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Extraction and Fractionation of Anti-Cholesterol Compounds from Water Chestnut (*Eleocharis dulcis*)

Hemi Adi Putra, Amir Husni* & Alim Isnansetyo
Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Sleman Regency, Special Region of Yogyakarta, Indonesia
*Corresponding author, email: a-husni@ugm.ac.id
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ABSTRACT Cholesterol is a significant contributor to mortality rates worldwide. The quest for effective cholesterol treatments continues, aiming to develop preventive agents with minimal adverse effects. This study elucidates the anti-cholesterol properties of extracts and components fractionated from water chestnut (*Eleocharis dulcis*). The research encompassed several phases: sampling, extraction and fractionation, qualitative and quantitative flavonoid analysis, anti-cholesterol activity assays, and gas chromatography-mass spectrometry (GC-MS) analysis. The quantitative flavonoid assessment revealed that the highest flavonoid concentration was present in the ethyl acetate: methanol (5:5) fraction, totaling 79.224 mg/mL. The water chestnut extract demonstrated notable anti-cholesterol activity with an IC_{50} value of 13016.27 ± 5648.31 ppm. In contrast, the water chestnut fraction exhibited enhanced anti-cholesterol activity with an IC_{50} value of 42.22 ± 9.79 ppm, comparable to simvastatin. The bioactive compounds in water chestnut attributed to its anti-cholesterol effects include 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, n-Hexadecanoic acid, Octadecanoic acid ethyl ester, 9-Octadecenamide (Z)-, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, 3,4-Dimethoxycinnamic acid, and 2H-1-Benzopyran-2-one, 7-methoxy-. These findings suggest that the fraction derived from *E. dulcis* exhibits significant anti-cholesterol activity and holds promise for further development in treating hypercholesterolemia.

Keywords: Anti-cholesterol; chromatography-mass spectrometry (GC-MS); *Eleocharis dulcis*; hypercholesterolemia; flavonoids

INTRODUCTION

Cardiovascular disease (CVD) ranks as a predominant cause of mortality worldwide, with dyslipidemia, particularly elevated levels of LDL cholesterol, serving as a significant risk factor. While pharmacological interventions such as statins demonstrate efficacy, their application is frequently accompanied by adverse effects and substantial costs, necessitating the exploration of safer and more economical natural alternatives. Phytochemicals derived from plants, including flavonoids, polyphenols, and plant sterols, have been shown to exhibit hypocholesterolemic properties through mechanisms such as the inhibition of cholesterol absorption and the enhancement of lipid metabolism (Bachheti *et al*., 2022).

Eleocharis dulcis, known as water chestnut, is a plant that lives in tidal and muddy swamp waters (Yulita *et al*., 2022), belongs to the Cyperaceae family, originates from the Asian region, but is also found in tropical Africa and Australia (Zhang *et al*., 2022). The plants are widely used in China as a source of high-carbohydrate food and have been used as traditional medicine to treat hypertension, pharyngitis, constipation, and chronic nephritis (Zhan *et al*., 2016). Studies have identified bioactive compounds in its tubers and skin, including flavonoids such as luteolin and apigenin, and plant sterols such as daucosterol, which have potential in lowering cholesterol levels (Zhang *et al*., 2022). However, specific exploration of anti-cholesterol compounds in *E. dulcis* is still limited, especially regarding optimal extraction and fractionation methods to obtain these active compounds (Yang *et al*., 2024).

Extraction is a pivotal step in isolating bioactive compounds from natural sources, and the choice of extraction method and solvent significantly influences the efficiency and selectivity of the process. Reflux extraction, combined with ethanol as a solvent, is widely employed due to its effectiveness in extracting a broad spectrum of phytochemicals (Zhang *et al*., 2018). The active compounds of water chestnut can be obtained from a multistage extraction method using n-hexane, ethyl acetate, and ethanol solvents (Baehaki *et al*., 2021). Ethanol is a preferred solvent due to its polarity, safety, and ability to extract a wide range of polar and non-polar compounds. It is especially effective in extracting phenolic compounds, flavonoids, and terpenoids, which are known for their antioxidant properties (Lee *et al*., 2023), Previous studies have shown that ethanol extracts of *E. dulcis* have high phenolic and flavonoid contents, as well as significant antioxidant activity (Zhang *et al*., 2022). However, studies targeting the isolation and characterization of anti-cholesterol compounds from *E. dulcis* through extraction and fractionation approaches are still rare. This study aims to investigate the anti-cholesterol potential of water chestnut extracts.

MATERIALS AND METHODS

Materials

The raw materials used in this study were water chestnuts obtained from swamp waters in East OKU, South Sumatra. The obtained samples were then diced and air-dried to prepare for extraction and fractionation. The chemicals used are ethanol p.a (Merck, Germany), n-hexane p.a (Merck, Germany), ethyl acetate p.a

(Merck, Germany), Na_2CO_3 (Merck, Germany), phosphate buffer (Merck, Germany), technical methanol and p.a. (Merck, Germany), Fe. (Merck, Germany), $FeCl_3$ (Merck, Germany), $AlCl_3$ (Merck, USA), anhydrous acetic acid (BASF, Germany), concentrated sulfuric acid (BASF, Germany), chloroform (Sigma-Aldrich, Germany), quercetin (Sigma-Aldrich, Germany), H_2SO_4 (Sigma-Aldrich, Germany). Tools for chemical testing were beaker glass (Iwaki Pyrex, Indonesia), Erlenmeyer (Iwaki Pyrex, Indonesia), freeze dryer (Zirbus, Germany), spatula (Iwaki Pyrex, Indonesia), analytical balance (Ohaus. Germany), funnel (Iwaki Pyrex, Indonesia), Herb Grinder (Cuisinart, USA), rotary evaporator (DLAB RE100 PRO, China), micropipette (Eppendorf, Germany), volume pipette (Iwaki Pyrex, Indonesia), test tube rack (Iwaki Pyrex, Indonesia), spatula (Iwaki Pyrex, Indonesia), shaker (IKA, Germany), oven (Salvis, Switzerland), porcelain cup (Haldenwanger, Germany), UV-Vis spectrophotometer (Amtast, Turkey), column chromatography (Iwaki Pyrex, Indonesia), and a GC MS set (Shimadzu, Japan).

