CHEMICAL ENGINEERING TRANSACTIONS
VOL. 115, 2025
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Carlo Pirola, Antonio Espuña, Sabrina Copelli

ISBN 979-12-81206-16-8; **ISSN** 2283-9216
DOI: 10.3303/CET25115004

Phytochemical Composition and Comparative Antioxidant Activity of Leaf and Stem Bark Extracts of *Glochidion arborecens* Using DPPH and ABTS Assays

Haryoto[a], Edtano Trinanda[a], Broto Santoso[a], Muhammad Dohir Siaga Nagara[a], Novian Liwanda[b], Waras Nurcholis[b,c,*]

[a]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Kartasura, Central Java 57169, Indonesia
[b]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java 16680, Indonesia
[c]Tropical Biopharmaca Research Center, IPB University, Bogor, West Java 16680, Indonesia
wnurcholis@apps.ipb.ac.id

This study explores the phytochemical composition and antioxidant activity of ethanol extracts from the leaves and stem bark of *Glochidion arborescens*. Extraction yielded 2.68% from leaves and 1.72% from stem bark. Phytochemical screening revealed the presence of flavonoids, tannins, and saponins, while steroids and triterpenoids were absent. Antioxidant activity was evaluated using DPPH and ABTS assays, with vitamin C as the positive control. The extracts demonstrated significant antioxidant potential, with IC_{50} values of 75.25 µg/mL and 74.92 µg/mL in the DPPH assay for leaves and stem bark, respectively. For the ABTS assay, IC_{50} values were 42.40 µg/mL for leaves and 34.00 µg/mL for stem bark. These findings indicate that the antioxidant activity of *G. arborescens* depends on the plant part and assay method used. The results provide valuable insights into the antioxidant properties of *G. arborescens*, emphasizing its potential pharmacological applications as a natural source of antioxidants. Future research should investigate the mechanisms underlying these antioxidant effects and assess their therapeutic potential in in vivo models. This study contributes to the growing body of knowledge on plant-derived antioxidants and their relevance in combating oxidative stress-related diseases.

1. Introduction

The rise in global pollution has significantly increased human exposure to free radicals, leading to severe health risks (Fenger, 2009; Shaddick et al., 2020). Free radicals, molecules with unpaired electrons, are highly reactive and induce the formation of reactive oxygen species (ROS) such as hydroxyl radicals (OH•), superoxide anions (O2−2−), and hydrogen peroxide (H22O22) (Kumar et al., 2007). These ROS can damage cellular components, including proteins, nucleic acids, and lipids, contributing to conditions like cancer, neurodegenerative diseases, and chronic inflammation (Ma, 2010). To mitigate such damage, antioxidants play a critical role by donating electrons to neutralize free radicals, thereby preventing oxidative stress (Jacob and Burri, 1996). Consequently, antioxidants have been widely studied for their potential in reducing the risks of chronic diseases such as cardiovascular disorders and cancer (Prakash et al., 2001).

Antioxidants are categorized into enzymatic and non-enzymatic types (Mandal et al., 2009). Enzymatic antioxidants, including catalase, superoxide dismutase (SOD), and glutathione reductase, function by breaking down ROS enzymatically. Non-enzymatic antioxidants, such as α-tocopherol, ascorbic acid (vitamin C), and phenolic compounds, scavenge free radicals directly. Common methods to assess antioxidant capacity include the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay (Thaipong et al., 2006). The DPPH assay measures radical scavenging through oxidation-reduction reactions, while the ABTS assay evaluates antioxidant activity through interactions with free radical compounds. These methods provide complementary insights, as their results may vary depending on sample preparation and the structural properties of antioxidants (Shah et al., 2015).

