



Secondary metabolites, antioxidant activity, and acute toxicity of ethanol extract of *Artocarpus elasticus* leaves

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ABSTRACT

Artocarpus elasticus belongs to Moraceae; a family best known for their fruits that support human life. Along with its nutritive components, different parts of *A. elasticus* are traditionally used to treat fever, hypertension, digestive problems, infections, and female fertility. However, scientific data of this plant, especially its leaves, are still limited, requiring further research. The present study investigated secondary metabolites, antioxidant, and acute toxicity of its leaves using methods on ethanolic maceration, phytochemical screening, determination of phenolics and flavonoids content, qualitative and quantitative DPPH radical scavenging activity, and brine shrimp lethality test. The yield of ethanolic extract of *A. elasticus* leaves was 6.52 gram extract per 100 gram of dried leaves. The study found the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins in the extract. The TPC (28.29 ± 1.46 mg GAE/g of extract) and TFC (7.72 ± 0.34 mg QE/g of extract) found high in diluted extract at 10 and 100 $\mu\text{g/mL}$, respectively. The extract showed moderate free radical scavenging activity with an SC_{50} value of 8.04 $\mu\text{g/mL}$ and an AAI of 0.99. The extract also displayed toxicity towards *A. salina* nauplii with an LC_{50} of 116.24 $\mu\text{g/mL}$. The study concludes that leaves of *A. elasticus* could be further explored for antioxidant and cytotoxic agents.

Article history:

Received 01 Feb 2026

Revised 25 Feb 2026

Accepted 27 Feb 2026

Available online 28 Feb 2026

Keywords:

Artocarpus elasticus
phytochemical screening
antioxidant activity
acute toxicity

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DOI: 10.5614/crbb.2026.7.2/48NN15AB

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1. Introduction

Indonesia has the potential to become a country that produces medicine from plants, considering that it has diverse natural resources (Siregar et al., 2020). This country is estimated to have 25% of the world's flowering plant species and is the seventh largest, with 20,000 species, 40% of which are native plants (Kusuma and Hikmat, 2015). One of the flowering plants that produce edible fruits is the Moraceae family, comprising 47 accepted genus, including *Artocarpus*. Plants of the genus *Artocarpus* consist of 76 accepted species that are native to tropical and subtropical Asia to western Pacific (Plants of the World Online, 2024). In Southeast Asia countries, including Indonesia, plants of this genus are often used as traditional medicine and food such as jackfruit (*Artocarpus heterophyllus*), cempedak (*A. integer*), and breadfruit (*A. altilis*) (Solichah et al., 2021).

The genus *Artocarpus* produces secondary metabolites that are important for medicinal drug development. These compounds include phenolics such as prenylated flavonoids and stilbenoids. They show pharmacological actions such as antioxidant, antidiabetes, anti-inflammatory, and cytotoxic (Jagtap and Bapat, 2010). One rare species, *A. elasticus* locally known as "kumbou" in Muna Island (Southeast Sulawesi). Some studies have reported chemical entities and pharmacological activity of this plant from different regions, yet, scientific reports from Sulawesi Island are

rarely found. Hence, the present study reported the phytochemical compounds, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and acute toxicity of *A. elasticus* leaves extracted using ethanol, with a purpose of adding the knowledge of the plant.

2. Materials and methods

2.1. Materials

Materials used include pharmaceutical grade ethanol (96%), deionized water (Waterone), Folin-Ciocalteu (FC) reagent (Merck), sodium carbonate (Merck) aluminum chloride hexahydrate (LobaChemie), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Himedia), ascorbic acid (Sigma-Aldrich), gallic acid (Sigma-Aldrich), quercetin (Sigma-Aldrich), pre-coated TLC sheet Alugram Sil G/UV254 0.2 mm (Macherey-Nagel), potassium dichromate (Sigma-Aldrich), and *Artemia salina* cysts (EG).