Preparation and identification of water chestnut

Water chestnut was collected at coordinates 4.142393S and 104.610963E, Tambak Boyo Village, Buay Madang Timur District, East OKU Regency, South Sumatra Province, in January 2024. Fresh samples of 10 kg were collected and put into a container, and taken to the laboratory. Fresh samples were then washed to remove dirt such as sand and mud. Wet samples were dried indoors (without sunlight) for 4-7 days until the water content reached 11-14% (Nurfahmi *et al*., 2018). The dried samples were then cut into small pieces and blended into powder.

Water analysis

The assessment of water content was conducted utilizing a moisture analyzer. A precise quantity of 5 grams of water chestnut sample was meticulously weighed and placed into the apparatus, followed by recording the sample's initial weight. The water chestnut specimen was then heated until all moisture was thoroughly evaporated. Upon the drying process's completion, the sample's water content was calculated and presented as the final result.

Extraction of water chestnut

Water chestnut (*E. dulcis*) was extracted using the reflux method as delineated in the research by Nursamsiar *et al*. (2023). A total of 40 g of powdered sample was subjected to reflux extraction with 400 mL of 70% ethanol maintained at a temperature of 60°C for 3 hours. This reflux extraction procedure was reiterated three times. The resultant extract was subsequently desiccated using a freeze dryer. The yield of the extract was determined by comparing the final weight (extract weight) to the initial weight (weight of the sample employed), subsequently multiplied by 100% (Tang *et al*., 2024).

Fractionation of water chestnut extract by column chromatography

The fractionation of water chestnut extract via column chromatography is elucidated in the methodology presented by Qomariyah & Mursiti (2019). The preparation of the column chromatography commences with the activation of 100 g of silica gel 60 (0.2-0.5 mm), which was subjected to heating in an oven at 110ºC for 60 minutes. Subsequently, the silica gel was amalgamated with n-hexane to create a slurry-like mixture. To initiate the filling of the column, a layer of glass wool was placed at the base as a foundational filler. Thereafter, the silica gel slurry was meticulously introduced through the column wall, ensuring that it stirs gently to avert the formation of air pockets within the column's midsection. It was allowed to rest overnight. The thickness of the silica gel slurry layer within the column was calibrated to extend to three-quarters of the column's height.

The subsequent step involved dissolving 2 g of water chestnut extract in 20 mL of n-hexane. Furthermore, a gradual elution process was executed utilizing a n-hexane and ethyl acetate mixture, totaling 20 mL, in the following ratios: (9:1), (8:2), (7:3), (6:4), (1:1), (4:6), (3:7), (2:8), (1:9), culminating in 20 mL of ethyl acetate. This was followed by elution employing a mixture of ethyl acetate and methanol, also totalling 20 mL, adhering to the same ratios: (9:1), (8:2), (7:3), (6:4), (1:1), (4:6), (3:7), (2:8), (1:9), concluding with 20 mL of methanol. The extract was absorbed onto the silica gel within the column until it reached the upper limit of the silica gel, achieved by a gradual introduction while simultaneously opening the column tap. The elution solution (eluate) was collected in 20 mL vials for each fraction. The resultant fractions were subsequently evaluated quantitatively for flavonoid content and anti-cholesterol activity, utilizing the Liebermann-Burchard method (Qomariyah & Mursiti, 2019).

Qualitative and quantitative analysis of the extract and fractions

Qualitative flavonoid analysis was conducted by dissolving 0.1 g of the extract in 10 mL of 96% ethanol. Subsequently, 1 mL of 10% NaOH was introduced to 2 mL of water chestnut extract (Puspasari & Puspita, 2023). In parallel, total flavonoid levels were assessed utilizing the $AlCl_3$ colorimetric method. The evaluation of total flavonoid content in this investigation is based on the research conducted by Alagan *et al*. (2017), albeit with minor modifications.

A quercetin standard solution was analyzed to derive a standard curve. A 10 mg of quercetin was solubilized in 10 mL of 80% methanol, yielding a stock solution concentration of 1000 ppm. This stock solution was subsequently subjected to a series of dilutions, producing 20, 40, 60, 80, and 100 ppm concentrations. A precise volume of 0.5 mL from each concentration of the standard solution was meticulously pipetted into separate test tubes, which were then combined with 1.5 mL of 95% methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The resultant mixture was incubated at ambient temperature for 30 minutes before measuring its absorbance utilizing a UV-Vis spectrophotometer at a wavelength of 415 nm. Furthermore, the assessment of total flavonoid content in extracts and fractions of water chestnut was executed analogous to the evaluation of the standard quercetin

solutions. The determination of total flavonoid content was performed using a quercetin standard curve (QE) at a concentration of 1 mg/mL, expressed as QE (mg/g), by incorporating the sample absorbance values into the quercetin standard curve equation represented as $y = ax + b$.

Analysis of anti-cholesterol activity

A stock solution of water chestnut extract was meticulously measured to 25 mg and subsequently placed into a 25 mL volumetric flask, which was then filled with chloroform. A stock solution of 300 ppm of the fraction was prepared by accurately measuring 3 mL and transferring it into a 10 mL volumetric flask, with the volume completed using chloroform. A simvastatin stock solution at 140 ppm was generated by weighing 5 mg of simvastatin and dissolving it in a 50 mL volumetric flask filled with chloroform. Furthermore, a series of test concentrations, specifically 0.625, 1.25, 2.5, 5, 10, and 20 mg/mL, were formulated; each concentration was placed into a 10 mL volumetric flask and subsequently adjusted to volume with chloroform. An aliquot of 2.5 mL from each concentration was transferred into a vial. Following this, 2.5 mL of a 100 ppm cholesterol solution was added, along with 2 mL of acetic anhydride and 0.1 mL of concentrated sulfuric acid, resulting in a colorimetric transformation to green, which was allowed to stand for 15 minutes. The blank solution was prepared by taking 2.5 mL of the cholesterol solution. To this, 2 mL of acetic anhydride and 0.1 mL of concentrated sulfuric acid were added, and then similarly allowed to stand for 15 minutes. The absorbance of the test solution was subsequently measured using a UV-Vis spectrophotometer at a wavelength of 420 nm (Musa *et al*., 2019). The absorbance values of the cholesterol standard solution, alongside the absorbance measurements of the sample acquired through a UV-Vis spectrophotometer, were employed to ascertain the percentage inhibition of cholesterol utilizing the following formula:

$$\text{Inhibition } (\%) = \left(\frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \right) \times 100\%$$

