



Article

Investigation of standardized *Alocasia cordifolia* rhizomes extract for its phyto-chemical screening and antioxidant potential

Manuscript received: 14 Nov 2023
 Revised version: 20 December 2023
 Accepted: 23 December 2023
 Published: 24 December 2023

Dong Shui^{1,2}, Sreemoy Kanti Das^{1*}, Nadiah Syafiqah
 Nor Azman¹, Rong-Hui Geng²

Edited by:

Prof. Md. Moklesur Rahman Sarker
 Gono Bishwabidyalay (University)
 Bangladesh

Reviewed by:

Dr. Shihab Uddin
 University of Asia Pacific, Bangladesh
 Dr. Souvik Mukherjee
 Central University, Bilaspur, India
 Md. Rejaul Islam
 Daffodil International University,
 Bangladesh

Citation:

Shui D, Das SK, Azman NSN, Geng R-H. Investigation of standardized *Alocasia cordifolia* rhizomes extract for its phyto-chemical screening and antioxidant potential. Healthmed J Pharm Sci 2023; 1(1):34-43.



Copyright: © 2023 by the authors.
 Licensee HMS Media International,
 Dhaka, Bangladesh
 This article is an open access article
 distributed under the terms and
 conditions of the Creative Commons
 Attribution 4.0 (CC BY 4.0)
 International license.

Abstract

Alocasia cordifolia, locally known in Malaysia as “Birah negeri”, is one of the most abundant natural resources in Malaysia. The aim of this study was to investigate the phytochemical constituents which possess antioxidant potential from *A. cordifolia* rhizomes, and provide possible sources for future novel antioxidant substances in food and pharmaceutical formulations. The rhizomes of *A. cordifolia* were extracted with different solvents by increasing the polarity from chloroform, acetone, and ethanol. The phytochemical analysis of extracts was conducted by standard phytochemical methods and thin-layer chromatography (TLC). Different solvent extracts were then used for the evaluation of total phenolic content, total flavonoid content and *in vitro* antioxidant activities. Correlation between the antioxidant activity and total phenolic and flavonoid contents was analyzed using Pearson’s coefficient. Preliminary qualitative phytochemical investigations showed the presence of alkaloids, flavonoids, phenols, glycosides, phytosterols, steroids, tannins, saponins, terpenoids, and fixed oils and fats. Ethanol extract showed the highest total phenolic content (134.2±3.4 mg gallic acid equivalent/g) and total flavonoid content (71.8±1.8 mg rutin equivalent/g). All the extracts had antioxidant activity whereas the highest activity was observed with ethanol extract. Both total phenols and total flavonoids were significantly positively correlated with the radical scavenging activity on DPPH, superoxide anion, ABTS, and hydroxyl. *A. cordifolia* rhizomes can be used as a potent source of antioxidants, and specific bioactive ingredients and mechanisms of action are still required to be further studied.

Keywords: *Alocasia cordifolia*, phytochemical, antioxidant, phenolic compounds, flavonoid.

¹Faculty of Pharmacy, Lincoln University College, Selangor, Malaysia; ²Center of Scientific Research, Nanyang Medical College, Nanyang, China

*Correspondence: Dr. Sreemoy Kanti Das, Faculty of Pharmacy, Lincoln University College, Selangor, Malaysia; Email: sreemoy@lincoln.edu.my, Phone: +601112809033

Introduction

Bioactive compounds or extracts from plants have been used for treating and preventing diseases since ancient times. Traditional medicines were mostly used in combination to achieve healing of the disease with the characteristics of multi-components, multi-target and multi-channels, which makes them obtain a better therapeutic advantage (Song et al., 2019). Despite synthetic drugs occupy an important position for curing various diseases in recent years, they have several limitations, such as side effects and high cost (Badisa et al., 2014). Therefore, traditional medicines attract more and more attention all over the world due to their good therapeutic effects with less toxic side effects, low cost and easy availability, particularly in developing countries (Watcho et al., 2019).

Reactive oxygen species (ROS) include hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen (1O_2) and ozone (O_3). It is well recognized that oxidative stress, defined as an imbalance between radicals and antioxidant defense, is closely related to pathogenesis and pathophysiology of various diseases, including aging, cancer, atherosclerosis, diabetes and neurodegenerative disorders (Hwang et al., 2013). Over the years, a large number of medicinal plants have been investigated for their antioxidant potential. Natural antioxidants, including phenolics, flavonoids, tannins and anthocyanidins, have high biological activity and safety. Thus, in recent years, researchers and scientists have devoted themselves to discovering natural antioxidants for health maintenance, or prevention and treatment of diseases.

