



Phytochemical screening, identification of compounds, and antioxidant activity test of sirsak extract (*Annona muricata* L.) leaf grown in North Sumatra, Indonesia

Indah Yani Br. Tambunan¹, Esther Siringo-Ringo¹, Mastiur Julianti Butar-Butar¹, Rini Febrianti¹, and Kasta Gurning^{2*}

¹Department of Midwifery, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan-20141, Indonesia.

²Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan-20141, Indonesia.

*Correspondence E-mail: kastagurning@gmail.com

Abstract

The research is to identify and analyze the secondary metabolite content of sirsak (*Annona muricata* L.) leaves, which are extracted with ethanol and water solvents, and determine their potential activity as antioxidants. Extraction using ethanol (Merck) and water as solvent; phytochemical screening uses standard reagents: FeCl₃ 5% in water, FeCl₃ 1%, Dragendrof, Mayer, Wagner, Mg ribbon, HCl (concentrated), Liberman-Buchard. Analysis of secondary metabolite compound components from the extract using GC-MS Shimadzu (QP-2010S Shimadzu, Japan) and determination of potential antioxidant activity using the DPPH method. Phytochemical screening of the ethanol extract of *A. muricata* L. leaf contains phenolic secondary metabolites, flavonoids, saponins, tannins, alkaloids, and triterpenoids. According to phytochemical screening, *A. muricata* L. leaf water extract contains phenolic compounds, tannins, flavonoids, alkaloids, and steroids. The results of ethanol extract analysis using GC-MS obtained 24 types of secondary metabolite compounds with the three highest secondary metabolite compounds, namely dodecanoic acid, 1,2,3-propanetriyl ester (16.76%), dodecanoic acid, 1,2,3-propanetriyl esters (16.52%), and glycerol trilaurate (15.07%); *A. muricata* leaf water extract contains 15 metabolite compound components with the three highest secondary metabolite compounds, namely n-hexadecoic acid (37.40%), 9-Hexadecenoic acid (16.59%), and benzeneethanol, 4-hydroxy (6.76%). The antioxidant activity value extracted with water solvent has an IC₅₀ of 99.96 ppm, and that extracted with ethanol has an IC₅₀ of 264.51 ppm. *A. muricata* L. extract leaf contains various secondary metabolites, and samples extracted with water show better antioxidant activity compared to samples extracted with ethanol.

Keywords: *Annona muricata* L., DPPH, GC-MS, Sirsak leaf, secondary metabolites

Introduction

Research on the exploration and use of plants is something that is promising. Apart from assessing relatively small side effects because it is sourced from natural ingredients, it also has potential activity that is useful in treating various diseases (Gurning *et al.*, 2021a; Mutakin *et al.*, 2022; Puthi and Jomduang, 2021). Many diseases occur due to exposure to free radicals, such as cancer, diabetes, hypertension, cholesterol, heart disease, obesity, arthritis, dementia, osteoporosis, and metabolic syndrome (Tan *et al.*, 2018). Free radicals are atomic or molecular species that have unpaired electrons, which make the atom or molecule unstable in high-energy conditions. Radicals have two sources, namely endogenous and exogenous. Endogenous radicals are produced from the working conditions of cells in the mitochondria that occur in the body, while exogenous radicals are produced from outside the body, such as exposure to sunlight, cigarette smoke, chemicals, radioactivity, and so on. The types of radicals generally come from reactive nitrogen species (RNS) and reactive oxygen

species (Gurning and Hariyadi, 2022; Baliyan *et al.*, 2022; Guan and Lan, 2018; Pomatto and Davies, 2018). Free radicals in low conditions in the body are needed, such as in the metabolic breakdown of fatty acids by acyl CoA oxidase (Pomatto and Davies, 2018). If there is an increase in the concentration of free radicals, it will trigger various problems and the emergence of various diseases. Free radicals can be disabled with antioxidant compounds. The balance of free radicals and antioxidants in the body is very necessary for the continuity of physiological functions in the body (Ifeanyi, 2018).

Antioxidants play the role of deactivating free radicals by donating electrons to free radical atoms or molecules so that they do not cause further reactions and minimize them (Sinaga *et al.*, 2022a; Gulcin, 2020). Antioxidants are naturally obtained from the use of natural ingredients, one of which sirsak (*Annona muricata* L.) leaf. The *Annona muricata* L. (*A. muricata*) leaf have traditionally been used in society by boiling them and then drinking the boiled water, which is believed to strengthen the body's immune system and treat various cancers. The leaf of this plant are rich in secondary metabolites such as alkaloids, essential oils, flavanol triglycosides, phenolics, polysaccharides, lactones, flavonoids, and carotenoids (Wahab *et al.*, 2018; Lubis *et al.*, 2023). Based on this information, the aim of this research is to determine the secondary metabolite content of *A. muricata* leaf extract (water and ethanol solvents) using gas chromatography-mass spectrometry (GC-MS) and determine the antioxidant activity value using the 2,2-diphenyl-1-picrylhydrazil (DPPH) method.

Material and Methods

Materials

The tools used in this research include a macerator made of glass, a panic for decoction extraction made of stainless steel, Whatman number 1 filter paper, a buchner funnel, a vacuum pump, a vacuum rotary evaporator, a UV-Vis spectrometer (Spectronic 3000, Genesis 10 made in Japan), and a GC-MS Shimadzu (QP-2010S Shimadzu, Japan). Sample extraction used ethanol (Merck), distilled water, and standard reagents for phytochemical screening.

Plant sample preparation

250 g of the first simplicia powder from *A. muricata* leaf was extracted using 2 liters of ethanol (Merck) in a macerator vessel for 3 days with occasional stirring, then filtered using Whatman number 1 filter paper. 1 liter of new ethanol solvent for 1 day, then filtered. 250 g of simplicia powder from both *A. muricata* leaf was extracted using water as a solvent in a decoction vessel with heating for 30 minutes to a temperature of 90°C, then filtered using Whatman number 1 filter paper with the help of a buchner filter and vacuum. The ethanol extract was concentrated using a vacuum rotary evaporator at a temperature of 60 °C, while the water extract was concentrated using an oven at the same temperature, so that each crude extract was concentrated and ready for further use.