Determination of IC_{50} (inhibitor concentration 50%) value

The measurement of the IC_{50} value was conducted through linear regression analysis, elucidating the correlation between the percentage inhibition value (y) and the variation in concentration (x). This process yielded a linear equation represented as: $y = ax + b$. The derived linear regression equation was subsequently employed to ascertain the IC_{50} value of the sample by setting the y value to 50, thereby enabling the determination of the corresponding x value from the equation. The IC_{50} value states the concentration of the sample solution (isolated compound or simvastatin) to reduce cholesterol by 50% (Anggraini & Nabillah, 2018).

Identification of compounds using Gas Chromatography-Mass Spectrometry (GC-MS).

The identification of compounds via GC-MS was conducted by Hasan et al. (2019) with minor modifications. The analysis was performed on a fraction of *E. dulcis*. Initially, the sample was dissolved in DMSO to a volume of 20 µL. Subsequently, 10 µL of the sample was injected into the injection port at a temperature of 3000°C, with the interface also maintained at 3000°C, and the source temperature set at 250°C. The split ratio was configured at 153.0, utilizing a splitless mode injector. The gaseous or volatilized samples were transported by helium gas at a flow rate of 1 mL per minute through the GC column. The temperature during injection was established at 70°C, which was then incrementally raised by 10°C per minute until reaching a final temperature of 300°C. Upon separation, the components underwent ionization. The detector subsequently captured the resultant ion fragments, generating a fragmentation pattern. The mass fragments were compared against the WILEY and NIST standard reference data, as indicated by the Similarity Index (SI) percentage.

Statistical analysis

Data were analyzed using regression analysis, which relates cause and effect between one variable and another. Data obtained using a spectrophotometer in the form of absorbance values were analyzed using a linear regression equation (x,y) and IC_{50} to determine the relationship between variables in the form of cholesterol-lowering effects using the Microsoft Excel program and IBM SPSS Statistics 25, analyzed by Tukey HSD.

RESULTS AND DISCUSSION

Water content

The water content in water chestnut was measured prior to the extraction process. The objective of assessing the water content in water chestnut powder is to ascertain that the powder is in optimal condition and can be preserved for an extended duration. The water content of water chestnut powder was determined to be 6.44 ± 1.18%. This finding aligns with the literature, which stipulates that the water content in sample extracts should not exceed 10% (Zahara *et al*., 2024). A reduced water content in the sample powder diminishes the likelihood of fungal contamination. Water content is a critical parameter that influences the longevity of the extract and mitigates microbial activity during storage. Extracts with elevated water content are more susceptible to microbial proliferation. Conversely, low-water-content extracts exhibit greater stability during long-term storage compared to their high-water-content counterparts (Angelina *et al*., 2015).

Yield

The yield of the water chestnut extract was calculated to be 3.41 ± 0.44%. This yield contrasts with the findings of Baehaki *et al*. (2021), who conducted the extraction of water chestnut through maceration utilizing ethanol as a solvent, yielding 2.01 ± 0.22%. The yield is subject to many influencing factors, including extraction temperature, duration of the extraction process, and the circulation of solvents and simplex components. Excessively high temperatures and prolonged extraction durations can lead to the oxidation and subsequent degradation of compounds within the solution. Conversely, insufficient temperatures and abbreviated

extraction times may result in suboptimal extraction of bioactive constituents, yielding a diminished quantity of compounds (Nurfahmi *et al.*, 2018).

Flavonoid content

The results of the extraction and fractionation of column chromatography were analyzed for flavonoid levels qualitatively and quantitatively. The results of qualitative analysis through phytochemical tests of the extract and fraction of water chestnut can be seen in Table 1. Table 1 shows that the results of the qualitative flavonoid test indicate the formation of a yellow color. This finding aligns with research conducted by Puspasari & Puspita (2023), which revealed that the qualitative test of flavonoids on suruhan leaves produced color variations from yellow to reddish yellow. Adding 10% NaOH to the extract containing flavonoids functions as an alkaline solvent, which facilitates the release of flavonoids from other compounds contained in the water chestnut extract. The flavonoids then react with alkali, producing compounds that provide a distinctive color that can vary from bright yellow to reddish yellow, depending on the type and concentration of flavonoids contained in water chestnut.

The highest concentration of flavonoids was observed in the 15th eluent (an ethyl acetate: methanol fraction in a 1:1 ratio), measuring at 79.25 mg/mL (Table 1). The findings from the quantitative flavonoid analysis revealed a marked contrast when compared to the research conducted by Baehaki *et al.* (2021), which involved the extraction of water chestnut via the maceration method utilizing ethanol as a solvent, yielding a flavonoid content of 48.56 mg/mL. The implementation of column chromatography for the fractionation of water chestnut extract presents distinct advantages in achieving a more optimal and efficient separation. Column chromatography can regulate the elution rate and employ more suitable solvents for the segregation of flavonoids. In contrast, the reflux and maceration techniques often result in complex mixtures that hinder the separation process. In contrast, column chromatography remains unaffected by high temperatures throughout the separation process, thereby preserving the integrity of flavonoid compounds exceptionally well (Turatbekova *et al.*, 2023).

Anti-cholesterol activity

The anti-cholesterol activity assay was performed on extracts and fractions exhibiting the highest flavonoid concentrations, specifically the ethyl acetate: methanol (1:1) fraction, alongside simvastatin as a positive control. The percentage of inhibition and the IC50 value were subsequently calculated using the absorbance values obtained. Data illustrating the relationship between concentration and anti-cholesterol activity is depicted in Figure 1, while the IC_{50} value is detailed in Table 2.