2.2. Sample collection and preparation

Leaves of *A. elasticus* were collected from the villagers of Waara village of Loghia subdistrict of Muna regency, Indonesia, in July 2024. Samples were washed with tap water, drained, chopped into small, and sun-dried under shade. The dried leaves are ground into powder and stored in a tightly closed container. A voucher

specimen was made with number AE-001 and determined by the Direktorat Pengelolaan Koleksi Ilmiah.

2.3. Sample extraction

The powder (300 g) was extracted by the maceration method using ethanol (96%) twice the sample height for 3×72 hours in room temperature. The extract was then filtered to separate the filtrate and residue. The filtrate was then evaporated to obtain a thick extract using a rotary evaporator. The thick extract was weighed and stored in the refrigerator.

2.4. Phytochemical screening

Phytochemical screening was carried out based on previously reported studies (Sabandar et al., 2020) to identify the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins in the ethanol extract of *A. elasticus* leaves. Qualitative observations were also carried out based on the results of color intensity for alkaloids (reddish orange), flavonoids (red), tannins (brownish green/dark blue), terpenoids (reddish brown), steroids (greenish yellow fluorescence), and saponins (foam formation) with a high scale (+++), medium (++), and low (+) presence in the extract.

2.5. Total phenolic content (TPC) assay

Determination of TPC values of ethanol extract of *A. elasticus* leaves was evaluated spectrophotometrically using the Folin-Ciocalteu method according to previous research (Molole et al., 2022) with some modifications.

2.5.1. Determination of maximum absorption wavelength and time of incubation

Gallic acid solution (5 and 10 µg/mL) and extract solution (20 and 40 µg/mL) each was pipetted as much as 150 µL into a microtube containing 750 µL of Folin-Ciocalteu solution (10%, w/v in water). The mixture was then vortexed until homogeneous and then incubated for 5 minutes. The mixture was added with 600 µL sodium carbonate (7.5%, w/v in water) and then vortexed until homogeneous. The mixture was pipetted as much as 1.5 mL into a cuvette and then scanned for its maximum wavelength in the range of 400-900 nm. The optimum incubation time was measured every 5-minute interval for 1 hour at the averaged maximum wavelength.

2.5.2. Determination of TPC

Determination of the TPC value of the ethanol extract of *A. elasticus* leaves was carried out with three repetitions of each extract concentration (10, 50, and 100 µg/mL). Gallic acid solutions (0, 2.5, 5, 10, and 25 µg/mL) for the calibration curve were made three times. The mixture was then prepared according to the previous procedure, and the absorbance was measured at the maximum wavelength (763 nm) and incubation time (60 minutes) determined previously. A linear calibration curve was plotted using absorbance and concentrations of gallic acid. The concentration of gallic acid from each extract concentration was calculated using the regression equation of its absorbance. Yield the conversion to TPC in milligrams of gallic acid equivalents per gram of extract (mg GAE/g) using Eq. (1).

$$C = C1 \times V/m \quad \text{Eq. (1)}$$

where C is the TPC in mg/g, in GAE (gallic acid equivalents), C1 is the gallic acid concentration from the gallic acid calibration curve in mg/mL, V is the extract volume in mL, and m is the weight of the extract in gram.

2.6. Total flavonoid content (TFC) assay

Determination of TFC values of the ethanol extract of *A. elasticus* leaves was evaluated spectrophotometrically using the aluminum chloride method according to previous research (Molole et al., 2022; Wahyuni et al., 2024), with some modifications.

2.6.1. Determination of maximum absorption wavelength and time

Quercetin solution (6.3 and 12.5 µg/mL) and extract solution (500 and 1000 µg/mL) were each pipetted as much as 750 µL into a microtube containing 750 µL of AlCl₃ (10%, w/v in water). The mixture was then vortexed until homogeneous and then pipetted as much as 1.5 mL into a cuvette and scanned for its maximum wavelength in the range of 325-600 nm. The optimum incubation time was measured every 5-minute interval for 40 minutes at the maximum wavelength of quercetin.