Alocasia cordifolia (*A. cordifolia*) belonging to the family of Araceae is locally known in Malaysia as “Birah negeri”, which is used as a household decorative plant. This is a lofty succulent herbaceous plant that can grow up to 4.5 meters in height. It possesses a sizable elongated stem, and its leaves are arrow-shaped with shallow and rounded lobes (Karnawat et al., 2015). The leaves of *A. cordifolia* have been claimed to have antioxidant, antinociceptive, anti-inflammatory, antifungal, hepatoprotective, antidiabetic, and hypolipidemic activities (Islam et al., 2013; Arbain et al., 2022; Ongpoy, 2017). The rootstocks of this plant have been found to possess anthelmintic activity (Islam et al., 2013). However, few studies have been undertaken on the rhizomes of *A. cordifolia*, especially concerning the relationship between phytochemical compounds and antioxidant capacity. The present study was designed to analyze phytoconstituents present in *A. cordifolia* rhizomes extract and to evaluate its antioxidant potential.

Materials and Methods

Collection and authentication of plant materials

The fresh whole plant of *A. cordifolia* was purchased from Katsura Garden Center (Petaling Jaya, Selangor, Malaysia). The plant was authenticated by Dr. Sreemoy Kanti Das, assigned the voucher specimen (No. SBID: 001/21), and deposited in the Faculty of Pharmacy, Lincoln University College, Malaysia.

Drying and grinding

The rhizomes of *A. cordifolia* were washed with tap water, cut into small pieces, dried first under sunlight for 3 days and then placed in a hot air oven at 50°C until completely dry. The dried plant rhizomes were ground into a fine powder with the help of a Philips HR2221/01 blender. In order to be used in the future, the powder was stored in an airtight container.

Extraction of plant

The extraction of the rhizomes of *A. cordifolia* was made by continuous hot percolation process in the Soxhlet apparatus. And sequential extraction of *A. cordifolia* rhizomes was performed with three solvents: chloroform, acetone and ethanol, with an increasing order of polarity, described as follows.

The powdered rhizomes of *A. cordifolia* (200 g) were extracted sequentially with chloroform (800 mL) at 65°C, acetone (800 mL) at 60°C and 70% ethanol (800 mL) at 60°C until the solvent became colourless. After completion extraction, the extract solution was evaporated under a water bath to obtain chloroform extract (2.1 g), acetone extract (3.7 g) and ethanol extract (5.6 g), respectively.

Phytochemical Analysis

Standard phytochemical methods

Test for alkaloids

Extracts were dissolved in dilute Hydrochloric acid and filtered. a) Mayer's Test: Filtrates obtained were treated with Mayer's reagent (Potassium Mercuric Iodide). The presence of alkaloids was confirmed by the formation of a yellow-colored precipitate. b) Filtrates were treated with Wagner's reagent (1.27 g Iodine in 2 g of Potassium Iodide). The formation of brown or reddish-brown precipitate indicated the presence of alkaloids.

Test for flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of 2% sodium hydroxide solution. Intense yellow colour appeared which became colourless with addition of few drops of dilute acid (HCl), indicating the presence of flavonoids. b) Lead acetate Test: Extracts were treated with few drops of 10% lead

acetate solution. Yellow color precipitate formation indicated the presence of flavonoids.

Test for phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of 5% neutral ferric chloride solution. Appearance of bluish-black colour was confirmed as a positive presence of phenols.

Test for glycosides

Keller Killiani Test: Extracts were treated with few drops of glacial acetic acid and ferric chloride solution. Formation of two layers was observed after adding concentrated sulphuric acid. Lower reddish brown layer and upper acetic acid layer which turns bluish green indicated the presence of glycosides.

Test for phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered through filter paper. After adding a few drops of concentrated sulphuric acid, it was vortexed and allowed to stand. Appearance of golden yellow color indicated the presence of phytosterol.

Test for steroids

Libermann Burchard's Test: Extracts were dissolved in 2 mL of chloroform and followed by the addition of 2 mL concentrated sulphuric acid, the appearance of red colour in the lower chloroform layer gave an indication of the presence of steroids.

Test for tannins

Gelatin Test: Extracts were treated with 1% gelatin solution containing sodium chloride, the formation of a white precipitate indicated the presence of tannins.

Test for saponins

Foam Test: Extracts were mixed with 5 mL of distilled water, and it was shaken vigorously. The formation of stable foam was taken as evidence of the presence of saponins.

Test for terpenoids

When the extracts were mixed with chloroform and concentrated sulfuric acid, a reddish-brown color appeared at the interface indicating the presence of terpenoids.

Test for fixed oils and fats

The dry powdered extracts were pressed with filter paper. The appearance of oily stain on filter paper indicated the presence of the fixed oils and fats.