Figure 1 shows that simvastatin has the most vigorous anti-cholesterol activity, followed by the rat purun fraction, and lastly, the rat purun extract. The water chestnut fraction showed an IC_{50} value of 42.22 ± 9.79 ppm, while simvastatin had an IC_{50} value of 12.41 ± 3.74 ppm (Table 1). Although the water chestnut fraction showed anti-cholesterol activity comparable to simvastatin, the water chestnut extract showed much

Table 1. Qualitative and quantitative analysis of flavonoid content from the extract and fractions of water chestnut.

Extract/fractions	Qualitative	Quantitative (mg/mL)
Reflux extract	+	45.24 ± 1.56
Hexane	-	0.59 ± 0.63
H:E (9:1)	-	1.13 ± 0.84
H:E (8:2)	-	4.48 ± 0.91
H:E (7:3)	-	6.19 ± 1.06
H:E (6:4)	+	13.95 ± 0.60
H:E (1:1)	+	15.98 ± 0.86
H:E (4:6)	+	17.43 ± 0.86
H:E (3:7)	+	22.38 ± 0.78
H:E (2:8)	+	34.88 ± 1.94
H:E (1:9)	+	40.44 ± 1.67
Ethyl acetate	+	48.83 ± 2.58
E:M (9:1)	+	58.11 ± 4.73
E:M (8:2)	++	76.45 ± 3.80
E:M (7:3)	++	77.88 ± 1.65
E:M (6:4)	++	78.10 ± 1.12
E:M (1:1)	++	79.25 ± 1.73
E:M (4:6)	++	78.46 ± 1.18
E:M (3:7)	++	69.89 ± 1.33
E:M (2:8)	++	65.14 ± 2.29
E:M (1:9)	++	64.31 ± 2.33
Methanol	++	61.27 ± 3.06

Description: H:E = Hexane: ethyl acetate ratio,
E:M = Ethyl acetate: Methanol ratio,
(+) = contain flavonoid,
(-) = does not contain flavonoid

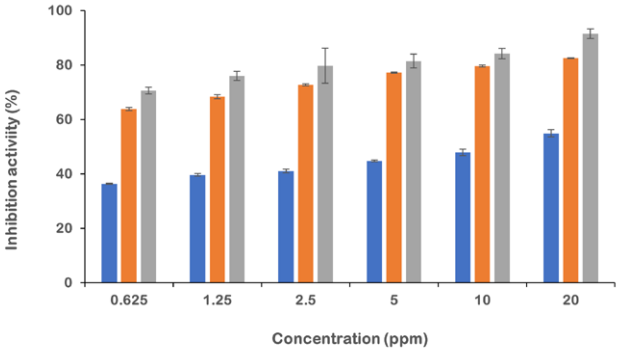

Figure 1. Effect of concentration of extract (■), ethyl acetate:methanol (1:1) fraction (■) of water chestnut and simvastatin (■) on anticholesterol activity.

lower activity (13016.27 ± 5648.31 ppm). The extract and fraction of water chestnut are thought to have anti-cholesterol activity due to their flavonoid content. This is in line with the concentration of flavonoids in each treatment; the higher the flavonoid content in an extract isolate, the higher the anti-cholesterol activity (Anggraini & Nabillah, 2018). The ethanol reflux method is practical, but it can reduce the bioactivity of thermolabile compounds. To increase the potency of fractions to equal

or exceed simvastatin, low temperature methods such as ultrasonic extraction, solvent modification, and further fractionation and purification should be used (Mehta *et al*., 2022; Cao *et al*., 2025).

Table 2. Anti-cholesterol activity (IC_{50}) of Simvastatin, water chesnut fraction, and water chestnut extract.

Sample	IC_{50} (ppm)
Simvastatin	12.41 ± 3.74[a]
Water chestnut fraction	42.22 ± 9.79[a]
Water chestnut extract	13,016.27 ± 5,648.31[b]

Note: Each value is expressed as mean ± SD in triplicate experiments. Values [a-b] with different letters indicate significant differences between treatments ($p<0.05$), analyzed using Tukey HSD.

Cholesterol reacts with concentrated sulfuric acid, yielding cholestadine sulfonic acid, which subsequently interacts with flavonoids via the hydroxyl group present in cholesterol. This intricate process facilitates the establishment of bonds with the substituent groups of flavonoids, thereby playing a significant role in the reduction of cholesterol levels. The mechanism by which flavonoids mitigate cholesterol can be elucidated through the interaction between the hydroxyl group of cholesterol and the ketone group of flavonoids, culminating in the formation of a hemiacetal. Moreover, a hydrogen bond transpires when flavonoids possess a carbonyl group associated with cholesterol at the hydroxyl group (Anggraini & Nabillah, 2018).

GC-MS analysis

The water chestnut fraction exhibiting the most pronounced anti-cholesterol activity was discerned through GC-MS analysis. The chromatogram of the water chestnut fraction is illustrated in Figure 2. A multitude of peaks indicates the presence of compounds that can be classified as flavonoids based on their chemical structures; however, numerous other compounds appear to be more closely associated with fatty acids, siloxanes, or various other organic substances. Fatty acid compounds were also identified in the GC-MS results, with several emerging as predominant constituents, including octadecanoic acid, 9-octadecenamide, and octadecanamide. The water chestnut compounds identified using GC-MS and their biological activity can be seen in Table 3.

The outcomes of the column chromatography analysis for the identification of water chestnut via GC-MS unveiled two constituents of flavonoid compounds, specifically 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione and 2H-1-Benzopyran-2-one, 7-methoxy-. The compound 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione is an organic entity categorized within the realm of flavonoid compounds, characterized by the molecular formula C17H2403 (Alraddadi *et al*., 2024). The compound 2H-1-Benzopyran-2-one, 7-methoxy- is a derivative of benzopyran—the fundamental structure of flavonoids—distinguished by the presence of a methoxy group at the seventh position. Flavonoids typically exhibit a foundational benzopyran structure, demonstrating variations in substitutions (such as methoxy or hydroxyl groups) on their benzene rings (Ganpatrao & Venkatesham, 2023). Biological activity for the compounds 1,4-Cyclohexanediol, (Z)-, TMS derivative, 3-Methylsalicylic acid, 2TMS