Phytochemical screening

The phytochemical components identified included phenolics, flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids by following previous research methods using standard reagents (Lubis *et al.*, 2023; Gurning *et al.*, 2021b; Sinaga *et al.*, 2022b).

Identification and analysis of secondary metabolite content

Each thick extract from *A. muricata* leaf (ethanol and water extracts) was analyzed using GC-MSQP2010S Shimadzu with column type Agilent DB-5MS, length 30 meters, ID 0.25mm, film 0.25 µm, and operates at an electron impact (EI) energy of 70 eV. The carrier gas uses helium gas with oven temperature conditions of 70.0°C, injection temperature of 300.0°C, sampling time of 1.00 min, pressure of 30.0 kPa, total flow time of 35.6 mL/min, flow time in column 0, 65 mL/min, and anchoring time of 5 minutes, starting with a temperature of 100–300°C with a temperature change rate of 5°C. The identified active compounds were compared by peak retention time with a Chromeleon similarity library MS instrument (Gurning *et al.*, 2020; Situmorang *et al.*, 2022; Tambunan *et al.*, 2023).

Determination antioxidant activity

Each thick extract from *A. muricata* leaf (ethanol and water extract) was made into a stock solution with a concentration of 100 ppm. Concentration variations were carried out in determining the antioxidant activity of each extract, namely 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, by pipetting a certain volume of the mother liquor to match the specified concentration. The solvent used in making the solution and at varying concentrations is ethanol (Merck). The DPPH concentration

used as a source of free radicals is 0.2 mM. 300 μ L of each extract was pipetted into a 5 mL measuring flask, added with 500 μ L of 0.2 mM DPPH, and filled up to the 5 mL mark with ethanol solvent. The solution mixture was incubated for 30 minutes to reach the reaction time, then measured at the maximum wavelength of the DPPH solution (517 nm) (Jakkratuwat and Kunchansombat, 2021; Sartagoda *et al.*, 2021). Measurements were carried out with three repetitions. The determination of antioxidant activity is obtained from the curve between percent inhibition (Y axis) and concentration (ppm) of the sample so that the linear regression equation $y = bx \pm a$ is obtained, where y represents the IC₅₀ of the sample in providing activity as an antioxidant (Sinaga *et al.*, 2022; Situmorang *et al.*, 2022).

Results:

Phytochemical screening of *A. muricata* L., leaf extracts

Phytochemical screening was carried out with the aim of qualitatively identifying various groups of secondary metabolites contained in each extract. The secondary metabolite group components identified included phenolics, flavonoids, tannins, saponins, alkaloids, and steroids and triterpenoids using standard reagents in Table 1.

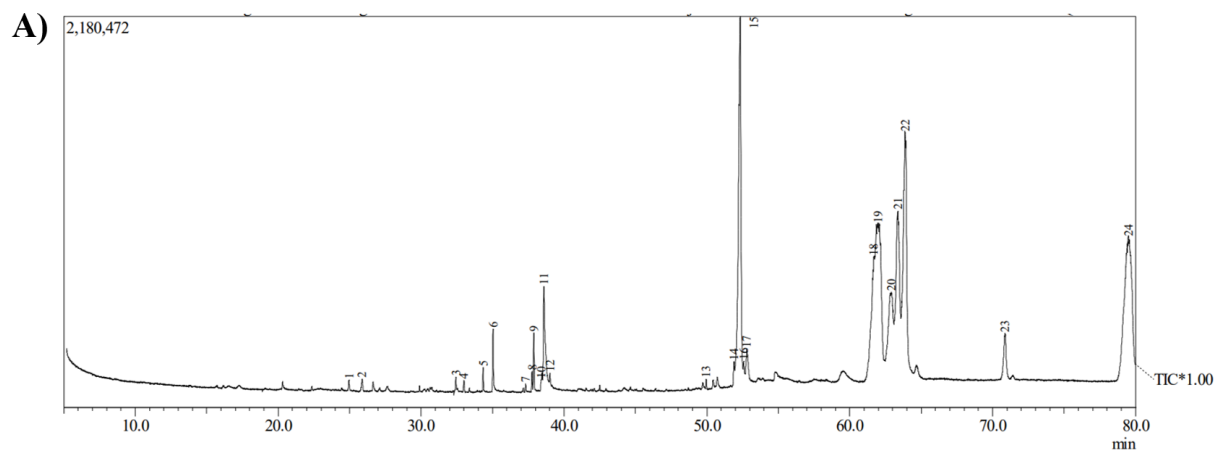
Table 1. Phytochemical screening results from *Annona muricata* L., leaf

No.	Compound Group	Method/Reagents	Results	
			Ethanol Extract	H ₂ O Extract
1.	Phenolics and polyphenolics	FeCl ₃ 5% (at ethanol)	+	+
2.	Flavonoids	Shinoda test	+	+
3.	Tannins	FeCl ₃ 1%	+	+
4.	Saponins	Foaming test	+	-
5.	Alkaloids	Dragendroff's	+	+
		Mayer	+	+
		Wagner	+	+
6.	Steroids/Triterpenoids	Libermann-Buchard	+/+	+/-

Note:(+) positive contains; and (-) negative contains

GC-MS identification of secondary metabolites compounds

Analysis using GC-MS aims to identify and determine the levels of various components of secondary metabolite compounds contained in each extract. The chromatogram of the analysis results using GC-MS is shown in Figure 1. The analysis results show that the ethanol extract of *Annona muricata*, L., leaf has 24 peaks (Table 2), and the water extract of *A. muricata* L., leaf has 15 peaks (Table 3). The number of peaks from each extract indicates the number of secondary metabolite components contained in the extract. These results indicate that in *A. muricata*, L., leaf extracted with an ethanol solvent contains more secondary metabolite compounds than the extraction process with a water solvent. Phytochemical screening results from *A. muricata* L., leaf.