Test for quinones

Diluted sodium hydroxide was added to the crude extract. A blue-green or red coloration was confirmed as a positive presence of quinones.

Test for coumarin

A mixture of 10 % sodium hydroxide and chloroform was added to the extract for the observation of yellow color, which indicates coumarin presence.

Qualitative analysis of phenolic acids and flavonoids by thin-layer chromatography (TLC)

The qualitative analysis of phenolic acids and flavonoids by TLC was performed as described earlier (Das *et al.*, 2015). TLC was carried out using silica gel 60, F254, and 20 × 20 cm aluminum sheets (Merck, Darmstadt, Germany). Solutions of investigated extracts, standard substances including phenolic acids (gallic, protocatechuic and vanillic acids) and flavonoids (quercetin and kaempferol) were prepared by dissolving 10 mg in 1 ml of methanol. Samples were spotted onto TLC plates, and plates were developed up to 19 cm in a hermetic glass chamber for at least 30 min. In this investigation ethyl acetate-methanol-water, 77:13:10 was used as the developing solvent. Visualization of phenolic acids was performed by spraying the plates with solution of iron III chloride (2% methanol) and aluminium chloride (1% in ethanol), a blue or light blue spot indicated the presence of phenolic acids. Whereas visualization of flavonoids was achieved by spraying with solution of aluminium chloride (1% in ethanol), the colour of spot changed from brown to yellow indicated the presence of flavonoids. The colour of the spots in each plate was recorded under UV at 254nm, and the R_f value was determined. Identification was achieved by comparing R_f values and spot colours with the selected standard compounds.

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content (Kim *et al.*, 2003; Chouhan *et al.*, 2022). Briefly, 1 mL of sample (1 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent for 5 minutes, followed by the addition of 10 mL of 7% Na_2CO_3 solution and 13 mL of distilled water. The solution was mixed well and incubated at room temperature for 1.5 h, after which absorbance was read at 750 nm. Gallic acid was used to establish standard calibration curve and the total phenolic content was expressed as milligrams gallic acid equivalent per gram extract.

Determination of total flavonoid content

Total flavonoid content was estimated using aluminium chloride colorimetric method with slight modification (Chouhan *et al.*, 2022; Part *et al.*, 2008). Briefly, 0.3 mL of sample (1 mg/mL), 3.4 mL of 30% methanol, 0.15 mL of NaNO_2 (0.5 M) and 0.15 mL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.3 M) were mixed in a 5 mL test tube for 5 minutes. Then 1 mL of 1 M NaOH was added. The solution was mixed thoroughly and kept at room

temperature for 15 minutes, after which absorbance was measured at 506 nm. Rutin was used for the standard calibration curve and the total flavonoid content was expressed as milligrams rutin equivalent per gram extract.

Antioxidant Potential

The extract was dissolved in methanol to obtain a stock solution with the concentration of 1 mg/mL and then diluted to produce the series concentrations for antioxidant assays. For all assays, ascorbic acid was used as an antioxidant standard for comparison. In the present study, the antioxidant activity was expressed as IC₅₀, which is the concentration of sample required to inhibit 50% of free radicals.

DPPH radical scavenging activity

The capacity of extracts to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted according to the method previously described, with some modifications (Wu *et al.*, 2020). The stock solution of DPPH was prepared by dissolving 24 mg of DPPH with 100 mL methanol and stored in the dark at -20°C until further use. The working solution of DPPH was obtained by diluting DPPH stock solution with methanol to attain an absorbance of about 0.95 ± 0.02 at 517 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). 100 µL of various concentrations of the sample extract was mixed with 3 mL of the working DPPH solution. After vigorously shaking, the reaction mixture was kept at room temperature for 15 minutes in the dark. Then, the absorbance was measured at 517 nm. In this assay, DPPH solution was used as a blank control. The percentage of DPPH radicals scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of blank control without samples and A_{sample} is the absorbance in the presence of the sample.

Superoxide anion radical scavenging activity

Using riboflavin-light-NBT system, the superoxide anion radical scavenging activity was investigated (14). The reaction mixture consisted of 0.5 mL of 50 mM phosphate buffer (pH 7.6), 0.3 mL of 50 mM riboflavin, 0.25 mL of 20 mM phenazine methosulphate (PMS), and 0.1 mL of 0.5 mM nitroblue tetrazolium (NBT), prior to the addition of 1.0 mL of various concentrations of the sample. After illuminating the reaction mixture using a fluorescent lamp for 20 min, the absorbance was taken at 560 nm. The reaction mixture without any test sample was used as the blank control. The percentage of superoxide anion radical scavenging activity was determined as follows:

Superoxide anion radical scavenging activity (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$

where A_{control} is the absorbance of the blank control reaction (containing all reagents except the sample) and A_{sample} is the absorbance in the presence of the sample.