2.6.2. Determination of TFC

Determination of the TFC value of the ethanol extract of *A. elasticus* leaves was carried out with three repetitions of each extract concentration (100, 500, and 1000 µg/mL). Quercetin solutions (0, 2.5, 5, 10, and 12.5 µg/mL) for the calibration curve were made three times. The mixture was then prepared according to the previous procedure, and the absorbance was measured at the maximum wavelength of quercetin (419 nm) and the incubation time (5 minutes) determined previously. A linear calibration curve of quercetin was plotted using its absorbance versus concentration. The concentration of quercetin from each extract concentration was calculated using the regression equation of its absorbance. Yield the conversion to TFC in milligrams of quercetin equivalents per gram of extract (mg QE/g) using Eq. (1).

$$C = C1 \times V/m \quad \text{Eq.(1)}$$

where C is TPC in mg/g, in QE (quercetin equivalents), C1 is the quercetin concentration from the quercetin calibration curve in mg/mL, V is the volume of extract in mL, and m is the weight of the extract in gram.

2.7. Antioxidant assay

The antioxidant activity of the ethanol extract of *A. elasticus* leaves was analyzed qualitatively using the TLC dot-blot staining method and quantitatively using the UV-Vis spectrophotometric method according to previous studies (Qodrie et al., 2022; Sabandar et al., 2023), with some modifications. The qualitative analysis was performed by spotting 20 µL of the extract series solution (in 96% ethanol) on the TLC plate, and then the plate was dipped into DPPH solution (0.5 mM in 96% ethanol) for 5 seconds. The presence of a yellow zone around the spot against the purple DPPH indicates antioxidant activity or free radical scavenging activity. The final concentration of the extract on the plate ranged from 6.3 to 100 µg/spot, where ascorbic acid was used as the positive control of the assay. For quantitative analysis, 750 µL of the extract solution (0.8 to 100 µg/mL, in 96% ethanol) was mixed with 750 µL of DPPH solution (0.02 mM in 96% ethanol) in a microtube and then incubated for 15 minutes in a dark room. Then, the absorbance of the reaction mixture was measured at a wavelength of 515 nm. The percentage of radical scavenging activity (%RSA) was calculated using Eq. (2). The SC₅₀ (Half-maximal Scavenging Concentration) and AAI (Antioxidant Activity Index) values of the extract and positive controls (ascorbic acid, gallic acid, and quercetin) were obtained from a nonlinear regression (curve fit) using GraphPad Prism 5 and Eq. (3).

$$\%RSA = (\text{Abs of DPPH} - \text{Abs of sample}) / \text{Abs of DPPH} \times 100\% \quad \text{Eq. (2)}$$

$$AAI = \text{Concentration of DPPH } (\mu\text{g/mL}) / \text{SC}_{50} (\mu\text{g/mL}) \quad \text{Eq. (3)}$$

2.8. Acute toxicity assay

Acute toxicity assay of the ethanol extract of *A. elasticus* leaves was carried out using the brine shrimp lethality test (BSLT) method according to previous studies (Yamin et al., 2020), with some modifications. The extract solution (10 to 1000 $\mu\text{g/mL}$, in seawater), as much as 100 μL , was added to 100 μL of seawater on a microplate containing ten *A. salina* nauplii aged 48 hours. Then, the mixture was incubated for 24 hours at room temperature, and then the number of nauplii deaths per well was microscopically counted. Potassium dichromate was used as the positive control of the assay. The percentage of deaths was calculated using Eq.(4). The lethal concentration 50% (LC_{50}) values of the extract and positive control were obtained from probit analysis using Minitab 17.

$$\%Mortality = (\text{Total dead nauplii} / \text{Total nauplii}) \times 100\% \quad \text{Eq. (4)}$$

2.9. Data analysis

Data from TPC, TFC, antioxidant, and acute toxicity assays were obtained from three replicate experiments ($n = 3$) and are presented as mean \pm standard deviation (SD). SC_{50} values were calculated using GraphPad Prism 5, and LC_{50} values were determined using Minitab 17.