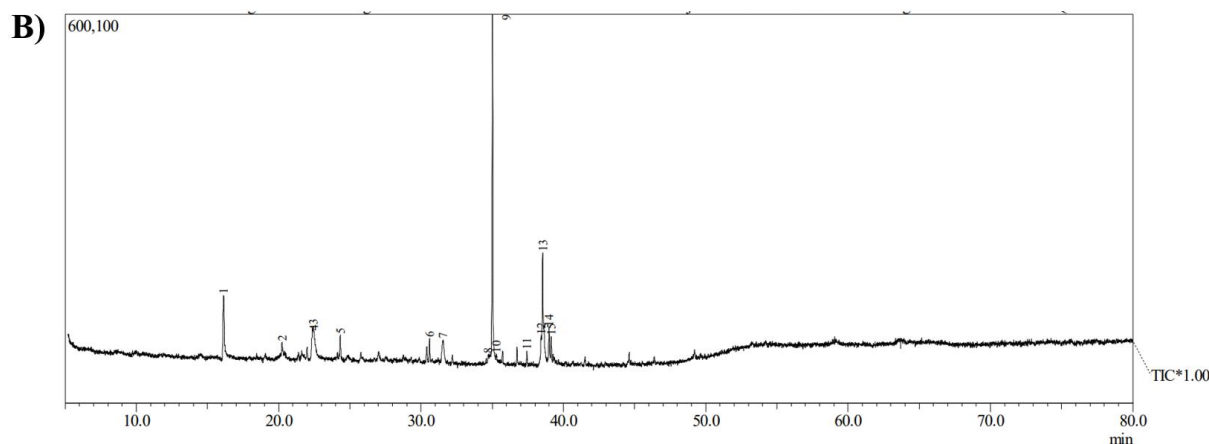


Figure 1. Chromatogram of *A. muricata*, L. leaf extract; (a) extracted with ethanol solvent; and (b) extracted with water solvent.

Table 2. Results of GC-MS identification of the ethanol extract of *Annona muricata*, L., leaf

No.	Compounds Name	Molecular Formula	Rt (min)	% area
1.	Dodecanoic acid, methyl ester	C ₁₃ H ₂₆ O ₂	25.05	0.16
2.	Decanoic acid, methyl ester	C ₁₁ H ₂₂ O ₂	25.97	0.20
3.	Oxirane, tetradecyl	C ₁₆ H ₃₂ O	32.50	0.15
4.	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	33.01	0.18
5.	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	34.43	0.27
6.	Hexadecanoic acid (CAS) palmitic acid	C ₁₆ H ₃₂ O ₂	35.17	0.85
7.	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	37.40	0.11
8.	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	C ₁₉ H ₃₄ O ₂	37.83	0.26
9.	Linoleic acid chloride	C ₁₈ H ₃₁ ClO	38.01	0.78
10.	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	38.52	0.39
11.	9-Octadecen-1-ol, (Z)- (CAS) cis-9-Octadecen-1-ol	C ₁₈ H ₃₆ O	38.90	3.30
12.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	39.13	0.31
13.	Squalene	C ₃₀ H ₅₀	50.04	0.12
14.	3-Methyl-2-nitrobenzyl alcohol	C ₈ H ₉ NO ₃	51.96	0.44
15.	Dodecanoic acid, 1,2,3-propanetriyl ester	C ₃₉ H ₇₄ O ₆	52.53	16.52
16.	Nitrone, N-phenyl-.alpha.-p-tolyl	C ₁₄ H ₁₃ NO	52.67	0.42
17.	Vinyl laurate	C ₁₄ H ₂₆ O ₂	53.03	1.20
18.	Trilaurin	C ₃₉ H ₇₄ O ₆	61.77	7.37
19.	Glycerol trilaurate	C ₃₉ H ₇₄ O ₆	62.43	15.07
20.	Hexatriacontane	C ₃₆ H ₇₄	63.10	7.44
21.	Dodecanoic acid, 1,2,3-propanetriyl ester	C ₃₉ H ₇₄ O ₆	63.58	10.87
22.	Hexatriacontane	C ₃₆ H ₇₄	64.35	14.99
23.	4-N-propylheptadecane	C ₂₀ H ₄₂	71.13	1.85
24.	Dodecanoic acid, 1,2,3-propanetriyl ester	C ₃₉ H ₇₄ O ₆	79.98	16.76

Table 3. Results of GC-MS identification of the H₂O extract of *Annona muricata*, L., leaf

No.	Compounds Name	Molecular Formula	Retention time	% area
1.	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)	C ₉ H ₈ O ₃	16.26	9.80
2.	N-methylpyrrole	C ₅ H ₇ N	20.33	1.90
3.	Benzeneethanol, 4-hydroxy	C ₈ H ₁₀ O ₂	22.48	6.76
4.	Benzeneethanol, 2-hydroxy	C ₈ H ₁₀ O ₂	22.68	2.86
5.	n-Dodecane	C ₁₂ H ₂₆	24.41	2.10
6.	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	30.69	2.10
7.	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	C ₁₃ H ₂₈ O ₃	31.73	5.16
8.	Nonylic acid	C ₉ H ₁₈ O ₂	34.92	3.09

9.	<i>n</i> -Hexadecenoic acid	C ₁₆ H ₃₂ O ₂	35.17	37.40
10.	Cyclohexane, 1,1'-[1-(2,2-dimethylbutyl)-1,3-propanediyl]bis-	C ₂₁ H ₄₀	35.56	2.10
11.	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	37.54	1.71
12.	9,12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	38.48	2.96
13.	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	38.78	16.59
14.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	39.08	3.23
15.	9,12-Octadecadienoyl chloride, (Z,Z)	C ₁₈ H ₃₁ ClO	39.22	2.25

Determination of antioxidant activity of *A. muricata* L., leaf extract

According to the literature, *A. muricata* leaf have potential activity as an antioxidant. Determination of antioxidant activity using the DPPH method uses UV-Vis spectrophotometry, which is measured at the maximum wavelength of DPPH. The analysis curve for determining antioxidant activity is shown in Figure 2. The positive control used in this study used vitamin C. The measurement results in determining the antioxidant activity of each extract from the linear regression equation for the ethanol extract of *A. muricata* L., leaf obtained $y = 0.01343x + 14.477$; $R^2 = 0.9841$; the activity value (IC₅₀) is 264.51 ppm. A water extract of *A. muricata* L., leaf obtained $y = 0.4656x + 3.518$; $R^2 = 0.9622$, obtained an activity value (IC₅₀) of 99.96 ppm. The linear regression equation for vitamin C as a positive control is $y = 0.2877x + 60.375$; $R^2 = 0.9625$, obtaining an antioxidant activity value (IC₅₀) of 36.02 ppm.