ABTS radical scavenging activity

The 2,2'-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), also known as the ABTS cation scavenging assay was carried out to estimate the free radical scavenging activity of plant extracts (Shah *et al.*, 2013). A dark-coloured solution containing ABTS radical cation was made by mixing ABTS (7 mM) with potassium persulfate (2.45 mM) solution and kept in the dark overnight. Then, the ABTS radical cation working solution was diluted with 50% methanol until an absorbance of 0.70 ± 0.02 at 745 nm. Free radical scavenging activity was evaluated by mixing 3.0 mL of ABTS working solution with 300 µL of test sample in a microcuvette. The decrease in absorbance was measured exactly 1 minute after mixing the solution, the final absorbance was noted up to 6 min. The scavenging activity was determined according to the following equation: ABTS radical scavenging activity

$$\text{ABTS radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of blank control without samples and A_{sample} is the absorbance in the presence of the sample.

Hydroxyl radical scavenging activity

The scavenging activity against the hydroxyl radical was determined by the Fenton reaction (Shah *et al.*, 2013). The reaction mixture consisted of: 200 µL of premixed 100 mM ferric chloride and 100 mM ethylenediaminetetraacetic acid (EDTA) solution (1:1; v/v), 500 µL of 2.8 mM 2-deoxyribose in 50 mM phosphate buffer (pH 7.4), 100 µL of 200 mM H₂O₂ without or with the 100 µL of the sample solution. The reaction was triggered by adding 100 µL of ascorbate (300 mM) and then kept for 1 h at 37°C. A 0.5 mL aliquot of the reaction mixture was taken and added to 1 mL of 2.8% trichloroacetic acid (TCA) aqueous solution, then 1.0 mL of 1% thiobarbituric acid (TBA) aqueous solution was added to the reaction mixture. The reaction mixture was heated on a boiling water bath for 15 min (100°C). The mixture was cooled and the absorbance was then measured at 532 nm. The percentage inhibition was estimated according to the formula: Hydroxyl radical scavenging activity

$$\text{Hydroxyl radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

A_{control} is the absorbance of blank control without samples, whereas A_{sample} is the absorbance in the presence of the sample.

Results and Discussion

In order to extract bioactive ingredients of a wide range of polarity from crude plant material completely, the rhizomes of *A. cordifolia* were extracted with different solvent by increasing the polarity from chloroform, acetone, and ethanol, respectively. The extractives value was reported in Table 1. It can be seen that extraction with ethanol resulted in the highest amount of total extractable compounds whereas the extraction yield with chloroform was only small in comparison with that of acetone.

The result of preliminary phytochemical screening was tabulated in Table 2, which revealed the variation in phytochemicals of various solvents extracts of *A. cordifolia* rhizomes. Alkaloids, flavonoids and phenols were commonly present in all the extracts, while quinones and coumarin were not detected in any of the extracts. Glycosides was detected in only acetone and ethanol extracts, and terpenoids was found in only chloroform and ethanol extracts. In addition, only the ethanolic extract was observed for the presence of phytosterols, steroids, tannins and saponins, but lacked fixed oils and fats. The result confirms the presence of active ingredients which are well known to exhibit medicinal as well as physiological properties, and ethanolic extract has the most abundant compounds as compared to the other two extracts.

Previous studies found that phenolic compounds, including phenolic acids and flavonoids, possess strong antioxidant activity (Kumari et al., 2017). TLC is a simple, fast, and accurate method, which can be used for qualitative analysis of constituents (Mohamed et al., 2017). In the present study, phenolic acids and flavonoids in different rhizomes extracts were investigated via TLC (Table 3). The result shows the presence of phenolic acids and flavonoids in all studied extracts, whereas different extracts presented differences in phytochemical compound. The ethanolic extract contained gallic acid, protocatechuic acid, vanillic acid, quercetin, and kaempferol (Figure 1), which was the most abundant phenolic acids and flavonoids followed by acetone and chloroform extract. The presence of phenolic acids and flavonoids supports the antioxidant activity of *A. cordifolia* rhizomes.

The content of total phenolic and flavonoid in different solvent extracts was estimated from standard curve calibration of gallic acid and rutin respectively. Table 4 summarizes that total phenolic content in different solvent extracts varied widely, ranging from 79.5 ± 2.5 to 134.2 ± 3.4 mg/g. Total phenolic content of ethanol extract was significantly higher ($P < 0.05$) than that of the chloroform and acetone extract. However, no significant difference in total phenolic content was

found between the chloroform and acetone extract ($P > 0.05$). Total flavonoid compounds are also widely distributed in different solvent, ranging from 31.3 ± 1.8 to 71.8 ± 1.8 mg/g. The ethanol extract showed the highest amount of flavonoid content followed by acetone extract and chloroform extract. The results indicated that ethanol extract had the highest total phenolic and flavonoid contents, and ethanol was the most appropriate solvent to extract the maximal amounts of phenolic and flavonoid components.