3. Results and discussion

3.1. Yield of extraction and phytochemical screening

Maceration of *A. elasticus* leaves using ethanol as the solvent produced a dark brown extract with a yield of 6.52gram extract per 100 gram of dried leaves. The results of phytochemical screening (Table 1) showed that the extract contained secondary metabolite compounds of alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins. This was also found in a study of *A. elasticus* stem bark from methanol extract, ethyl acetate fraction and water fraction (Yamin et al., 2020).The presence of tannins (brownish green) and flavonoids (red) in the extract shows high intensity (+++) where alkaloids (reddish orange), terpenoids (reddish brown), steroids (greenish yellow fluorescence) show low intensity (+) based on the color formed. In addition, the presence of saponins shows moderate intensity (+ +) based on the foam formed. Two groups of phenolics (flavonoids and tannins) observed as dominant phytochemicals of the leaves extract, indicating its potent antioxidant activity. Some studies have reported the occurrence of prenylated dihydrochalcones (a group offlavonoid) from dichloromethane extracts of the leaves, barks, and root bark of this plant (Daus et al., 2017; Jenis et al., 2019; Ko et al., 2005).

Table 1. Phytochemical screening of 96% ethanol extract of *A. elasticus* leaves

Phytochemical compounds	Result	Qualitative observation
Alkaloid	Positive	+
Flavonoid	Positive	+++
Tannin	Positive	+++
Terpenoid	Positive	+

Steroid	Positive	+
Saponin	Positive	++

3.2. Total phenolic content

Phenolics are kind of compounds with powerful antioxidant activity (Kumar and Goel, 2019). Their presence in extract is often presented as total phenolic content (TPC). Determination of TPC in *A. elasticus* leaf extract was carried out using the Folin-Ciocalteu (F-C) method based on the oxidation reaction of phenolic compounds with reagents to form a blue complex compound which absorbance can be measured at a maximum wavelength of 760 nm (Molole et al., 2022). The absorption spectrum of *A. elasticus* leaf extract was compared with the standard compound gallic acid. All extract concentrations showed peak absorption similar to gallic acid, with the highest absorption in the range of 740 nm to 780 nm (Fig. 1). Based on these results, an averaged wavelength of 763 nm was chosen for determining the TPC of *A. elasticus* extract. The optimum time for the reaction was determined using the selected maximum wavelength (763 nm). Fig. 1 (right) shows the increase in absorbance from the 5th minute to the 60th minute for all test samples. Their absorbance increased steadily, although the increment was not much different. This indicates that the reaction proceeded perfectly between the phenolic compound and the F-C reagent. The results indicate an incubation time of 60 minutes for further TPC determination in the extract.

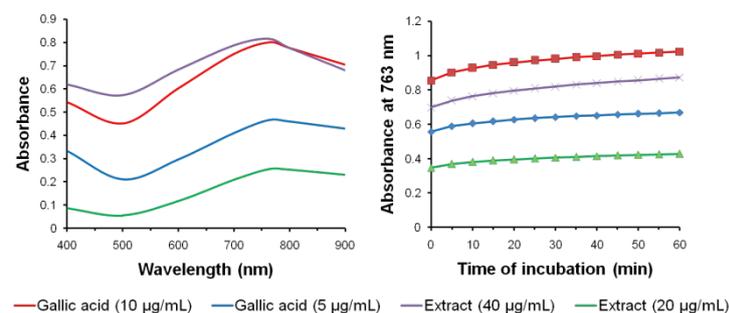


Fig. 1. Absorption spectra (left) and absorbance change during incubation time (right) of Folin-Ciocalteu reagent reaction with gallic acid and *A. elasticus* extract.

The total phenolic content of *A. elasticus* leaf extract was determined from the gallic acid calibration curve equation (0 to 25 $\mu\text{g/mL}$). The coefficient of determination (R^2) of the resulting calibration curve ($y = 0.1016x + 0.0964$) was 0.9962, indicating good linearity. The TPC value of ethanol extract of *A. elasticus* leaves was reported at concentrations of 10, 50, and 100 $\mu\text{g/mL}$ (Table 2).