Paper Received: 22 July 2024; Revised: 15 November 2024; Accepted: 05 February 2025
Please cite this article as: Haryoto H., Trinanda E., Santoso B., Nagara M.D.S., Liwanda N., Nurcholis W., 2025, Phytochemical Composition and Comparative Antioxidant Activity of Leaf and Stem Bark Extracts of Glochidion arborecens Using DPPH and ABTS Assays, Chemical Engineering Transactions, 115, 19-24 DOI:10.3303/CET25115004

Natural sources, particularly plants, are rich in antioxidants like flavonoids, alkaloids, and terpenoids, making them valuable for pharmacological research (Purwanto et al., 2017). Among these, *Glochidion arborescens* Blume, locally known as *mareme* in Indonesia, has garnered attention due to its preliminary reported antioxidant activity (Indra et al., 2019). The *Glochidion* genus is known to produce bioactive compounds, including sesquiterpenoids, triterpenoids, and glycosides (Sudhakar and Swetha, 2010). Despite its potential, comprehensive studies on the antioxidant properties of *G. arborescens* remain limited.
This study aims to evaluate the antioxidant activity of ethanol extracts from the leaves and stem bark of *G. arborescens*using DPPH and ABTS assays, with ascorbic acid as a positive control. By assessing these activities, the study highlights the pharmacological potential of *G. arborescens* and provides foundational data for future research on its bioactive properties.

2. Material and Methods

2.1 Sample preparation and extraction

The plant sample (*Glochidion arborescens* Blume) was identified at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Pharmaceutical Biology Study Program, Padjadjaran University, under reference number 50/HB/01/2022. Ethanol extract samples were prepared by washing the leaves and stem bark thoroughly with running water. The samples were then oven-dried at 40°C for 3 × 24 hours. The dried samples were ground and sieved through a 60-mesh sieve to obtain a fine powder. The powder was macerated using 500 mL of 96% ethanol for 3 × 24 hours. The resulting solution was filtered with Whatman No. filter paper, and the solvent was evaporated to produce a crude extract. The final volume of the extract was reduced to 20 mL by evaporating the remaining solvent.

2.2 Phytochemical qualitative test

Phytochemical screening was conducted to identify the presence of bioactive compounds, specifically flavonoids, tannins, saponins, triterpenoids, and steroids, in the extracts of *Glochidion arborescens*. Flavonoids were detected by mixing 3–7 drops of the extract with concentrated H22SO44, which produced a distinct yellow or dark red color change, indicative of the compound's presence (Puspa et al., 2017). The identification of tannins involved adding 5 drops of the extract to 10 mL of hot distilled water, followed by the addition of 3–4 drops of FeCl33. The formation of a blue-black color confirmed the presence of pyrogallol tannins, while a blue-green color indicated catechol tannins (Muthmainnah, 2019).
For saponins, 0.1 g of the extract was dissolved in 10 mL of distilled water and subjected to heating, filtering, and vigorous shaking for 30 seconds. The persistence of foam with a height of at least 1 cm was taken as a positive result for saponins (Abbasari and Haryoto, 2022). The screening for triterpenoids and steroids was conducted by dissolving 0.05 g of extract in a mixture of 0.5 mL chloroform and 0.5 mL acetic anhydride, followed by the careful addition of 2 mL concentrated H22SO44. The emergence of a bluish-green coloration confirmed the presence of steroids, whereas brownish or violet-colored rings at the interface indicated triterpenoids (Sarker et al., 2006; Evans, 2009).

2.3 DPPH assay

The antioxidant activity of the *G. arborescens* extracts was analyzed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following a protocol adapted from Kaedeides et al. (2019). A volume of 100 µL of extract was mixed with 100 µL of DPPH solution (125 µM in ethanol) in a 96-well microplate. The reaction mixture was then incubated at 25°C in the dark for 30 minutes to prevent light-induced degradation of DPPH. After incubation, absorbance was measured at 515 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany). The decrease in absorbance indicated the scavenging activity of the extracts. The results were compared with ascorbic acid, which was used as the standard control, and IC_{50} values were calculated to determine the concentration required for 50% radical scavenging activity.