Several *in vitro* antioxidant activity measurement techniques have been used to rapidly screen antioxidant substances. However, free radicals are proven to play a critical role in a variety of pathological events, so the most widely used methods for evaluating the antioxidant activity are those that produce free radical species which are then neutralized by antioxidant substances (Masoko et al., 2007). This study used four different *in vitro* antioxidant assays: DPPH radical scavenging activity, superoxide anion radical scavenging activity, ABTS radical scavenging activity and hydroxyl radical scavenging activity. Results are shown in Figure 2 and in Table 5.

Antioxidants can quench DPPH by electron donation or hydrogen atom transfer and decrease the purple color of the DPPH solution measured at 517 nm (Mardani-Ghahfarokhi et al., 2020). The reaction mix with a low absorbance indicates a greater DPPH radical scavenging activity. The DPPH radical scavenging activity of various solvent extracts can be ranked as ethanol extract > acetone extract > chloroform extract. Although DPPH radical scavenging activity of various extracts was found to be low ($P < 0.05$) than that of ascorbic acid, each extract possessed antioxidant activity in a dose-dependent manner (Figure 2A, Table 5). The results indicated that the *A. cordifolia* rhizomes contains phytochemical constituents which are capable of donating hydrogen to scavenge the free radicals that might cause potential damage.

Superoxide anion radical is proven to be one of the most dangerous reactive oxygen species among free radicals. Alternatively, superoxide anion is a weak oxidant that can produce highly toxic radicals, including hydroxyl radicals and singlet oxygen, which both contribute to oxidative stress (Bajpai et al., 2017). Figure 2B and Table 5 show that each extract has the scavenging effects on superoxide anion radical, and the order of activity was ethanol extract > acetone extract > chloroform extract. ABTS radical scavenging assay is based on the generation of a blue/green $ABTS^+$, which can be decolorized by antioxidant components. The results reveal that ethanol extract has higher scavenged activity on ABTS radical compared

Table 1. The extractives value of *A. cordifolia*

Plant Name	Part Used	Method of Extraction	Percentage yield %		
			Chloroform	Acetone	Ethanol
<i>A. cordifolia</i>	Rhizomes	Hot percolation using soxhlet apparatus	1.05	1.85	2.8

Table 2. Preliminary phytochemical screening of different solvent extracts of *A. cordifolia* rhizomes

Phytoconstituents	Chloroform extract	Acetone extract	Ethanol extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Glycosides	-	+	+
Phytosterols	-	-	+
Steroids	-	-	+
Tannins	-	-	+
Saponins	-	-	+
Terpenoids	+	-	+
Fixed oils and fats	+	+	-
Quinones	-	-	-
Coumarin	-	-	-

+ (plus) sign indicates the presence of phytochemical constituents; – (minus) sign indicates the absence of phytochemical constituents..

Table 3. Qualitative analysis of phenolic acids and flavonoids in different solvent extracts of *A. cordifolia* rhizomes by TLC

Phytoconstituents	R _f of standard	Chloroform extract	Acetone extract	Ethanol extract
Gallic acid	0.45	-	+	+
Protocatechuic acid	0.56	-	+	+
Vanillic acid	0.71	+	-	+
Quercetin	0.63	-	+	+
Kaempferol	0.92	+	-	+

+ (plus) sign indicates the presence of phytochemical compounds; – (minus) sign indicates the absence of phytochemical compounds.

Table 4. Total phenolic and flavonoid contents in different solvent extracts of *A. cordifolia* rhizomes

	Total phenolic content (mg gallic acid equivalent/g of extract)	Total flavonoid content (mg rutin equivalent/g of extract)
Chloroform extract	79.5±2.5 ^b	31.3±1.8 ^c
Acetone extract	85.6±6.0 ^b	65.0±2.4 ^b
Ethanol extract	134.2±3.4 ^a	71.8±1.8 ^a

Values are presented as mean ± SD (n = 3). Means with different superscript (a-c) letters in the same column are significantly ($P < 0.05$) different from each other via ANOVA and Duncan's test.