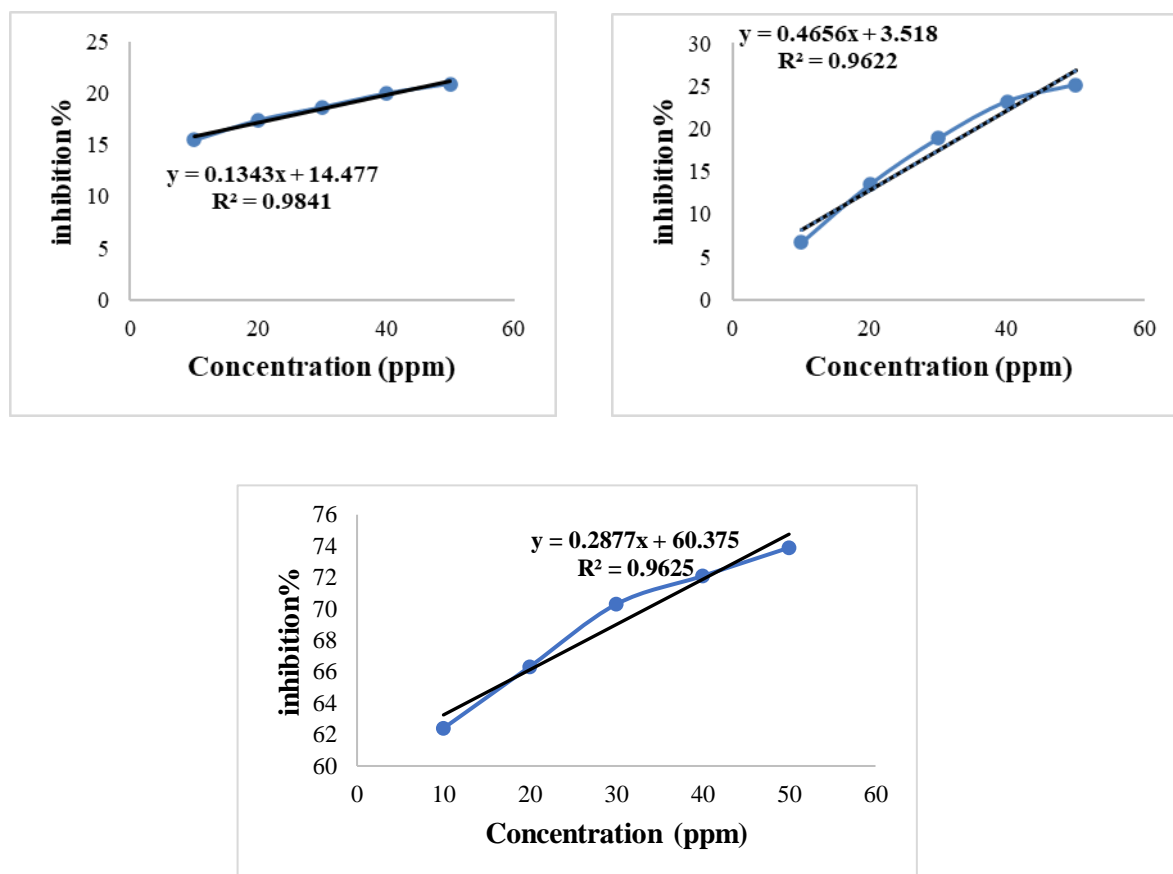


Figure 2. Curve to determine the antioxidant activity of *A. muricata*, L. leaf; (a) extracted with ethanol solvent; (b) extracted with water solvent; and (c) vitamin C.

Discussion

The extraction process for *A. muricata* L. leaf samples was carried out with two variations of solvents with the aim of comparing the secondary metabolite content and antioxidant activity produced from each extract. Qualitatively, the results of phytochemical screening show that each *A. muricata* L., leaf extract using both ethanol and water solvents is able to screen various groups of secondary metabolites contained in the sample. The ability of the ethanol solvent to filter various secondary metabolite groups and various secondary metabolite compounds is more abundant when compared

to the water solvent. This can be seen from the results of the phytochemical screening of the ethanol extract of *A. muricata* L. leaf, which was able to screen for secondary metabolites of phenolics, flavonoids, saponins, tannins, alkaloids, and triterpenoids, which was supported by data from analysis using GC-MS which obtained 24 types of secondary metabolite compounds. The results of the phytochemical screening of *A. muricata* leaf water extract were able to screen for phenolic compounds, tannins, flavonoids, alkaloids, and steroids, and data from analysis using GC-MS obtained 15 components of secondary metabolite compounds. The three highest secondary metabolite contents of *A. muricata* leaf extract with ethanol solvent were dodecanoic acid, 1,2,3-propanetriyl ester (16.76%), dodecanoic acid, 1,2,3-propanetriyl ester (16.52%), and glycerol trilaurate (15.07%); with water solvent, namely n-Hexadecanoic acid (37.40%), 9-Hexadecenoic acid (16.59%), and benzeneethanol, 4-hydroxy (6.76%). The content of various phytochemical compounds resulting from GC-MS analysis in the ethanol and water extracts of *A. muricata* leaf which have various potential bioactivities, is shown in Table 4 and Table 5.