2.4 ABTS assay

The ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay was conducted following a method adapted from Kaedeides et al. (2019) with minor modifications. ABTS reagent was prepared by reacting 7.7 mM ABTS stock solution with 2.4 mM potassium persulfate in a 2:1 ratio. The mixture was incubated in the dark at room temperature (25°C) for 12–16 hours to generate ABTS radical cations. For the assay, 20 µL of *G. arborescens* extract was added to 180 µL of ABTS reagent in a 96-well microplate (Biologix, Germany). The reaction mixture was incubated for 6 minutes at 25°C, after which the absorbance was measured at 734 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany). The radical scavenging activity was determined by comparing the absorbance reduction to that of the standard control (ascorbic acid), and IC_{50} values were calculated.

2.5 Data analysis

All experiments were performed in triplicate to ensure reliability and reproducibility of results. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) in SPSS software version 25 (IBM, Armonk, NY, USA) to determine significant differences ($p < 0.05$) between treatments. Graphs and IC_{50} calculations were prepared using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

The ethanol extracts of *G. arborescens* leaves and stem bark yielded 2.68% and 1.72%, respectively (Table 1), values derived by dividing the extract weight by the sample weight, as per Molyneux (2004). These yields, while indicative of bioactive compounds, fall below the 10% benchmark considered optimal (Bravdo et al., 1984). Factors such as solvent polarity, extraction duration, sample preparation, and storage conditions likely influenced these results (Komes et al., 2011; Raya et al., 2015). The structural differences between leaves and stem bark, which can affect secondary metabolite concentrations, may explain the observed variations (Nurhayati et al., 2009; Nawaz et al., 2020). Ethanol was effective as a solvent due to its ability to dissolve both polar and non-polar compounds, making it suitable for extracting diverse phytochemicals, including flavonoids, tannins, and saponins (Raya et al., 2015). While the yields were low, they align with studies by Nurhayati et al. (2009), who associated low yields with concentrated bioactive constituents. Optimizing extraction parameters, such as temperature and solvent-to-sample ratios, as recommended by Komes et al. (2011), could enhance recovery efficiency. Environmental factors, such as soil composition and climate, also influence phytochemical content (Nawaz et al., 2020). These results emphasize the need for advanced compositional analysis to identify specific bioactive compounds, as suggested by Rohaeti et al. (2022), providing a baseline for improving extraction methodologies and evaluating pharmacological potential.

Table 1. Extraction results of leaves and stem bark of G. arborescens plants

Plant parts	Average sample weight (g)	Average extract weight (g)	Yield (%)
Leaves	105.23	2.82	2.68
Stem bark	70.50	1.21	1.72

The ethanol extract of *G. arborescens* leaves and stem bark tested positive for flavonoids, tannins, and saponins, while testing negative for steroids and triterpenoids (Table 2). These findings align with Saefudin et al. (2018), who reported a high presence of flavonoids and saponins in the bark and leaves of plants within the same genus, supporting their role as major secondary metabolites. Rahmiyani and Fitriana (2020) emphasized that the extraction efficiency of such phytochemicals depends on solvent polarity, as polar solvents like ethanol excel at dissolving polar compounds such as flavonoids, tannins, and saponins. Conversely, non-polar compounds like steroids and triterpenoids are less soluble in polar solvents, as per the "like dissolves like" principle described by Salehi et al. (2019). The observed results demonstrate that ethanol is a selective solvent for polar compounds, making it a suitable choice for targeting antioxidant-rich constituents in *G. arborescens*. This selectivity could explain the absence of non-polar phytochemicals in the extract. Furthermore, the positive detection of tannins and saponins in both leaves and stem bark reinforces their widespread distribution across different plant parts. These metabolites are known to contribute to the plant's biological activities, including antioxidant properties, as highlighted in previous studies (Rahmiyani and Fitriana, 2020). The observed trends provide insights into the chemical diversity of *G. arborescens*, offering a basis for optimizing extraction techniques and exploring its pharmacological potential further.

