavonoids, standard quercetin was made as a comparison. Quercetin was chosen because it is one of the flavonoids (flavonols) groups (Ukieyanna, 2012). It can be seen in Table 1.

3.2 Minimum inhibitory concentration

Based on Table 2, bacterial growth inhibition has several levels of inhibition diameter and growth inhibition response rate. A diameter < 6 mm indicates that there is no weak resistance response, a diameter of 11 – 20 mm indicates a moderate resistance response and a diameter of 21–30 mm indicates a strong resistance response (Morales *et al*., 2003).

Based on Table 2, the antibacterial activity was indicated by the formation of an inhibitory zone around the paper disc. The formation of an inhibition zone indicates an indication of antibacterial activity. This can be seen from the results of antibacterial activity tests on extracts with varying concentrations of 300, 200, 100, 50, and 25 mg/mL for the test bacteria *S. aureus*, *S. epidermidis, P. acnes* and *P. aeruginosa*. The content of saponins, tannins, alkaloids, flavonoids, and steroids, has antibacterial potential. The mechanism of action of saponins as an antibacterial is to reduce surface tension, resulting in increased permeability of cell leakage and resulting in the release of intracellular compounds. Tannins have antibacterial activity related to their ability to inactivate microbial cell adhesins as well as inactivate enzymes and interfere with protein transport in the inner layer of cells (Cavalieri *et al*., 2005). The mechanism of action of alkaloids as an antibacterial is predicted through inhibition of cell wall synthesis which will cause cell lysis so that cells will die. Flavonoids are known to have antibacterial properties where the mechanism of action is to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds. The mechanism of action of steroids in inhibiting microbes is by damaging the plasma membrane of microbial cells, causing leakage of the cytoplasm out of the cell which in turn causes cell

Table 1. Result of antioxidant activity (IC_{50}) with DPPH, total phenolic, and total flavonoid method

Extract	IC_{50}	Total phenolic	Total flavonoid
Artocarpus altilis Leaves	29.60±0.14 µg/mL	295.22±3.20 mg GAE/g	28.55±0.54 mg QE/g

https://doi.org/10.26656/fr.2017.7(3).834

Table 2. The minimum inhibitory concentration

Bacteria	Inhibition zone diameter (Concentration mg/mL)				
	300	200	100	50	25
Staphylococcus aureus	12.20±0.10	11.93±0.06	11.37±0.06	11.14±0.15	9.67±0.16
Staphylococcus epidermidis	12.30±0.20	11.73±0.12	11.50±0.10	10.83±0.06	10.43±0.05
Propionibacterium acnes	13.47±0.06	11.87±0.12	11.60±0.10	10.73±0.12	10.07±0.15
Pseudomonas aeruginosa	12.27±0.15	12.07±0.06	11.67±0.12	10.77±0.16	10.47±0.12

death (Madduluri *et al*., 2013).

Based on this research, the leaf extract of *A. altilis* has the potential as an antioxidant and antibacterial. This plant contains many efficacious chemical compounds, one of which is flavonoid compounds that have antioxidant and antibacterial abilities. So that the leaves of *A. altilis* can be used as a traditional medicine to improve body health.

4. Conclusion

Based on the results obtained, it can be concluded that the ethanol extract of *A. altilis* leaves has a strong antioxidant activity of 29.60±0.14 µg/mL and the concentration of 300 mg/mL up to a concentration of 25 mg/mL can inhibit the growth bacterial.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We gratefully thank the Research Center University of Sumatera Utara through Hibah Talenta "Basic Research" Research Grant 2021.

References

Agarwal, A., Saleh, R.A. and Bedaiwy, M.A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*, 79(4), 829–843. https://doi.org/10.1016/s0015-0282(02)04948-8

Al-Jaber, N., Awaad, A. and Moses, J. (2011). Review on some antioxidant plants growing in Arab world. *Journal of Saudi Chemical Society*, 15(15), 293-307. https://doi.org/10.1016/j.jscs.2011.07.004

Al-Rifai, A., Aqel, A. and Al-Warhi, T. (2017). Antibacterial, antioxidant activity of ethanolic plant extracts of some Convolvulus species and their DART-ToF-MS profiling. *Evid Based Complement Alternative Medicine*, 2017, 5694305. https://doi.org/10.1155/2017/5694305

Amarowicz, R. (2007). Tannins: The new natural antioxidants? *European Journal of Lipid Science Technology*, 109(6), 549–551. https://doi.org/10.1002/ejlt.200700145

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature,* 181, 1199–1200. http://doi.org/10.1038/1811199a0

Boateng, J. and Diunase, K.N. (2015). Comparing the Antibacterial and Functional Properties of Cameroonian and Manuka Honeys for Potential Wound Healing-Have We Come Full Cycle in Dealing with Antibiotic Resistance. *Molecules,* 20, 16068. https://doi.org/10.3390/molecules200916068

Cavalieri, S.J., Rankin, I.D., Harbeck, R.J., Sautter, R.S., McCarter, Y.S., Sharp, S.E., Ortez, J.H. and Spiegel, C.H. (2005). Manual of Antimicrobial Susceptibility Testing. USA: American Society for Microbiology.