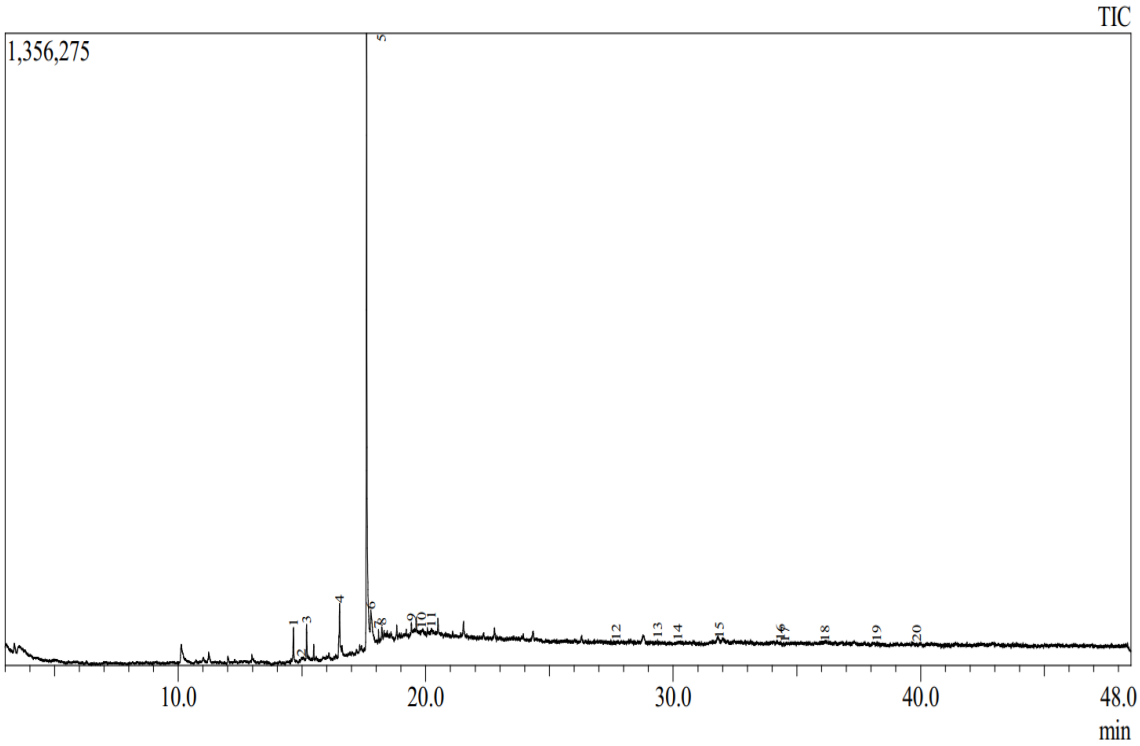

Figure 2. Chromatogram of the ethyl acetate: methanol (1:1) fraction.

Table 3. Compound identification from the ethyl:methanol (1:1) fraction of water chestnut and its biological activity.

Peak	RT	Compound name	Molecular formula	MW	Area (%)	Biological activity
1	14.655	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	3.70	Antimicrobial and antifungal (Alraddadi *et al.*, 2024), antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic, anti-inflammatory, antihypertensive, antihistamine, and antifungal (Singh *et al.*, 2024)
2	14.980	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.48	Anti-inflammatory, antiandrogenic, antioxidant, antibacterial, hypocholesterolemic, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, antipsychotic (Alraddadi *et al.*, 2024), antioxidant, anticholesterol, antiandrogenic, hemolytic, 5-alpha reductase inhibitor (Manoranjitha & Malarvizh, 2023)
3	15.185	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	3.18	Antioxidant and antimicrobial (Tavanappanavar *et al.*, 2024)
4	16.518	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	6.36	Anti-cholesterol (Manoranjitha & Malarvizh, 2023)
5	17.611	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	64.92	Anti-inflammatory and anti-cholesterol(Landeta *et al.*, 2024)
6	17.783	Octadecanamide	$C_{18}H_{37}NO$	283	9.46	Inhibition of α-amylase and α-glucosidase (Klomsakul & Chalopagorn, 2024)
7	18.095	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1.12	Antifungal, antibacterial, antioxidant and anticancer (Setyawati *et al.*, 2023)
8	18.225	1-Monopalmitin, 2TMS derivative	$C_{25}H_{54}O_4Si_2$	474	2.25	Antimicrobial and anticancer (Sonosy *et al.*, 2023)
9	19.421	1,4-Cyclohexanediol, (Z)-, TMS derivative	$C_9H_{20}O_2Si$	188	1.71	-
10	19.830	3-Methylsalicylic acid, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	296	0.90	-
11	20.225	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{36}O_4$	352	1.34	Antifungal (Hameed & Al-Muhsin, 2024), anti-inflammatory, hypocholesterolemic, anticancer, hepatoprotective, antihistamine, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary (Manoranjitha & Malarvizh, 2023)
12	30.194	3-(4-Hydroxy-3-methoxyphenyl)propionic acid, 2TMS derivate	$C_{16}H_{28}O_4Si_2$	340	0.51	Antidiabetes and anticancer (Ohue-Kitano *et al.*, 2023)

Table 3. (Continued)

Peak	RT	Compound name	Molecular formula	MW	Area (%)	Biological activity
13	29.380	3,4-Dimethoxycinnamic acid, TMS derivativ	$C_{14}H_{20}O_4Si$	280	0.50	Antioxidant, anti-inflammatory and anti-cholesterol (Theodosis *et al*., 2023)
14	30.194	2H-1-Benzopyran-2-one, 7-methoxy-	$C_{10}H_8O_3$	176	0.50	Antioxidant, anti-inflammatory and anti-cholesterol (Gupta *et al*., 2024)
15	31.865	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O_2$	222	0.61	Antihypertension (Wawrzyniak *et al*., 2024)
16	34.335	Quinoline, 4-chloro-6-methoxy-2-methyl-	$C_{11}H_{10}ClNO$	207	0.48	-
17	34.520	5-Aminosalicylic acid, O,O'-bis(trimethylsilyl)	$C_{13}H_{23}NO_3Si_2$	297	0.63	Anti-inflammatory (Kim *et al*., 2024)
18	36.135	2-Acetylthiophene, 5-(2-thienyl)-	$C_{10}H_8OS_2$	208	0.46	-
19	38.221	10,11-Dihydro-10-hydroxycarbamazepine, N-trimethylsilyl-, trimethylsilyl ether	$C_{21}H_{30}N_2O_2Si_2$	398	0.46	-
20	39.839	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, tris-(O-trimethylsilyl)-	$C_{18}H_{34}O_4Si_3$	398	0.45	-

derivative, Quinoline, 4-chloro-6-methoxy-2-methyl-, 2-Acetylthiophene, 5-(2-thienyl)-, 10,11-Dihydro-10-hydroxycarbamazepine, N-trimethylsilyl-, trimethylsilyl ether, and 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, tris-(O-trimethylsilyl)- in the water chestnut fraction are compounds whose activity has not been reported.