Table 2. Results of qualitative identification of secondary metabolite compounds in G. arborescens ethanol extract

Plant parts	Phytochemical compound				
	Flavonoids	Tannin	Saponin	Steroids	Triterpenoids
Leaves	++	+	+	-	-
Stem bark	++	+	+	-	-

The antioxidant activity of *G. arborescens* extracts was evaluated using DPPH and ABTS assays, with vitamin C as a positive control due to its established antioxidant capacity and lower IC_{50} values compared to vitamins E and A (Luqman and Destiani, 2017). Antioxidant activity is classified as very strong ($IC_{50} < 50$ µg/mL), strong

(IC_{50} 50–100 µg/mL), moderate (IC_{50} 100–150 µg/mL), weak (IC_{50} 150–200 µg/mL), and very weak (IC_{50} > 200 µg/mL) (Yuanita et al., 2020).

The DPPH assay measures free radical scavenging activity through a color change from purple to yellow and a decrease in absorbance at 515 nm (Munteanu et al., 2021; Mishra et al., 2012). *G. arborescens* ethanol extracts exhibited strong antioxidant activity, with IC_{50} values of 75.25 ± 1.32 µg/mL for leaves and 74.92 ± 1.41 µg/mL for stem bark, compared to 37.07 ± 0.85 µg/mL for vitamin C (Figure 1). Statistical analysis showed no significant difference ($p > 0.05$) between the extracts, but both were significantly weaker than vitamin C ($p < 0.05$).

The ABTS assay, which detects antioxidant activity based on the formation of a green-blue color measurable at 734 nm (Prior et al., 2003; Calvindi et al., 2020), demonstrated stronger activity than the DPPH method. The IC_{50} values were 42.40 ± 1.27 µg/mL for leaves and 34.00 ± 1.12 µg/mL for stem bark, while vitamin C showed an IC_{50} of 4.51 ± 0.64 µg/mL (Figure 1). Statistical analysis indicated significantly higher activity ($p < 0.05$) in stem bark compared to leaves, although both were weaker than vitamin C ($p < 0.05$).

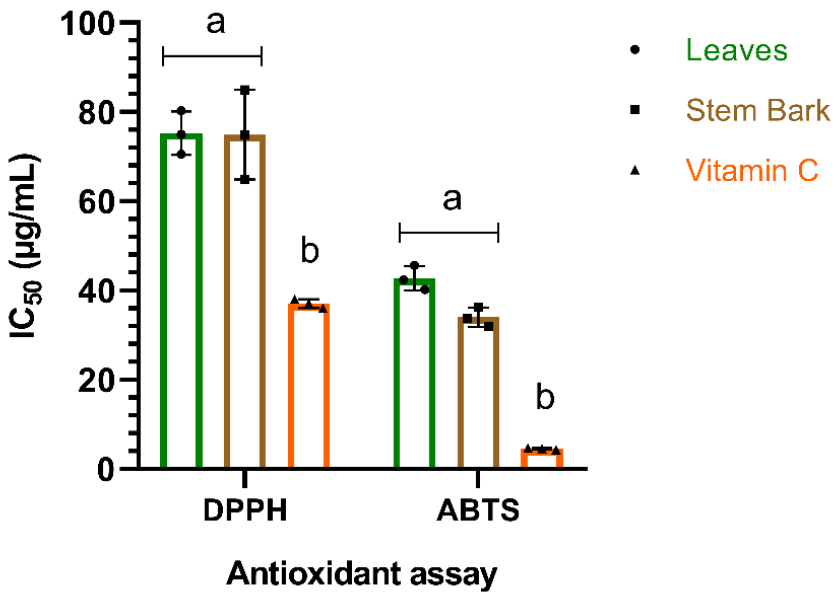

Figure 1. Results of quantitative antioxidant analysis of G. arborescens ethanol extract. DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); each value is presented as the mean of three replicates ± standard deviation (SD); the mean value marked with different letters represents statistically different results at p<0.05 on each method.

4. Conclusions

This study demonstrated that ethanol extraction effectively obtained bioactive compounds with antioxidant properties from *G. arborescens*. The leaves yielded higher extract percentages, while both leaves and stem bark contained flavonoids, tannins, and saponins. Antioxidant tests showed stronger activity with the ABTS method than the DPPH method. The stem bark exhibited greater antioxidant activity than the leaves in both assays. These findings highlight *G. arborescens* as a potential source of natural antioxidants for herbal medicine and therapeutic applications.