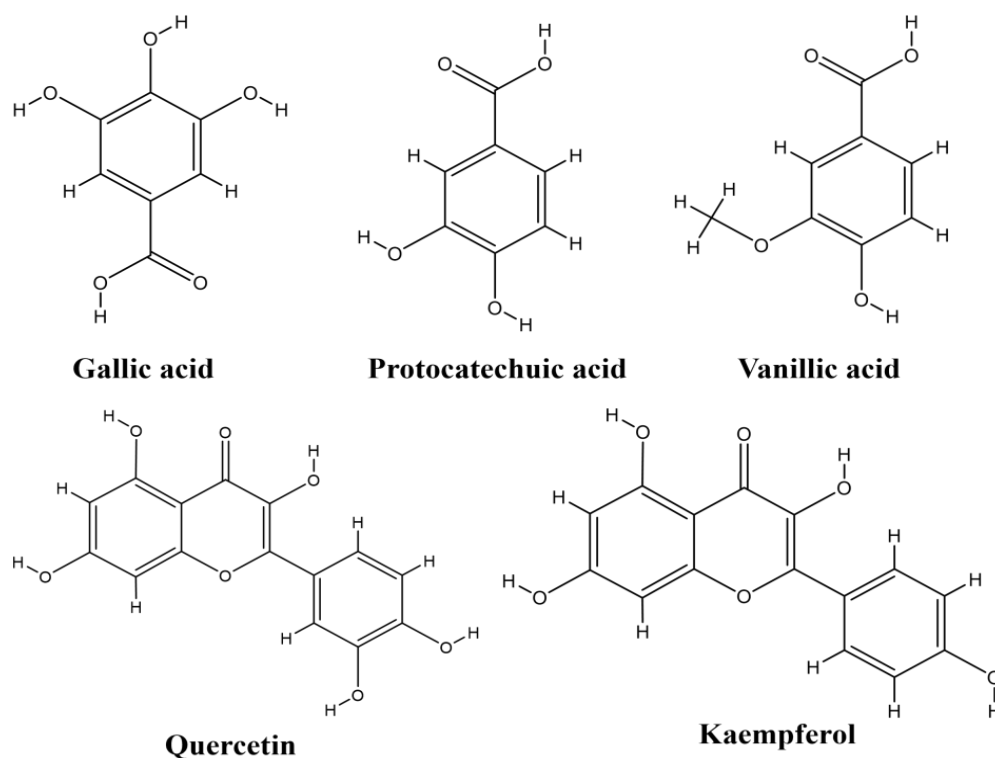


Figure 1. Chemical structure of phenolic acids and flavonoids in *A. cordifolia* rhizomes

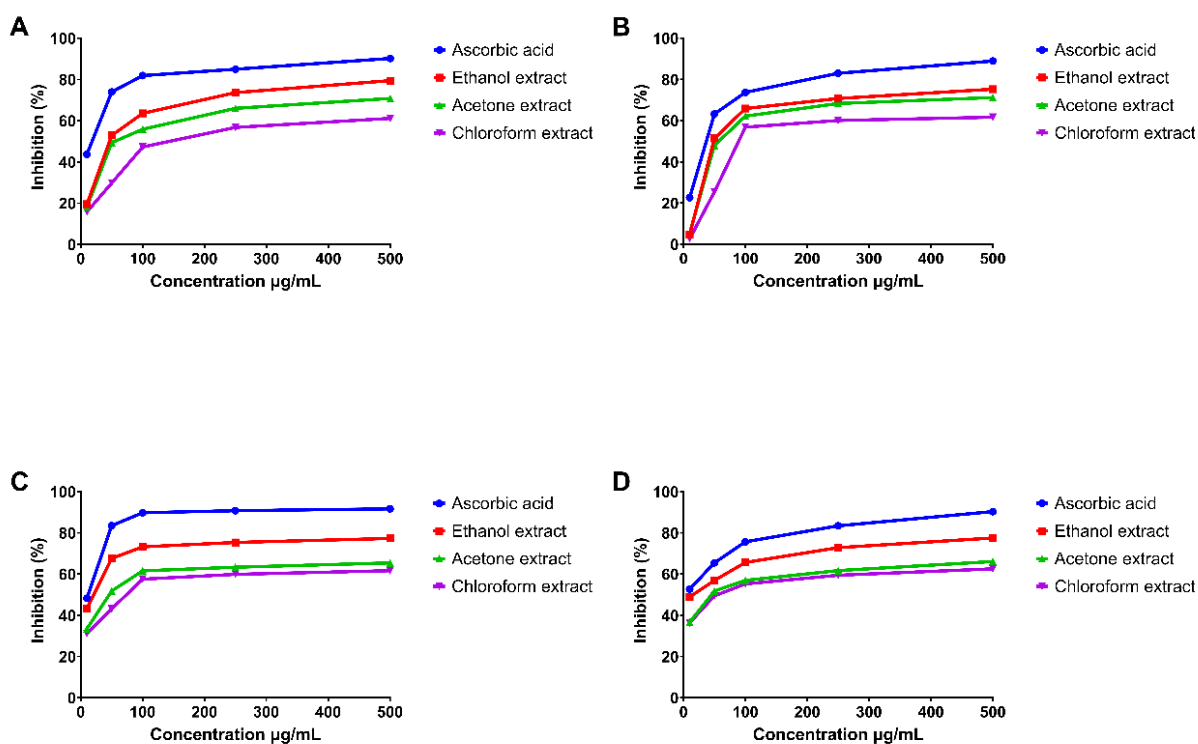


Figure 2. Antioxidant activities of different solvent extracts of *A. cordifolia* rhizomes: (A) DPPH radical scavenging activity; (B) Superoxide anion radical scavenging activity; (C) ABTS radical scavenging activity; (D) Hydroxyl radical scavenging activity.