Table 4. Phytochemical components and bioactivity of the ethanol extract of *A. muricata*, L., leaf

No.	Phytochemical components	Class of natural compounds	Potential bioactivity	References
1.	Dodecanoic acid, methyl ester	Saturated fatty acids	Antibacterial, antifungal, and antiviral	Astiti et al., 2021
2.	Decanoic acid, methyl ester	Saturated fatty acids	Antibacterial	Karthik et al., 2023
3.	Oxirane, tetradecyl	Oxirane	Antimicrobial	Musa et al., 2015
4.	Pentadecanoic acid, 14-methyl-, methyl ester	Saturated fatty acids	Antioxidant and antimicrobial	Muzahid et al., 2023
5.	Octadecanoic acid, methyl ester	Saturated fatty acids	Antibacterial, antifungal	Astiti et al., 2021
6.	Hexadecanoic acid (CAS) palmitic acid	Saturated fatty acids	Antioxidant, hypocholesterolemic, nematocide, and pesticide	Siswadi and Saragih, 2021
7.	9-Octadecenoic acid (Z)-, methyl ester	Linoleic acid ester	antioxidant, anti-cancer, and anti-inflammatory	Muzahid et al., 2023; Krishnamoorthy and Subramaniam, 2014
8.	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	Linoleic acid ester	Analgesic, anti-inflammatory and ulcerogenic	Hadi et al., 2016
9.	Linoleic acid chloride	Linoleic acid chloride	Anti-inflammatory	Kolar et al., 2019
10.	Octadecanoic acid, methyl ester	Stearic acid ester	Antioxidant, anti-inflammatory	Ganesh et al., 2017
11.	9-Octadecen-1-ol, (Z)-	Alcohol	Anti-neoplastic, Antioxidant, Natural moisturizer	Bantho et al., 2022
12.	Octadecanoic acid	Saturated fatty acids	Lowers cholesterol, antimicrobial, antioxidant, and anticancer activity	Bantho et al., 2022; Petkova et al., 2023
13.	Squalene	Linear triterpene	Antioxidant, antistatic, antibacterial, anticancer, antitumor	Ganesh et al., 2017; Willie and Uyoh et al., 2021; Padmashree et al., 2018
14.	3-Methyl-2-nitrobenzyl alcohol	Nitrogen compound	Unknown	
15.	Dodecanoic acid, 1,2,3-propanetriyl ester	Saturated fatty acids	b5- α reductase inhibitor, antiviral, antibacterial, antifungal	Achi and Ohaeri, 2015; Sharma et al., 2018
16.	Nitrone, N-phenyl-.alpha.-p-tolyl	Nitrogen compound	Unknown	
17.	Vinyl laurate	Saturated fatty ester	Antioxidant	Jiang et al., 2021
18.	Trilaurin	Saturated fatty ester	Antimicrobial	Kabara et al., 1972

19.	Glycerol trilaurate		Saturated fatty ester	Antimicrobial	Kabara et al., 1972
20.	Hexatriacontane		Alkane hydrocarbon	Antibacterial, antioxidant	Albratty et al., 2023; Marrufo et al., 2013
21.	Dodecanoic acid, 1,2,3-propanetriyl ester		Saturated fatty acids	b5- α reductase inhibitor, antibacterial, antifungal	Achi and Ohaeri, 2015; Sharma et al., 2018
22.	Hexatriacontane		Alkane hydrocarbon	Antibacterial, antioxidant	Albratty et al., 2023; Marrufo et al., 2013
23.	Hexatriacontane		Alkane hydrocarbon	Antibacterial, antioxidant	Albratty et al., 2023; Marrufo et al., 2013
24.	Dodecanoic acid, 1,2,3-propanetriyl ester		Saturated fatty acids	b5- α reductase inhibitor, antibacterial, antifungal	Achi and Ohaeri, 2015; Sharma et al., 2018

Table 5. Phytochemical components and bioactivity of the H₂O extract of *A. muricata*, L., leaf

No.	Phytochemical components		Class of natural compounds	Potential bioactivity	References
1.	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)		Phenolic compound	Antibacterial, anesthetic, antimutagenic, cancer preventive.	Guerrero et al., 2017
2.	N-methylpyrrole		Nitrogen compound	Antimalaria, antioxidant	Kocaoğlu et al., 2019
3.	Benzeneethanol, 4-hydroxy		Phenolic compound	Antioxidant and Antimicrobial	Makky et al., 2021
4.	Benzeneethanol, 2-hydroxy		Phenolic compound	Antioxidant	Sopalun et al., 2021
5.	n-Dodecane		Alkane hydrocarbon	Antimicrobial	Octarya et al., 2021
6.	Tetradecanoic acid		Saturated fatty acids	Antioxidant, cancer preventive, lubricant, cosmetics	Vats and Gupta, 2017
7.	2-Cyclohexen-1-one, hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	4-	Terpenoids	Antitherbicides	Hossen et al., 2021
8.	Nonylic acid		Saturated fatty acids	Antibacterial, acaricidal, insecticidal, antibiofilm, antifungal	Hu et al., 2021; Lipińska et al., 2023
9.	n-Hexadecanoic acid		Saturated fatty acids	Antioxidants, hypocholesterolemic, antibacterial	Siswadi and Saragih, 2021
10.	Cyclohexane, 1,1'-[1-(2,2-dimethylbutyl)-1,3-propanediyl]bis-		hydrocarbon	Unknown	
11.	9-Hexadecenoic acid		Unsaturated fatty acids	Antiinflammatory	Astudillo et al., 2018
12.	9,12-Octadecadienoic acid (Z,Z)		Linoleic acid ester	Anticarcinogenic, antiatherogenic, anti-inflammatory	Vats and Gupta., 2017
13.	9-Hexadecenoic acid		Unsaturated fatty acids	Antiinflammatory	Astudillo et al., 2018
14.	Octadecanoic acid		Saturated fatty acids	Lowers cholesterol, antimicrobial, and anticancer activity	Bantho et al., 2022
15.	9,12-Octadecadienoyl chloride, (Z,Z)		Unsaturated fatty chloride	Hypocholesterolemia, hepatoprotective, antiandrogenic, antihistaminic, and anticancer	Soltan et al., 2023

The results of determining the antioxidant activity of *A. muricata* leaf extract using water as a solvent produced an antioxidant activity value (IC₅₀) of 99.96 ppm (strong category), while using ethanol as a

solvent, an antioxidant activity value (IC₅₀) was obtained as high as 264.51 ppm (weak category). The antioxidant activity value of the two *A. muricata* leaf extracts is still lower when compared to the antioxidant activity value (IC₅₀) of vitamin C as a positive control of 36.02 ppm (very strong category). The results of determining the antioxidant activity of *A. muricata* leaf using water as a solvent are in line with the assumption that people who consume boiled water from leaf samples can maintain and improve the body's immune system and can be used as an antidote and treatment for cancer, although this assumption still needs to be proven experimentally. The ability of the antioxidant activity of the water extract to be stronger compared to the ethanol extract from *A. muricata* leaf is also supported by the results of GC-MS analysis, where the water extract contains flavonoid and phenolic compounds, while the ethanol extract does not produce them. The content of flavonoid and phenolic compounds found in natural ingredients provides activity as an antioxidant (Lubis et al., 2023; Situmorang., 2022).