Acknowledgments

All authors would like to thank the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Muhammadiyah University of Surakarta for providing administrative and technical assistance in completing this research.

References

Ambarsari N., Haryoto, 2022, Isolation and identification of ethanol extract of mareme leaves (*Glochidion arborescens* Blume), *Medical Sains: Jurnal Ilmiah Kefarmasian*, 7(3), 429-438. (in Indonesian).

Bravdo B., Hepner Y., Loinger C., Cohen S., Tabacman H., 1984, Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard, *American Journal of Enology and Viticulture*, 35(4), 247-252. Doi: 10.5344/ajev.1984.35.4.247.

Calvindi J., Syukur M., Nurcholis W., 2020, Investigation of biochemical characters and antioxidant properties of different winged bean (*Psophocarpus tetragonolobus*) genotypes grown in Indonesia, *Biodiversitas Journal of Biological Diversity*, 21(6). Doi: 10.13057/biodiv/d210612.

Evans C.W., 2009, *Pharmacognosy Trease and Evans*, Saunders Elsevier, London, UK, 356 p.

Fenger J., 2009, Air pollution in the last 50 years–From local to global, *Atmospheric Environment*, 43(1), 13-22. Doi: 10.1016/j.atmosenv.2008.09.061.

Indra I., Nurmalasari N., Kusmiati M., 2019, Total phenolic, flavonoid content, and antioxidant activity of ethanol extract of mareme leaves (*Glochidion arborescens* Blume), *Jurnal Sains Farmasi & Klinis*, 6(3), 206-212. Doi: 10.25077/jsfk.6.3.206-212.2019.

Jacob R.A., Burri B.J., 1996, Oxidative damage and defense, *The American Journal of Clinical Nutrition*, 63(6), 985S-990S.

Kaderides K., Papaoikonomou L., Serafim M., Goula A.M., 2019, Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasound extraction, *Chemical Engineering and Processing-Process Intensification*, 137, 1-11. Doi: 10.1016/j.cep.2019.01.006.

Komes D., Belščak-Cvitanović A., Horžić D., Rusak G., Likić S., Berendika M., 2011, Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis, *Phytochemical Analysis*, 22(2), 172-180. Doi: 10.1002/pca.1264.

Kumar V., Cottran R.S., Robins S.L., 2007, *Buku Ajar Patologi Robbins*, Penerbit EGC, Jakarta, Indonesia, 456 p. (in Indonesian).

Lung J.K.S., Destiani D.P., 2017, Antioxidant activity test of vitamins A, C, and E using DPPH method, *Farmaka*, 15(1), 53-62. (in Indonesian).

Ma Q., 2010, Transcriptional responses to oxidative stress: pathological and toxicological implications, *Pharmacology & Therapeutics*, 125(3), 376-393. Doi: 10.1016/j.pharmthera.2009.11.004.

Mandal S., Yadav S., Yadav S., Nema R.K., 2009, Antioxidants: a review, *Journal of Chemical and Pharmaceutical Research*, 1(1), 102-104.

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.

Munteanu I.G., Apetrei C., 2021, Analytical methods used in determining antioxidant activity: A review, *International Journal of Molecular Sciences*, 22(7), 3380. Doi: 10.3390/ijms22073380.

Muthmainnah B., 2019, Phytochemical screening of secondary metabolites from pomegranate (*Punica granatum* L.) ethanol extract using colorimetric method, *Media Farmasi*, 13(2), 36-41. (in Indonesian).

Nawaz H., Shad M.A., Rehman N., Andaleeb H., Ullah N., 2020, Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds, *Brazilian Journal of Pharmaceutical Sciences*, 56, e17129. Doi: 10.1590/s2175-97902019000417129.

Nurhayati T., Aryanti N.D., 2009, Preliminary study of sponge extract as antioxidant, *Jurnal Kelautan Nasional*, 2(2), 43-51.