Several studies have elucidated that flavonoids possess considerable anti-cholesterol properties. Within the human body, flavonoids can dissolve cholesterol deposits adhering to the walls of coronary blood vessels. By mitigating cholesterol accumulation in these vessels, the risk of various cholesterol-related diseases, such as stroke, hypertension, and cardiovascular ailments, can be significantly reduced. Furthermore, flavonoids function as bioactive compounds that can diminish triglyceride levels (TGA) while augmenting High-Density Lipoprotein (HDL) concentrations. Additionally, flavonoids play a pivotal role in lowering blood cholesterol levels by inhibiting the activity of the enzyme 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG Co-A Reductase) (Ilyas *et al*., 2020). Flavonoid compounds can potentially reduce triglyceride levels by increasing Lipoprotein lipase activity (Safe *et al*., 2021). According to Tian *et al*. (2011), flavonoid compounds can also inhibit Fatty Acid Synthase (FAS). Inhibition of FAS directly can reduce fatty acid synthesis, thus having implications for reducing triglyceride formation (Musa *et al*., 2019).

Cinnamic acid compounds such as 3,4-Dimethoxy-cinnamic acid also appear in GC-MS results. Cinnamic acid can inhibit lipoprotein lipase, which is a key enzyme that breaks down triglycerides from chylomicrons and very low-density lipoproteins (VLDL) into free fatty acids that carry cholesterol in the blood. In addition, cinnamic acid can inhibit the activity of the HMG Co-A Reductase enzyme. By inhibiting LPL activity, cinnamic acid can prevent the breakdown of triglycerides into fatty acids and glycerol. Decreased LPL activity due to inhibition of cinnamic acid can increase the amount of LDL lipoproteins in the circulation. This can stimulate increased expression of LDL receptors in the liver, thereby increasing LDL uptake from the circulation and reducing total cholesterol levels (Theodosis *et al*., 2023).

In addition to the presence of cinnamic acid, the results of GC-MS analysis also identified the presence of polyunsaturated fatty acids. Polyunsaturated fatty acid compounds detected in rat purun, which have anti-cholesterol activity, include octadecanoic acid, 9-Octadecenamide, and 9,12,15-Octadecatrienoic acid. These fatty acids play a role in lowering LDL levels and increasing HDL levels (Manoranjitha & Malarvizh, 2023). In addition, polyunsaturated fatty acids also contribute to an increase in apolipoprotein A-1 (Apo A-1), a major component in transporting extrahepatic cholesterol back to the liver for excretion. Decreased regucalcin expression and increased Apo A-1 significantly reduce cholesterol levels in the blood circulation (Ahmed *et al*., 2022). Polyunsaturated fatty acids can decrease the expression of enzymes involved in the synthesis of cholesterol, triglycerides, and fatty acids. In addition, polyunsaturated fatty acids also increase the process of beta-oxidation of fatty acids, effectively reducing the amount of substrate available for triglyceride and VLDL synthesis. Enzymes such as phosphatidic acid phosphatase and diacylglycerol acyltransferase, which play a crucial role in triglyceride synthesis in the liver, may be produced in smaller amounts due to the influence of these fatty acids. Ultimately, fatty acids contribute to an increase in plasma lipoprotein lipase (LPL) concentrations, which accelerate the removal of

triglycerides from VLDL and chylomicrons in the systemic circulation (Backes *et al*., 2016).

CONCLUSIONS AND RECOMMENDATION

Conclusion

Water chestnut extract and fraction have anti-cholesterol activity with IC50 values of 13016.27 ± 5648.31 and 42.22 ± 9.79 ppm, respectively. The anti-cholesterol activity of water chestnut fraction is equivalent to sivastatin. Water chestnut compounds that act as anti-cholesterol include 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, n-Hexadecanoic acid, Octadecanoic acid, ethyl ester, 9-Octadecenamide, (Z)-, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, 3,4-Dimethoxycinnamic acid, and 2H-1-Benzopyran-2-one, 7-methoxy-.

Recommendation

Based on the results obtained from this study, further research is needed on the cytotoxicity test of the water chestnut and the activity of squalene synthase inhibitors.

AUTHORS' CONTRIBUTIONS

Each author contributed to the manuscript's analysis technique, English grammar check, and proofreading.

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REFERENCES

Ahmed, A.A., K.A. Balogun, K.A. Bykova & S.K. Cheema. 2014. Novel regulatory roles of omega-3 fatty acids in metabolic pathways: A proteomics approach. Nutrition and Metabolism. 11 (1). https://doi.org/10.1186/1743-7075-11-6.

Alagan, V., R. Valsala, & K. Rajesh. 2017. Bioactive chemical constituent analysis, in vitro antioxidant and antimicrobial activity of whole plant methanol extracts of *Ulva lactuca Linn*. British Journal of Pharmaceutical Research. 15 (1): 1–14. https://doi.org/10.9734/bjpr/2017/31818.

Alraddadi, T. M., S.O. Bahaffi, L.A. Alkhateeb, & M.W. Sadaka. 2024. Analysis of bioactive compounds present in *Boerhavia elegans* seeds by GC-MS. Open Chemistry. 22 (1). https://doi.org/10.1515/chem-2024-0068.