Conclusion

The first step that needs to be taken into account is taking samples of *A. muricata* leaf from trees that have already produced fruit. Because of these conditions, it is certain that the metabolic processes in plants have produced the maximum number of secondary metabolites. Samples before maceration must be cleaned of impurities to minimize the interference of impurities in the research. Success in measuring antioxidant activity is largely determined by the researcher's accuracy in achieving the reaction time of the mixture before it is measured using UV-Vis spectrophotometry. Measuring antioxidant activity using the DPPH method has easier, faster steps, and better sensitivity. Extraction of *A. muricata* leaf using water as a solvent provides better antioxidant activity values (IC₅₀) compared to extraction using ethanol as a solvent. The IC₅₀ value of *A. muricata* leaf water extract was 99.96 ppm (strong category) and *A. muricata* leaf ethanol extract was 264.51 ppm (weak category).

Acknowledgement

The authors would like to thank the Indonesian government, through the Ministry of Education, Culture, Research, and Technology, for providing research funding through the 2023 Beginner Lecturer Scheme (054/LL1/AL.04.03/2023; 006/LPPM/STIKes-SENIOR/VII/2023).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Achi, N. K., & Ohaeri, O. C. (2015). GC-MS determination of bioactive constituents of the methanolic fractions of *Cnidioscolus aconitifolius*. *British Journal of Pharmaceutical Research*, 5(3), 163–172. <http://doi.org/10.9734/BJPR/2015/13893>
- Albratty, M., Alhazmi, H. A., Meraya, A. M., Najmi, A., Alam, M. S., Rehman, Z., & Moni, S. S. (2021). Spectral analysis and Antibacterial activity of the bioactive principles of *Sargassum tenerrimum* J. Agardh collected from the Red sea, Jazan, Kingdom of Saudi Arabia. *Brazilian Journal of Biology*, 83, 1-110. <http://doi.org/10.1590/1519-6984.249536>
- Astiti, N. P. A., & Ramona, Y. (2021). GC-MS analysis of active and applicable compounds in methanol extract of sweet star fruit (*Averrhoa carambola* L.) leaves. *HAYATI Journal of Biosciences*, 28(1), 12-12. <http://doi.org/10.4308/hjb.28.1.12>
- Astudillo, A. M., Meana, C., Guijas, C., Pereira, L., Lebrero, P., Balboa, M. A., & Balsinde, J. (2018). Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *Journal of Lipid Research*, 59(2), 237-249. <http://doi.org/10.1194/jlr.M079145>
- Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P., Chang, C.M. 2022. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*. 27(4): 1326. <http://doi.org/10.3390/molecules27041326>
- Bantho, S., Naidoo, Y., Dewir, Y. H., Bantho, A., & Murthy, H. N. (2022). Chemical Composition of Combretum *erythrophyllum* leaf and stem bark extracts. *Horticulturae*, 8(8), 1-16. <http://doi.org/10.3390/horticulturae8080755>
- Ganesh, M., & Mohankumar, M. (2017). Extraction and identification of bioactive components in *Sida cordata* (Burm. f.) using gas chromatography–mass spectrometry. *Journal of food science and technology*, 54, 3082-3091. <http://doi.org/10.1007/s13197-017-2744-z>