Prakash A., Rigelhof F., Miller E., 2001, Antioxidant activity, *Medallion Laboratories Analytical Progress*, Minnesota, USA, 19(2), 3.

Prior R.L., Hoang H.A., Gu L., Wu X., Bacchiocca M., Howard L., Hampsch-Woodill K., Huang D., Ou B., Jacob R., 2003, Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples, *Journal of Agricultural and Food Chemistry*, 51(11), 3273-3279. Doi: 10.1021/jf0262256.

Puspa O.E., Syahbanu I., Wibowo M.A., 2017, Phytochemical and toxicity tests of nutmeg essential oil (*Myristica fragans* houtt) from Lemukutan Island, *Jurnal Kimia Khatulistiwa*, 6(2). (in Indonesian).

Purwanto D., Bahri S., Ridhay A., 2017, Antioxidant activity test of purnajiwa fruit extract (*Kopsia arborea* Blume) with various solvents, *KOVALEN: Jurnal Riset Kimia*, 3(1), 24-32. (in Indonesian).

Rahmiyani I., Fitriana N.N., 2020, Influence of extraction method to total flavonoid content of mareme leaf extract (*Glochidion arborescens* Blume), *Atlantis Press*, Amsterdam, Netherlands, 56-60 p.

Raya K.B., Ahmad S.H., Farhana S.F., Mohammad M., Tajidin N.E., Parvez A., 2015, Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration, *Bragantia*, 74, 445-452. Doi: 10.1590/1678-4499.0469.

Rogers N.M., Seeger F., Garcin E.D., Roberts D.D., Isenberg J.S., 2014, Regulation of soluble guanylate cyclase by matricellular thrombospondins: implications for blood flow, *Frontiers in Physiology*, 5, 134. Doi: 10.3389/fphys.2014.00134.

Rohaeti E., Nurafifah D., Batubara I., 2022, Antidiabetic activity of manonjaya snakefruit skin extract with zebrafish (*Danio rerio*) as animal model, *Jurnal Jamu Indonesia*, 7(3), 102-110. Doi: 10.29244/jji.v7i3.201.

Saefudin S., Basri E., Sukito A., 2018, Antioxidant activity and toxicity effect of eleven types of bark extracts acquired from Euphorbiaceae, *Indonesian Journal of Forestry Research*, 5(2), 133-146. Doi: 10.20886/ijfr.2018.5.2.133-146.

Salehi H.S., Ramdin M., Moultos O.A., Vlugt T.J., 2019, Computing solubility parameters of deep eutectic solvents from Molecular Dynamics simulations, *Fluid Phase Equilibria*, 497, 10-18. Doi: 10.1016/j.fluid.2019.05.022.

Sarker S.D., Latif Z., Gray A.I., 2006, *Natural Products Isolation*, Humana Press, New Jersey, USA, 360 p.

Shaddick G., Thomas M.L., Mudu P., Ruggeri G., Gumy S., 2020, Half the world's population are exposed to increasing air pollution, *NPJ Climate and Atmospheric Science*, 3(1), 23. Doi: 10.1038/s41612-020-0124-2.

Shah P., Modi H.A., 2015, Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity, *International Journal of Research in Applied Science and Engineering Technology*, 3(6), 636-641.

Sudhakar K., Swetha R., 2010, An updated review on the *Genus Glochidion* Plant, *Archives of Applied Science Research*, 2(2), 309-322.

Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Byrne D.H., 2006, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, *Journal of Food Composition and Analysis*, 19(6-7), 669-675. Doi: 10.1016/j.jfca.2006.01.003.

Yuniarti R., Nadia S., Alamanda A., Zubir M., Syahputra R.A., Nizam M., 2020, Characterization, phytochemical screenings and antioxidant activity test of kratom leaf ethanol extract (*Mitragyna speciosa* Korth) using DPPH method, *Journal of Physics: Conference Series*, 1462(1), 012026. Doi: 10.1088/1742-6596/1462/1/012026.