Angelina, M., P. Amelia, M. Irsyad, L. Meilawati & M. Hanafi. 2015. Karakterisasi ekstrak etanol herba katumpangan air (*Peperomia pellucida* L. Kunth) (characterization of ethanol extract from katumpangan air herbs (peperomia). Biopropal Industri. 6 (2): 53-61.

Anggraini, D.I & L.F. Nabillah. 2018. Activity test of suji leaf extract (*Dracaena angustifolia Roxb*.) on in vitro cholesterol lowering. Jurnal Kimia Sains dan Aplikasi. 21 (2): 54-58. https://doi.org/10.14710/jksa.21.2.54-58.

Bachheti, R.K., L.A. Worku, Y.H. Gonfa, M. Zebeaman, Deepti, D.P. Pandey & A. Bachheti. 2022. Prevention and treatment of cardiovascular diseases with plant phytochemicals: A review. Evidence-Based Complementary and Alternative Medicine. 2022: 5741198. https://doi.org/10.1155/2022/5741198.

Backes, J., D. Anzalone, D. Hilleman & J. Catini. 2016. The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids in Health and Disease. 15 (1): 1-12. https://doi.org/10.1186/s12944-016-0286-4

Baehaki, A., I. Widiastuti, S. Lestari, M. Masruro & H.A. Putra. 2021. Antidiabetic and anticancer activity of Chinese water chestnut (*Eleocharis dulcis*) extract with multistage extraction. Journal of Advanced Pharmaceutical Technology & Research.12 (1): 40-44. https://doi.org/10.4103/japtr.JAPTR_67_20

Cao, S., J. Liang, M. Chen, C. Xu, X. Wang, L. Qiu, X. Zhao & W. Hu. 2025. Comparative analysis of extraction technologies for plant extracts and absolutes. Frontiers in Chemistry. 13: 1536590. https://doi.org/10.3389/fchem.2025.1536590.

Ganpatrao, H.S & A. Venkatesham. 2023. Design and synthesis of benzopyrone compounds with potential medicinal applications. Anveshana's International Journal of Research in Pharmacy and Life Sciences. 9 (2): 176-182.

Gupta, R., A. K. Chaudhary, & R. Sharma. 2024. Analgesic and anti-inflammatory potential of *Ricinus communis L.nn*.: evidence from pharmacology to clinical studies. Current Pharmacology Reports. 10 (1): 27–67. https://doi.org/10.1007/s40495-023-00347-7.

Hameed, M. F & A. A. AL-Muhsin. 2024. Estimation of the minimum inhibitory concentration (MIC) of the ethanolic extract of *S. monoica* as an antifungal agent for *Candida albicans*. Journal of Pharmacognosy and Phytochemistry. 13 (3): 450-455. https://doi.org/10.22271/phyto.2024.v13.i3f.14986.

Hasan, M.M., M.R. Al Mahmud & M.G. Islam. 2019. GC-MS analysis of bio-active compounds in ethanol extract of *Putranjiva roxburghii* wall. fruit peel. Pharmacognosy Journal, 11(1), 146–149. https://doi.org/10.5530/pj.2019.1.24.

Ilyas, A.N., Rahmawati & H. Widiastuti. 2020. Uji aktivitas antikolesterol ekstrak etanol daun gedi (Abelmoschus *manihot (l.) medik)* secara in vitro. Jurnal Kesehatan. 3 (1): 57–64.

Kim, J., G. Ryu, J. Seo, M. Go, G. Kim, S. Yi, S. Kim, H. Lee, J. Y. Lee, H. S. Kim, M. C. Park, D. H. Shin, H. Shim, W. Kim & S. Y. Lee. 2024. 5-aminosalicylic acid suppresses osteoarthritis through the OSCAR-PPARγ axis. Nature Communications. 15 (1). https://doi.org/10.1038/s41467-024-45174-6.

Klomsakul, P & P. Chalopagorn. 2024. In vitro α-amylase and α-glucosidase inhibitory potential of green banana powder extracts. The Scientific World Journal. 2024 (1). https://doi.org/10.1155/2024/5515855.

Landeta, C., N. S. Wallach, J. Munizaga, M. P. G. Troncoso, C. B. Díaz, L. A. Caldas, P. Sartorelli, I. Martínez & M. E. Lienqueo. 2024. Comprehensive nutritional and functional characterization of novel mycoprotein derived from the bioconversion of Durvillaea spp. Foods. 13 (15). https://doi.org/10.3390/foods13152376.

Lee, J., J.T. Jayakody, J. Kim, J. Jeong, K. Choi, T. Kim, C. Seo, I. Azimi, J. Hyun, & B. Ryu. 2023. The Influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. Foods. 13 (19): 3151. https://doi.org/10.3390/foods13193151.

Manoranjitha, M., & A. Malarvizhi. 2023. Gas chromatography and mass spectroscopic analysis of bioactive compounds from *Scoparia dulcis* whole plant extract. International Journal of Zoological Investigations. 9 (3): 76-82. https://doi.org/10.33745/ijzi.2023.v09ispl3.011.

Mehta, N., S. Jeyapriya, P. Kumar, A. K. Verma, P. Umaraw, S. K. Khatkar, A. B. Khatkar, D. Pathak, U. Kaka, & A. Q. Sazili. 2022.

Ultrasound-assisted extraction and the encapsulation of bioactive components for food applications. Foods. 11 (19): 2973. https://doi.org/10.3390/foods11192973

Musa, W.J.A., B. Situmeang & J. Sianturi. 2019. Anti-cholesterol triterpenoid acids from *Saurauia vulcani Korth*. (Actinidiaceae). International Journal of Food Properties. 22 (1): 1439-1444. https://doi.org/10.1080/10942912.2019.1650762

Nurfahmi, A.R., A. Husni & A. Isnansetyo. 2018. Effect of *Sargassum hystrix* powder on the biochemical profile of diabetic wistar rats. Pakistan Journal of Nutrition. 17 (5): 248–254. https://doi.org/10.3923/pjn.2018.248.254

Nursamsiar, N., M. Marwati & S. Nur. 2023. The effect of extraction method on flavonoid content and antioxidant activity of red betel and green betel extracts. Tropical Journal of Natural Product Research. 7 (8): 36203625. https://doi.org/10.26538/tjnpr/v7i8.7

Ohue-Kitano, R., Y. Masujima, S. Nishikawa, M. Iwasa, Y. Nishitani, H. Kawakami, H. Kuwahara & I. Kimura. 2023. 3-(4-Hydroxy-3-methoxyphenyl) propionic acid contributes to improved hepatic lipid metabolism via GPR41. Scientific Reports. 13 (1). https://doi.org/10.1038/s41598-023-48525-3

Puspasari, H & W. Puspita. 2023. Identifikasi senyawa flavonoid ekstrak kental etanol daun suruhan (*Peperomia pellucida* L. Kunth) dengan metode kromatografi kolom dan kromatografi lapis tipis. Jurnal Komunitas Farmasi Nasional. 3 (2): 573-581.