Dalimunthe, A., Hasibuan, P.A.Z., Silalahi, J., Sinaga, S.F. and Satria, D. (2018). Antioxidant activity of alkaloid compounds from Litsea cubeba Lour. *Oriental Journal of Chemistry*, 34(2), 1149-1152. https://doi.org/10.13005/ojc/340270

Djeussi, D.E., Noumedem, J.A.K., Seukep, J.A., Fankam, A.G., Voukeng, I.K., Taneko, S.B., Nkuete, A.H.L. and Kuete, V. (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complementary and Alternative Medicine*, 13, 164. https://doi.org/10.1186/1472-6882-13-164

Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine*, 6, 35. https://doi.org/10.1186/1472-6882-6-35

Ebong, P.E. (2015). Phytomedicine: panacea for primary health care delivery in Nigeria, p. 63–74. Calabar, Nigeria: University of Calabar Press,

Ghasemzadeh, A. and Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, 5(31), 6697–6703. https://doi.org/10.5897/JMPR11.1404

Harahap, U., Hasibuan, P.A.Z., Sitorus, P., Arfian, N. and Satria, D. (2018). The anti-migration activity of an ethyl acetate fraction of *Zanthoxylum acanthopodium* DC. fruits in 4T1 breast cancer cells.

Asian Pacific Journal of Cancer Prevention, 19(2), 565-569. https://dx.doi.org/10.22034%2FAPJCP.2018.19.2.565

Hasibuan, P.A.Z., Harahap, U., Sitorus, P. and Satria, D. (2020). The anticancer activities of *Vernonia amygdalina* Delile. Leaves on 4T1 breast cancer cells through phosphoinositide-3-kinase (PI3K pathway). *Heliyon*, 6, e04449. https://doi.org/10.1016/j.heliyon.2020.e04449

Jamuna, S., Pulsamy, S. and Karthika, K. (2012). Screening of in vitro antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae). *Journal of Applied Pharmaceutical Science*, 2, 149-154. https://doi.org/10.7324/JAPS.2012.2722.

Johnson, O.O. and Ayoola, G.A. (2015). Antioxidant activity among selected medicinal plants combinations (multi-component herbal preparation). *International Journal of Research in Health Sciences*, 3, 526–532.

Liang, N. and Kitts, D.D. (2014). Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action. *Molecules*, 19, 19180 -19208. https://doi.org/10.3390/molecules191119180

Madduluri, S., Rao, K.B. and Sitaram, B. (2013). In vitro evaluation of antibacterial activity of five indegenous plants extract against five bacterial pathogens of human. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(4), 679-684.

Medina, A.L., Lucero, M.E. and Holguin, F.O. (2005). Composition and Antimicrobial Activity of *Anemopsis californica* Leaf Oil. *Journal of Agricultural and Food Chemistry*, 53(22), 8694–8698. https://doi.org/10.1021/jf0511244

Mercy, N., Jemmy, A. and Vanda, S. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (*Pometia pinnata*) terhadap Bakteri *Staphylococcus aureus* secara *In vitro*. *Jurnal MIPA*, 2(2). https://doi.org/10.35799/jm.2.2.2013.3121 [In Bahasa Indonesia].

Molyneux, P. (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology,* 26(2), 211-219.

Morales, G., Sierra, P., Mancilla, A., Paredes, A., Loyola La Gallardo, O. and Borquez, J. (2003). Secondary metabolites from four medicinal plants from northern Chile: antimicrobial activity and biotoxicity against Artemia salina. *Journal of the Chilean Chemical Society*, 48(2), 13–18. https://doi.org/10.4067/S0717-97072003000200002

Nazliniwaty, Olivia, A.H., Dewi, P., Denny, S. and Mahatir, M. (2021). Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Hydroalcoholic Extract of *Artocarpus lacucha* Buch-Ham. Leaves. *The International Conference on Chemical Science and Technology*, 2342, 080010. https://doi.org/10.1063/5.0045440

Pradhan, C., Mohanty, M. and Rout, A. (2012). Phytochemical screening and comparative bioefficacy assessment of *Artocarpus altilis* leaf extract for antimicrobial activity. *Frontier in Life Science*, 6(3-4), 71-76. https://doi.org/10.1080/21553769.2013.765811

Romero, C.D., Chopin, S.F., Buck, G., Martinez, E., Garcia, M. and Bixby, L. (2005). Antibacterial properties of common herbal remedies of the southwest. *Journal of Ethnopharmacology*, 99(2), 253-257. https://doi.org/10.1016/j.jep.2005.02.028

Rosidah, M., Yam, M.F., Sadikun, A. and Asmawi, M.Z. (2008). Antioxidant potential of *Gyunura procumbens. Pharmaceutical Biology*, 46(9), 616-25. https://doi.org/10.1080/13880200802179642

Ukieyanna, E., Suryani. and Roswiem, A.P. (2012). Aktivitas Antioksidan Kadar Fenolik dan Flavonoid Total Tumbuhan Suruhan. Bogor, Indonesia: Departemen Biokimia Institut Pertanian Bogor, BSc. Thesis. [In Bahasa Indonesia].

Urip, H., Aminah, D., Triana, H., Mahatir, M., Nasri. and Denny, S. (2021). Antioxidant and antibacterial activities of ethanol extract of *Vernonia amygdalina* Delile Leaves. *The International Conference on Chemical Science and Technology,* 2342, 080011. https://doi.org/10.1063/5.0045447