- Guan, G., & Lan, S. (2018). Implications of antioxidant systems in inflammatory bowel disease. *BioMed research international*, 2018. 1-7. <http://doi.org/10.1155/2018/1290179>
- Guerrero, R. V., Vargas, R. A., & Petricevich, V. L. (2017). Chemical compounds and biological activity of an extract from bougainvillea x buttiana (var. rose) holttum and standl. *International Journal of Pharmacy and Pharmaceutical Sciences*, 9(3), 42-46. <http://doi.org/10.22159/ijpps.2017v9i3.16190>
- Gulcin, İ. 2020. Antioxidants and Antioxidant Methods: An Updated Overview. *Arch Toxicol*. 94(3): 651–715. <http://doi.org/10.1007/s00204-020-02689-3>
- Gurning, K., Haryadi, W. 2022. Potential Antioxidants of Secondary Metabolite Isolates Ethyl Acetate Fraction *Coleus amboinicus* Lour. Leaves. *ScienceRise: Pharmaceutical Science*. 39(5): 100–5. <http://doi.org/10.15587/2519-4852.2022.266401>
- Gurning, K., Haryadi, W., Sastrohamidjojo, H. 2021. Isolation and Characterization of Antioxidant Compounds of Bangun-bangun (*Coleus amboinicus*, L.) leaves from North Sumatera, Indonesia. *Rasayan J Chem*. 14(1): 248–53. <http://doi.org/10.31788/RJC.2021.1416077>
- Gurning, K., Simanjuntak, H. A., & Purba, H. (2020). Identification of the chemical compound of essential oil from ketumbar (*Coriandrum sativum* L.) leaves with GC-MS. *Pharmacognosy Journal*, 12(5), 1019–1023. <http://doi.org/10.5530/pj.2020.12.144>
- Gurning, K., Simanjuntak, H. A., Purba, H., Situmorang, R. F. R., Barus, L., & Silaban, S. (2021, March). Determination of total tannins and antibacterial activities ethanol extraction seri (*Muntingia calabura* L.) leaves. In *Journal of Physics: Conference Series* (Vol. 1811, No. 1, p. 012121). IOP Publishing. <http://doi.org/10.1088/1742-6596/1811/1/012121>
- Hadi, M. Y., Mohammed, G. J., & Hameed, I. H. (2016). Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 8(2), 8-24. <http://doi.org/10.5897/JPP2015.0364>
- Hossen, K., Iwasaki, A., Suenaga, K., & Kato-Noguchi, H. (2021). Phytotoxicity of the novel compound 3-hydroxy-4-oxo- β -dehydroionol and compound 3-oxo- α -ionone from *Albizia richardiana* (Voigt.) King & Prain. *Environmental Technology & Innovation*, 23, 1-9. <http://doi.org/10.1016/j.eti.2021.101779>
- Hu, J., Zhang, J., & He, J. (2021). Structures, synthesis and biological activities of nonactic acid and its derivatives. *Current Medicinal Chemistry*, 28(42), 8673-8691. <http://doi.org/10.2174/0929867328666210628144347>
- Ifeanyi, O. E. (2018). A review on free radicals and antioxidants. *Int. J. Curr. Res. Med. Sci*, 4(2), 123-133. <http://dx.doi.org/10.22192/ijcrms.2018.04.02.019>
- Ilango, S., Sahoo, D. K., Paital, B., Kathirvel, K., Gabriel, J. I., Subramaniam, K., ... & Nirmaladevi, R. (2022). A review on annona muricata and its anticancer activity. *Cancers*, 14(18), 1-31. <http://doi.org/10.3390/cancers14184539>
- Jakkranuhwat, N., & Kunchansombat, P. (2021). Effect of foam-mat drying conditions on antioxidant activity, total phenolic compound, anthocyanin content and color of purple-fleshed sweet potato powder. *CMUJ. Nat. Sci*, 20(2), 1-8. <http://doi.org/10.12982/CMUJNS.2021.045>
- Jiang, C., Wang, L., Huang, X., Zhu, S., Ma, C., & Wang, H. (2021). Structural characterization and antioxidant property of enzymatic-transesterification derivatives of (-)-epigallocatechin-3-O-gallate and vinyl laurate. *Journal of Food Science*, 86(10), 4717-4729. <http://doi.org/10.1111/1750-3841.15894>
- Kabara, J. J., Swieczkowski, D. M., Conley, A. J., & Truant, J. P. (1972). Fatty acids and derivatives as antimicrobial agents. *Antimicrobial agents and chemotherapy*, 2(1), 23-28. <http://doi.org/10.1128/aac.2.1.23>
- Karthik, Y., Ishwara Kalyani, M., Krishnappa, S., Devappa, R., Anjali Goud, C., Ramakrishna, K., ... & Mushtaq, M. (2023). Antiproliferative activity of antimicrobial peptides and bioactive compounds from the mangrove *Glutamicibacter mysorens*. *Frontiers in Microbiology*, 14, 1-20. <http://doi.org/10.3389/fmicb.2023.1096826>
- Kocaoğlu, E., Talaz, O., Çavdar, H., Şentürk, M., Supuran, C. T., & Ekinçi, D. (2019). Determination of the inhibitory effects of N-methylpyrrole derivatives on glutathione reductase enzyme. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 34(1), 51-54. <http://doi.org/10.1080/14756366.2018.1520228>
- Kolar, M. J., Konduri, S., Chang, T., Wang, H., McNerlin, C., Ohlsson, L., ... & Saghatelian, A. (2019). Linoleic acid esters of hydroxy linoleic acids are anti-inflammatory lipids found in plants and mammals. *Journal of Biological Chemistry*, 294(27), 10698-10707. <http://doi.org/10.1074/jbc.RA118.006956>
- Krishnamoorthy, K., & Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *International scholarly research notices*, 2014. 1–13. <http://doi.org/10.1155/2014/567409>