Table 5. Radical scavenging activity of different solvent extracts of *A. cordifolia* rhizomes

Sample	IC ₅₀ values (µg/mL) of radical scavenging			
	DPPH radical	Superoxide anion radical	ABTS radical	Hydroxyl radical
Chloroform extract	174.3±3.8 ^a	153.1±1.1 ^a	83.9±2.4 ^a	61.8±2.2 ^a
Acetone extract	76.4±2.4 ^b	79.5±2.8 ^b	48.5±3.6 ^b	48.5±1.5 ^b
Ethanol extract	52.7±2.3 ^c	67.2±1.7 ^c	13.7±1.1 ^c	14.1±2.0 ^c
Ascorbic acid	13.2±1.2 ^d	33.0±0.9 ^d	10.2±0.6 ^c	9.4±1.1 ^d

Values are presented as mean ± SD (n = 3). Means with different superscript (a–d) letters in the same column are significantly ($P < 0.05$) different from each other.

Table 6. Pearson's correlation coefficient (r) between the antioxidant activity and total phenolic and flavonoid contents

Analytes	Total phenolic content	Total flavonoid content
DPPH radical scavenging activity	0.713*	0.996**
Superoxide anion radical scavenging activity	0.675*	0.994**
ABTS radical scavenging activity	0.894**	0.923**
Hydroxyl radical scavenging activity	0.976**	0.812**

* indicates significance at $P < 0.05$, ** indicates significance at $P < 0.01$.

to acetone and chloroform extracts. Ethanol extract has a statistically similar antioxidant activity to ascorbic acid ($P > 0.05$), suggesting the presence of strong antioxidants in this extract (Figure 2C and Table 5).

Hydroxyl radical is an extremely reactive free radical that is formed in biological systems. It may destroy the biomolecules of living cells and cause serious damage as a result (Al-Huqail et al., 2018). In the present study, IC₅₀ values of the extracts in scavenging hydrogen radical were significantly different ($P < 0.05$) from the IC₅₀ values determined for ascorbic acid. Ethanol extract exhibited prominent hydrogen radical scavenging activity (Figure 2D and Table 5).

From the above results, we found that all the extracts have antioxidant activity and ethanol extract showed better antioxidant activity in all assays. It has formerly been reported that phenolic and flavonoid components are known as the bases of the antioxidant activity of plant, so the correlation coefficient between the antioxidant activity and total phenolic and flavonoid contents was firstly investigated (Table 6). We found that both total phenols and total flavonoids were significantly positively correlated with the radical scavenging activity on DPPH, superoxide anion,

ABTS, and hydroxyl ($P < 0.05$ or $P < 0.01$). Our results strongly suggest that phenol and flavonoid compounds are considered to be major contributors to the antioxidant activity of *A. cordifolia* rhizomes.

Conclusion

Over the past few years, antioxidant compounds from natural plant have gained much attention for their health benefits. The present study clearly demonstrates that *A. cordifolia* rhizomes is a rich source of active phytoconstituents, which possess biological and pharmacological activities. Different solvent extracts of *A. cordifolia* rhizomes have antioxidant activity, however, ethanol extract showed the highest antioxidant activity than acetone and chloroform extract. The correlation results indicate the crucial role of phenolic and flavonoid compounds as antioxidant constituents in the plant extract. Therefore, *A. cordifolia* rhizomes can be used as a potent source of antioxidants, and the specific active ingredients and mechanisms of action are still required to be further explored using modern technology means.

Acknowledgements

The authors would like to express their gratitude to the Lincoln University College authority to facilitate in conducting the study.

Conflicts of Interest

All the authors declare that they don't have any kind of conflict of interest in the publishing of the article.

Funding Information

This study was not supported by national or international research grant or any kind of financial support to conduct the study.