Table 2. TPC values of ethanol extract of *A. elasticus* leaves (values presented as mean \pm SD, $n = 3$) at different concentrations

Concentration of extract ($\mu\text{g/mL}$)	Concentration of phenolics from equation ($\mu\text{g/mL}$)	TPC (mg GAE/g of extract)
10	1.89 ± 0.12	28.29 ± 1.46
50	8.92 ± 0.09	26.75 ± 0.22
100	14.13 ± 0.66	21.20 ± 0.80

The TPC values were decreased along with the increment of extract concentrations. This trend indicates more solubility of phenolics at diluted concentrations of extract than the concentrated solutions of the extract. The decrease in TPC values in more concentrated samples is thought to occur due to the limited amount of Folin-Ciocalteu reagent relative to the concentration of phenolic compounds, so that not all phenolic

groups can react optimally and produce lower absorption. Similar findings were also found by Molole et al. (2022) where the lower TPC values obtained in samples with higher concentrations may be due to insufficient amounts of F-C reagents in the reaction medium.

3.3. Total flavonoid content

Determination of total flavonoid content was carried out using the aluminium chloride method based on the oxidation reaction of flavonoid compounds with aluminum to form a yellow complex compound whose absorbance can be measured at a maximum wavelength of 420 nm. The absorption spectrum of *A. elasticus* leaf extract was compared with the standard compound quercetin. All extract concentrations showed the same spectrum at the maximum wavelength of quercetin 419 nm (Fig. 2; left). Based on these results, the wavelength of 419 nm was chosen for determining the TFC of *A. elasticus* extract. The optimum time for the reaction was determined using the selected maximum wavelength. Fig. 2 (right) shows that the *A. elasticus* extract exhibited an increase in absorbance at 5 minutes, and then slightly decreased from 10 to 40 minutes. For quercetin, the absorbance found to be constant from 10 to 40 minutes. Hence, the optimum incubation time for determining the TFC of *A. elasticus* leaf extract was 5 minutes.

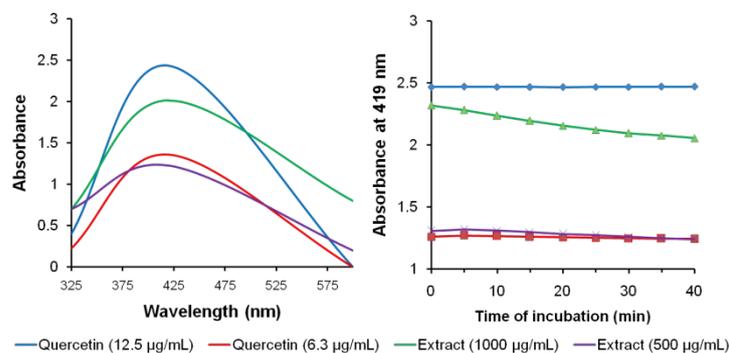


Fig. 2. Absorption spectra (left) and absorbance change during incubation time (right) of aluminium chloride reaction with quercetin and *A. elasticus* extract.

The total flavonoid content of *A. elasticus* leaf extract was determined from the quercetin calibration curve equation (0 to 12.5 µg/mL). The coefficient of determination (R^2) of the resulting calibration curve ($y = 0.2212x + 0.0332$) was 0.999, indicating a good linearity. The study found that about 36% of phenolics in the extract are flavonoids. In line with its TPC values, the lower the concentrations of extract, the higher the TFC values obtained (Table 3). This trend might be due to the limited number of reagents that react with flavonoid compounds. Another suggestion might be caused by matrix effects that can reduce extraction efficiencies of phenolic and flavonoids of the leaf extract of *A. elasticus*. In addition, some flavonoids such as prenylated flavonoids are less soluble in ethanol which results in low solubility in solution. Therefore, further study is required to establish optimum solubility of flavonoids in certain solvents which is important for pharmaceutical development regarding standardization of active compounds in the extract.