- Lipińska, M. M., Haliński, Ł. P., Gołębiowski, M., & Kowalkowska, A. K. (2023). Active Compounds with Medicinal Potential Found in Maxillariinae Benth.(Orchidaceae Juss.) Representatives—A Review. *International Journal of Molecular Sciences*, 24(1), 1-35. <http://doi.org/10.3390/ijms24010739>
- Lubis, M.F., Kaban, V.E., Gurning, K., Parhan, P., Syahputra, H., Juwita, N.A., Astyka, R., Zulfansyah, I. (2023). Phytochemicals and Biological Activities of Ethanolic Extract of *Garcinia atroviridis* Leaf Grown in Indonesia. *J Med Chem Sci*. 6(10):2456–2469. <http://doi.org/10.26655/JMCHEMSCI.2023.10.20>
- Makky, E. A., AlMatar, M., Mahmood, M. H., Ting, O. W., & Qi, W. Z. (2021). Evaluation of the antioxidant and antimicrobial activities of ethyl acetate extract of *Saccharomyces cerevisiae*. *Food technology and biotechnology*, 59(2), 127-136. <http://doi.org/10.17113/ftb.59.02.21.6658>
- Marrufu, T., Nazzaro, F., Mancini, E., Fratianni, F., Coppola, R., De Martino, L., ... & De Feo, V. (2013). Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules*, 18(9), 10989-11000. <http://doi.org/10.3390/molecules180910989>
- Musa, A. M., Ibrahim, M. A., Aliyu, A. B., Abdullahi, M. S., Tajuddeen, N., Ibrahim, H., & Oyewale, A. O. (2015). Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). *Journal of intercultural ethnopharmacology*, 4(2), 129-133. <http://doi.org/10.5455/jice.20150106124652>
- Mutakin, M., Fauziati, R., Fadhillah, F. N., Zuhrotun, A., Amalia, R., & Hadisaputri, Y. E. (2022). Pharmacological activities of soursop (*Annona muricata* Lin.). *Molecules*, 27(4), 1201, 1-17. <http://doi.org/10.3390/molecules27041201>
- Muzahid, A. A., Sharmin, S., Hossain, M. S., Ahamed, K. U., Ahmed, N., Yeasmin, M. S., ... & Bhuiyan, M. N. H. (2023). Analysis of bioactive compounds present in different crude extracts of *Benincasa hispida* and *Cucurbita moschata* seeds by gas chromatography-mass spectrometry. *Heliyon*, 9(1), 1-9. <http://doi.org/10.3390/molecules27041201>
- Octarya, Z., Novianty, R., Suraya, N., & SARYONO, S. (2021). Antimicrobial activity and GC-MS analysis of bioactive constituents of *Aspergillus fumigatus* 269 isolated from Sungai Pinang Hot Spring, Riau, Indonesia. *Biodiversitas Journal of Biological Diversity*, 22(4), 1839-1845. <http://doi.org/10.13057/biodiv/d220429>
- Padmashree, M., Ashwathanarayana, R., & Raja Naika, R. B. (2018). Antioxidant, cytotoxic and nutritive properties of Roem & Schult. *Ipomoea staphylina* plant extracts with preliminary phytochemical and GCMS analysis. *Asian Journal of Pharmacy and Pharmacology*, 4(4), 473-92. <http://doi.org/10.31024/ajpp.2018.4.4.16>
- Petkova, N., Bileva, T., Valcheva, E., Dobrevska, G., Grozeva, N., Todorova, M., & Popov, V. (2022). Non-polar fraction constituents, phenolic acids, flavonoids and antioxidant activity in fruits from Florina apple variety grown under different agriculture management. *Natural and Life Science Communications*, 22(1), 1-13. <http://doi.org/10.12982/NLSC.2023.012>
- Pomatto, L. C., & Davies, K. J. (2018). Adaptive homeostasis and the free radical theory of ageing. *Free Radical Biology and Medicine*, 124, 420-430. <http://doi.org/10.1016/j.freeradbiomed.2018.06.016>
- Punthi, F., & Jomduang, S. (2021). Effect of processing steps on bioactive compounds and antioxidant activities of high anthocyanin mulberry fruit powder. *CMUJ. Nat. Sci*, 20(2), 1-9. <http://doi.org/10.12982/CMUJNS.2021.043>
- Sartagoda, K. J., Ilano, M. C., Flandez, L. E., & Castillo-Israel, K. A. (2021). Evaluation of the antioxidant activity of bignay (*Antidesma bunius* (Linn.) Spreng var. Kalabaw) flesh and seeds as affected by maturity and processing method. *CMUJ. Nat. Sci*, 20(2), 1-10. <http://doi.org/10.12982/CMUJNS.2021.042>
- Sharma, D., Rani, R., Chaturvedi, M., & Yadav, J. P. (2018). Antibacterial efficacy and gas chromatography-mass spectrometry analysis of bioactive compounds present in different extracts of *Allium sativum*. *Asian Journal Pharm Clin Res*, 11, 280-286. <http://doi.org/10.22159/ajpcr.2018.v11i4.24053>
- Sinaga, S. P., Lumbangaol, D. A., Iksen, R. F. R., & Gurning, K. (2022). Determination of phenolic, flavonoid content, antioxidant and antibacterial activities of seri (*Muntingia calabura* L.) leaves ethanol extract from North Sumatera, Indonesia. *Rasayan Journal of Chemistry*, 15(02), 1534-1538. <http://doi.org/10.31788/RJC.2022.1526730>
- Siswadi, S., & Saragih, G. S. (2021, May). Phytochemical analysis of bioactive compounds in ethanolic extract of *Sterculia quadrifida* R. Br. In *AIP Conference Proceedings* (Vol. 2353, No. 1). AIP Publishing. 1-8. <http://doi.org/10.1063/5.0053057>
- Situmorang, R. F. R., Gurning, K., Kaban, V. E., Butar-Butar, M. J., & Perangin-Angin, S. A. B. (2022). Determination of total phenolic content, analysis of bioactive compound components, and antioxidant activity of ethyl acetate Seri (*Muntingia calabura* L.) leaves from North Sumatera Province, Indonesia. *Open Access Macedonian Journal of Medical Sciences*, 10(A), 240-244. <http://doi.org/10.3889/oamjms.2022.8362>
- Soltan, O. I., Gazwi, H. S., Ragab, A. E., Aljohani, A. S., El-Ashmawy, I. M., Batiha, G. E. S., ... & Abdel-Hameed, S. M. (2023). Assessment of bioactive phytochemicals and utilization of *Rosa canina* fruit extract as a novel natural antioxidant for mayonnaise. *Molecules*, 28(8), 1-19. <http://doi.org/10.3390/molecules28083350>

Sopalun, K., Laosripaiboon, W., Wachirachaikarn, A., & lamtham, S. (2021). Biological potential and chemical composition of bioactive compounds from endophytic fungi associated with thai mangrove plants. *South African Journal of Botany*, 141, 66-76. <http://doi.org/10.1016/j.sajb.2021.04.031>

Tambunan, I. Y. B., Siringo-Ringo, E., Butar-Butar, M. J., & Gurning, K. (2023). GC-MS analysis of bioactive compounds and antibacterial activity of nangka leaves (*Artocarpus heterophyllus* Lam). *Pharmacia*, 70(1), 67-72. <http://doi.org/10.3897/pharmacia.70.e97990>

Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Sulaiman Rahman, H. (2018). Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Frontiers in pharmacology*, 9, 1-28. <http://doi.org/10.3389/fphar.2018.01162>

Vats, S., & Gupta, T. (2017). Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. *Physiology and molecular biology of plants*, 23, 239-248. <http://doi.org/10.1007/s12298-016-0407-6>

Wahab, S.M.A., Jantan, I., Haque, M. A., & Arshad, L. (2018). Exploring the leaves of *Annona muricata* L. as a source of potential anti-inflammatory and anticancer agents. *Frontiers in pharmacology*, 9, 1-20. <http://doi.org/10.3389/fphar.2018.00661>

Willie, P., Uyoh, E. A., & Aikpokpodion, P. O. (2021). Gas chromatography-mass spectrometry (GC-MS) assay of bio-active compounds and phytochemical analyses in three species of apocynaceae. *Pharmacognosy Journal*, 13(2), 383–392. <http://doi.org/10.5530/pj.2021.13.49>