References

- Al-Huqail AA, Elgaaly GA, Ibrahim MM. Identification of bioactive phytochemical from two *Punica* species using GC-MS and estimation of antioxidant activity of seed extracts. *Saudi Journal of Biological Sciences*. 2018;25(7):1420-8.
- Arbain D, Sinaga LMR, Taher M, Susanti D, Zakaria ZA, Khotib J. Traditional uses, phytochemistry and biological activities of *Alocasia* Species: A systematic review. *Frontiers in Pharmacology*. 2022;13:849704.
- Badisa RB, Fitch-Pye CA, Agharahimi M, Palm DE, Latinwo LM, Goodman CB. Milk thistle seed extract protects rat C6 astroglial cells from acute cocaine toxicity. *Mol Med Rep*. 2014;10(5):2287-92.
- Bajpai VK, Alam MB, Quan KT, Kwon K-R, Ju M-K, Choi H-J, et al. Antioxidant efficacy and the upregulation of Nrf2-mediated HO-1 expression by (+)-lariciresinol, a lignan isolated from *Rubia philippinensis*, through the activation of p38. *Scientific reports*. 2017;7(1):46035.
- Chouhan KBS, Mukherjee S, Mandal V. Reconfiguring extraction of phenolics & flavonoids through a solvent-free gravity assisted model for the complete recovery of target analytes from moringa leaves: A complete overhauling attempt in the field of botanical extraction. *Sustainable Chemistry and Pharmacy*. 2022;29:100805.
- Hwang Y-J, Lee E-J, Kim H-R, Hwang K-A. In vitro antioxidant and anticancer effects of solvent fractions from *Prunella vulgaris* var. *lilacina*. *BMC complementary and alternative medicine*. 2013;13:1-9.
- Islam MK, Mahmud I, Saha S, Sarker AB, Mondal H, Monjur-Al-Hossain A, et al. Preliminary pharmacological evaluation of *Alocasia indica* Schott tuber. *Journal of Integrative Medicine*. 2013;11(5):343-51.
- Kanti Das S, Sengupta P, Shahimi Mustapha M, Kifayatullah M, Gousuddin M. Phytochemical Investigation and Antioxidant Screening of Crude Leaves Extract from *Epipremnum aureum*. *International Journal of Pharmacognosy and Phytochemical Research*. 2015;7(4):684-9.
- Karnawat BS, Joshi A, Narayan JP, Sharma V. *Alocasia macrorrhiza*: a decorative but dangerous plant. *International Journal of Scientific Study*. 2015;3(1):221-3.
- Kim D-O, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry*. 2003;81(3):321-6.
- Kumari R, Kumar S, Kumar A, Goel KK, Dubey RC. Antibacterial, antioxidant and Immuno-modulatory properties in extracts of *Barleria lupulina* Lindl. *BMC complementary and alternative medicine*. 2017;17(1):1-11.
- Mardani-Ghahfarokhi A, Farhoosh R. Antioxidant activity and mechanism of inhibitory action of gentisic and α -resorcylic acids. *Scientific Reports*. 2020;10(1):19487.
- Masoko P, Eloff JN. Screening of twenty-four South African *Combretum* and six *Terminalia* species (Combretaceae) for antioxidant activities. *African Journal of*

- Traditional, Complementary and Alternative Medicines. 2007;4(2):231-9.
- Mohamed HB, El-Shanawany SM, Hamad MA, Elsabahy M. Niosomes: a strategy toward prevention of clinically significant drug incompatibilities. *Scientific reports*. 2017;7(1):6340.
- Ongpoy RC. The medicinal properties of the alocasia genus: a systematic review. *Journal of Asian Association of Schools of Pharmacy*. 2017;6:25-33.
- Park Y-S, Jung S-T, Kang S-G, Heo BG, Arancibia-Avila P, Toledo F, et al. Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chemistry*. 2008;107(2):640-8.
- Shah NA, Khan MR, Ahmad B, Noureen F, Rashid U, Khan RA. Investigation on flavonoid composition and anti free radical potential of *Sida cordata*. *BMC Complementary and Alternative Medicine*. 2013;13:1-12.
- Song Y, Wang H, Pan Y, Liu T. Investigating the Multi-Target Pharmacological Mechanism of *Hedyotis diffusa* Willd Acting on Prostate Cancer: A Network Pharmacology Approach. *Biomolecules*. 2019;9(10):591.
- Watcho P, Lih F, Deeh PBD, Wankeu-Nya M, Ngadjui E, Bonsou GRF, et al. Aphrodisiac property of aqueous and methanolic extracts of *Raphia vinifera* (Arecaceae) in sexually experienced male rats. *International Journal of Reproductive BioMedicine*. 2019;17(6):413.
- Wu Y, Jiang H, Lin J-S, Liu J, Wu C-J, Xu R. Antioxidant, hypolipidemic and hepatic protective activities of polysaccharides from *Phascolosoma esculenta*. *Marine Drugs*. 2020;18(3):158.