Table 3. TFC values of ethanol extract of *A. elasticus* leaves (values presented as mean ± SD, n = 3) at different concentrations

Concentration of extract (µg/mL)	Concentration of flavonoids from the equation (µg/mL)	TFC (mg QE/g of extract)
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100	1.03 ± 0.05	7.72 ± 0.34
500	2.13 ± 0.09	3.20 ± 0.13
1000	3.51 ± 0.05	2.63 ± 0.28

3.4. Antioxidant activity

The DPPH method is based on a redox reaction in which the DPPH radical interacts with antioxidants through hydrogen atom transfer or electron donation, accepting a hydrogen atom to gain an electron pair and become stabilized (Aryanti et al., 2021; Zuraida et al., 2017). The results of the qualitative antioxidant assay of *A. elasticus* leaf extract showed the presence of DPPH radical scavenging activity, which was indicated by the appearance of a yellowish white stain with a purple DPPH background (Fig. 3; top image). The activity was compared with ascorbic acid as a positive control of the assay. Similarly, the quantitative assay showed potent DPPH radical scavenging activity, indicated by the low absorbance of DPPH when reacted with *A. elasticus* extract (Fig. 3; bottom image) with an SC_{50} value of 8.04 µg/mL and AAI of 0.99 (Table 4).

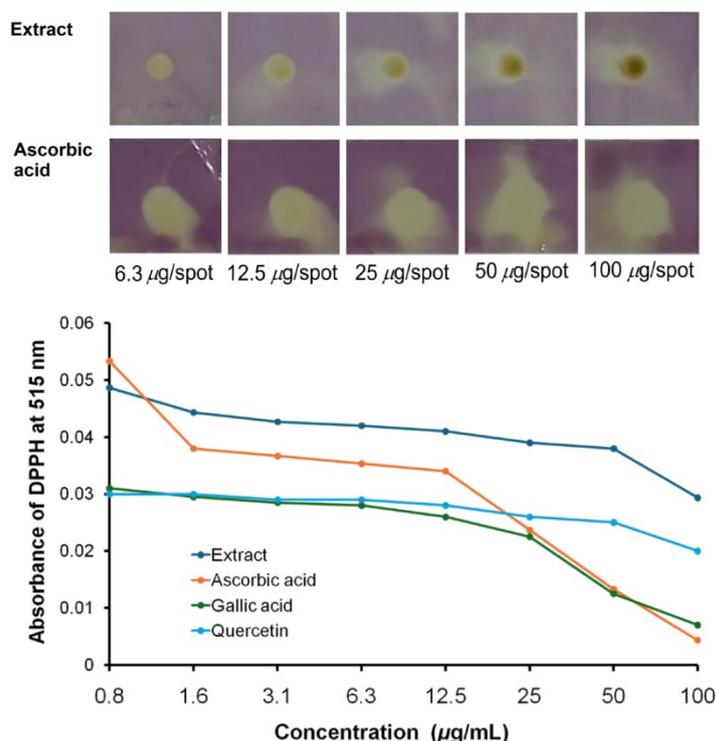


Fig. 3. Antioxidant activity of ethanol extract of *A. elasticus* leaves; yellowish white spots against purple background of DPPH (top image) and a decrease in DPPH absorbance as the extract and positive controls concentration increases (bottom image)

The antioxidant activity of *A. elasticus* was compared with ascorbic acid, gallic acid, and quercetin as the positive controls of the assay. The antioxidant activity of *A. elasticus* leaves is suggested due to the role of phenolics and flavonoids in the extract. Some antioxidant compounds are known to be present in *A. elasticus*, such as cycloartelastoxanthone, artelastoheterol, cycloartobiloxanthone, elastichalcone B, cycloartocarpesin, artonin E, elastixanthone, dan cycloartobiloxanthone (Lin et al., 2009; Ramli et al., 2016).