Qomariyah, M.E & S. Mursiti. 2019. Isolasi dan identifikasi senyawa flavonoid dari daun murbei (*Morus alba* Linn). Indonesian Journal of Chemical Science. 8 (1).: 16-22.

Safe, S., A. Jayaraman, R.S. Chapkin, M. Howard, K. Mohankumar & R. Shrestha. 2021. Flavonoids: structure–function and mechanisms of action and opportunities for drug development. Toxicological Research. 37 (2): 147-162. https://doi.org/10.1007/s43188-020-00080-z

Setyawati, A., E. Purwanto, A. Yunus, A.T. Sakya, M. Rahayu, D. Purnomo, R.B. Arniputri, G. Cahyo, Q.R. Dwiyanti, S. Egra, O. Talitha & A. Muthi. 2023. Effect of water availability on the growth and secondary metabolites of zodia (*Evodia suaveolens*). Agrosains : Jurnal Penelitian Agronomi. 25 (2): 78-84. https://doi.org/10.20961/agsjpa.v25i2.81528

Singh, K., K. Kishor, G. Kothiyal, R. Kamal, N. Kunar, S. Guru & R. Rai. 2024. GC-MS analysis for determination of bioactive compounds of *Phellinus pectinatus*: A species of wild mushroom from Uttarakhand Himalaya, India. Journal of Chemical Health Risks. 14 (3): 2005-2013. https://www.jchr.org/index.php/JCHR/article/view/4860

Sonosy, M., F. Abo-Elabbas & W. Elsayed. 2023. T34 biocontrol ® (*Trichoderma asperellum strain, t34*), a biocontrol agent reducing strawberry fruit rots. Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control. 15 (2): 121–140. https://doi.org/10.21608/eajbsf.2023.341967

Tang, Z., G. Huang & H. Huang. 2024. Ultrasonic-assisted extraction, analysis and properties of purple mangosteen scarfskin polysaccharide and its acetylated derivative. Ultrasonics Sonochemistry. 109. https://doi.org/10.1016/j.ultsonch.2024.107010

Tavanappanavar, A.N., S.I. Mulla, C.S. Seth, Z.K. Bagewadi, M. Rahamathulla, M.M. Ahmed, & S.A. Farhana. 2024. Phytochemical analysis, GC–MS profile and determination of antibacterial, antifungal, anti-inflammatory, antioxidant activities of peel and seeds extracts (chloroform and ethyl acetate) of *Tamarindus indica L*. Saudi Journal of Biological Sciences. 31 (1):1-9. https://doi.org/10.1016/j.sjbs.2023.103878

Theodosis, P., G. Papagiouvannis & E.A. Rekka. 2023. Ferulic, sinapic, 3,4-dimethoxycinnamic acid and indomethacin derivatives with antioxidant, anti-inflammatory and hypolipidemic functionality. Antioxidants. 12 (7): 1436 https://doi.org/10.3390/antiox12071436

Turatbekova, A., D. Mirzarakhmetova, J. Jumaniyozov, E. Khudayberganov, N. Toshpulatov, A. Rakhmatov & S. Muzafarov. 2023. A brief overview on the methods for extraction and identification of flavonoids. E3S Web of Conferences. 434. https://doi.org/10.1051/e3sconf/202343403037

Wawrzyniak, R., P. Grešner, E. Lewicka, S. Macioszek, A. Furga, B. Zieba, M. J. Markuszewski, & A. Dąbrowska-Kugacka. 2024. Metabolomics meets clinics: a multivariate analysis of plasma and urine metabolic signatures in pulmonary arterial hypertension. Journal of Proteome Research. 23 (8): 2795–2804. https://doi.org/10.1021/acs.jproteome.3c00255

Yang, X., Y. Gu, H. Liu, F. Shang & T. Koyama. 2024. Modulation of the gut microbiota alleviates insulin resistance in type 2 diabetic mice by daucosterol from Eleocharis dulcis peel. Journal of Functional Foods. 114: 106092. https://doi.org/10.1016/j.jff.2024.106092

Yulita, Y.Y., W. Winardi, & J. Jumiati. 2022. Remediasi air tercemar merkuri menggunakan purun tikus (*Eleocharis dulcis*) metode lahan basah buatan. Jurnal Reka Lingkungan, 10 (3): 212-221. https://doi.org/10.26760/rekalingkungan.v10i3.212-221

Zahara, E., Darmawi, U. Balqis, & C. Soraya. 2024. Characterization of candlenut leaf extract Simplicia (*Auleurites moluccanus*) Seulawah mountains. IOP Conference Series: Earth and Environmental Science. 1356 (1). https://doi.org/10.1088/1755-1315/1356/1/012077

Zhan, G., L. Pan, K. Tu & S. Jiao. 2016. Antitumor, antioxidant, and nitrite scavenging effects of Chinese water chestnut (*Eleocharis dulcis*) peel flavonoids. Journal of Food Science, 81 (10): H2578-H2586. https://doi.org/10.1111/1750-3841.13434

Zhang, QW., L.G. Lin & W.C. Ye. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 13: 20. https://doi.org/10.1186/s13020-018-0177-x

Zhang, Y., H. Xu, Z. Hu, G. Yang, X. Yu, Q. Chen, L. Zheng & Z. Yan. 2022. *Eleocharis dulcis* corm: Phytochemicals, health benefits, processing and food products. Journal of the Science of Food and Agriculture. 102 (1): 19-40. https://doi.org/10.1002/jsfa.11508

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