3.5. Acute toxicity

The BSLT (Brine Shrimp Lethality Test) method is one of the methods used to determine the possible toxicity of a compound to

cells (cytotoxic), on the basis of mortality rate of *A. salina* Leach shrimp nauplii (Prakash et al., 2013; Yashin et al., 2017). Results showed that *A. elasticus* leaf extract displayed toxicity with an LC₅₀ value of 116.24 µg/mL as compared to potassium dichromate at the positive control of the assay with an LC₅₀ value of 1.10 µg/mL (Table 5). Hence, the presence of toxic compounds in the extract can be developed as cytotoxic agents. Prenylated flavonoids are likely to contribute significantly to the cytotoxic effects of this plant.

Table 4. Percentage of DPPH radical scavenging activity (%RSA), SC₅₀, and AAI values of ethanol extract of *A. elasticus* leaves and positive controls (values presented as mean ± SD, n = 3)

Sample	Concentration (µg/mL)	%RSA	SC ₅₀ (µg/mL)	AAI
Extract	0.8	40.3 ± 2.4	8.04	0.99
	1.6	46.0 ± 5.2		
	3.1	47.9 ± 5.5		
	6.3	48.6 ± 5.9		
	12.5	50.0 ± 6.1		
	25	52.4 ± 5.1		
	50	53.7 ± 5.1		
	100	64.3 ± 2.7		
Ascorbic acid	0.8	52.3 ± 4.7	0.57	14.03
	1.6	65.5 ± 2.6		
	3.1	66.7 ± 2.4		
	6.3	67.9 ± 2.2		
	12.5	69.2 ± 2.0		
	25	78.0 ± 4.4		
	50	87.6 ± 4.7		
	100	96.1 ± 2.7		
Gallic acid	0.8	56.5 ± 3.8	0.65	12.31
	1.6	58.7 ± 4.0		
	3.1	60.1 ± 3.6		
	6.3	60.9 ± 2.0		
	12.5	64.0 ± 3.2		
	25	68.7 ± 1.8		
	50	83.0 ± 3.5		
	100	90.8 ± 3.6		
Quercetin	0.8	47.4 ± 0.1	4.17	1.92
	1.6	47.4 ± 2.4		
	3.1	49.1 ± 5.6		
	6.3	49.1 ± 2.9		
	12.5	50.9 ± 3.7		
	25	54.4 ± 4.7		
	50	56.1 ± 5.3		
	100	64.9 ± 5.2		

Table 5. Acute toxicity of ethanol extract of *A. elasticus* leaves (values presented as mean ± SD, n = 3)

Sample	Concentration (µg/mL)	%Mortality	LC ₅₀ (µg/mL)
Extract	10	40.00 ± 29.44	116.24
	100	50.00 ± 14.14	
	1000	96.67 ± 4.71	
Potassium dichromate (positive control)	10	70.00 ± 0.00	1.10
	100	100.00 ± 0.00	
	1000	100.00 ± 0.00	

4. Conclusion

In the present study, leaves of *Artocarpus elasticus* were investigated based on secondary metabolites composition and biological activities. The ethanol extract of the leaves contains alkaloids, flavonoids, tannins, terpenoids, steroids, and tannins. It also contains 25.41 mg of phenolics and 4.52 mg of flavonoids per gram of extract. The extract showed moderate free radical

scavenging activity with an SC₅₀ value of 8.04 µg/mL and an AAI of 0.99 and displayed toxicity towards *A. salina* nauplii with an LC₅₀ of 116.24 µg/mL. Therefore, *Artocarpus elasticus* leaves could potentially be a source of antioxidant and cytotoxic compounds.

Acknowledgements

Authors are grateful for a research grant scheme "Penelitian Dosen Pemula (PDP)" 2024 (contract number: 14/UN56.D.01/PN/03.00/2024) for partially financial support.

Conflict of interest

The authors declare no conflict of interest in this research.

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