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TOTAL PHENOLIC, TANNIN AND FLAVONOID CONTENTS OF *ARTOCARPUS ALTILIS* EXTRACTS

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ABSTRACT

The use of medicinal plants plays a vital role to maintain the human health. Due to the high demand for traditional medicines, there is a need for continuous research on medicinal plant for their therapeutic effects. Therefore, to investigate the phytochemicals present in the medicinal plants that are responsible for potential pharmacological action is necessary. *Artocarpus altilis* (family: Moraceae) has been traditionally used by the people in Indonesia to treat various human ailments. The aim of the present study was to determine the total phenolic, tannin and flavonoid contents in *A. altilis* leaf and fruit extracts. Ethanol and water extracts of *A. altilis* were prepared by cold maceration method and the extracts were subjected to preliminary phytochemical screening. The phytoconstituents such as carbohydrates and glycosides, proteins and free amino acids, phenolic compounds and tannins, flavonoids, volatile oils and terpenoids were found to be present in the extracts. The total tannin content, phenolic content and flavonoid content were determined by colorimetric method. The results showed that the total phenolic content in *A. altilis* leaf and fruit extracts ranged from 1.284 to 9.245 (mg Gallic acid equivalents / g dry crude extract) where the total tannin content ranged from 0.239 to 4.499 (mg Gallic acid equivalents / g dry crude extract) and total flavonoid content was ranged from 0.143 to 10.828 (mg Quercetin equivalents / g dry crude extract). The quantitative estimation of this study is a key reference to isolate the phytoconstituents for developing the novel herbal drugs and their standardization.

KEYWORDS

Artocarpus altilis, Total tannin, Total phenolic and Total flavonoids.

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INTRODUCTION

The use of medicinal plants as a remedy for human and animal diseases has been practiced for centuries. Since there are many scientific investigation of conventional remedies as an alternative medicine, there is a high demand for traditional medicines[1]. Phytochemicals are natural bioactive compounds found in plants which protects from various diseases[2]. Phytochemicals consists of primary and secondary metabolites. Primary metabolites comprised of carbohydrates, proteins

and amino acids while, secondary metabolites consist of anthraquinones, terpenoids, sterols, alkaloids, flavonoids and phenolic compounds and tannins[3]. Thus, it is essential to carry out the quantitative analysis on secondary metabolites to investigate the amount of phytochemicals present in plants that are responsible for their therapeutic use.

The plant genus, Artocarpus species (family: Moraceae) such as *A. heterophyllus* (jackfruit), *A. altilis* (breadfruit), *A. hirsutus* (wild jack), *A. lakoocha and A. camansi* (bread nut) are used as edible and conventional medicine. There are many scientific investigations on the phytoconstituents present in these genus due to their numerous health benefits to humans[4]. The plant native is New Guinea, Moluccas (Indonesia) and Philippines. *A. altilis* is currently cultivated in central and south America, Africa, India, Southeast Asia, Maldives, Indonesia, Srilanka and northern Australia[5]. Many parts of *A. altilis* such as leaves, fruits, roots and sap has been used as medicines. This plant has been used as a traditional medicine for the treatment of various diseases by people in Indonesia[6].

Leaf and stem bark extract of *A. altilis* showed the presence of phytosterols, anthraquinones, terpenoids, phenols, glycosides, flavonoids and diterpenes[7], while leaf extract has β-sitosterol, unsaturated triglycerides, squalene, polyprenol, lutein, unsaturated fatty acids[8], steroids, phytosterols, gums and resins[9]. *A. altilis* has been reported for the presence of flavonoids, stilbenoids, arylbenzofurons, jacalin (lectin)[10], amino acid, fatty acid and carbohydrate[11]. Potassium, calcium and vitamins such as vitamin C, thiamine, and pyridoxine has also been reported in breadfruit[12]. Since there is no scientific evaluation for the total phenolic, tannin and flavonoid contents in *A.altilis*, the present study was aimed to screen the phytoconstituents present in leaf and fruit of *A. altilis* qualitatively and quantitatively.

MATERIAL AND METHODS

Plant Material

The leaves and fruits of *Artocarpus altilis* were collected from Nilai, Negeri Sembilan, Malaysia on October 2017. The plant authentication was done by Miss. Rosni Ludin, Faculty of Forestry, University Putra Malaysia, Selangor, Malaysia (Ref No.: UPMFH/ Herbarium/ 08/ 17).

Preparation of Extracts

A. altilis fresh leaves and fruits were separated, washed and shade dried. Each part of the plant was coarsely powdered and uniformly divided into two portions and macerated separately with ethanol and water using cold maceration method for 7 days. Whatman filter paper was used to filter the extracts and the extracts were concentrated using rotary vacuum evaporator under reduced pressure[7]. The dry weight of the concentrated extracts were calculate to determine the extraction efficiency. The colour, consistency, and the percentage yield of the extracts are tabulated in Table No.1. The extracts were kept in refrigerator until further use[13].

Preliminary Phytochemical Screening of Various Extracts

Preliminary phytochemical screening of *A. altilis* leaf and fruit extracts was carried out using chemical tests as per the standard procedure[14] to identify the presence of secondary metabolites such as alkaloids, carbohydrates and glycosides, proteins and amino acids, phenolic compounds and tannins, flavonoids, volatile oils, terpenoids, fixed oils and fats, sterols, gums and mucilage. The results are recorded in Table No.2.

Quantitative Phytochemical Screening

Quantitative phytochemical screening was carried out to estimate the total phenolic content, total tannin content and total flavonoid content using UV-visible spectrophotometer.

Determination of total phenolic content

Folin-Ciocalteau reagent method was used to determine the total phenolic content of the extracts of *A. altilis*[15]. 0.2 mL of the diluted dried extract was added with 1.0 mL of Folin-Ciocalteau reagent (0.5 N) and incubated at room temperature for 15 min. To this content, 0.8 mL of 7.5% sodium carbonate was added and the volume was made upto 10mL using distilled water. The incubation was further continued at room temperature for 30 min. The absorbance was measured at 743 nm using a digital

UV-visible spectrophotometer against a blank. Gallic acid (mg/ 100mL) was used as a standard. The concentration of phenolic content in dried extract was determined from a standard curve of gallic acid ranging from 20 µg to 100 µg/L. The total phenolic content was expressed in terms of standard equivalent (mg/ g of the dried extract).

Determination of total tannin content

The tannin content in the extracts of *A. altilis* was determined by Folin- Ciocalteu method[16]. About 0.1mL of the sample was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35 % Na_2CO_3 solution. The volume was made up to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described above. Absorbance for test and standard solutions were read against the blank at 725 nm using digital UV-visible spectrophotometer. The tannin content was expressed in terms of mg of Gallic acid equivalent/ g of dried extract.

Determination of total flavonoid content

Aluminium chloride colorimetric method was used to determine the total amount of flavonoid content in the extracts of *A. altilis*[16]. 1.0 mL sample (1 mg/mL) of the extract was added into a volumetric flask (10 mL) containing 1.0 mL methanol, 0.5 mL aluminium chloride (1.2 %) and 0.5 mL potassium acetate (120 mM). The volume was made up to 10 mL using distilled water. The mixture was shaken well and incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a digital UV-visible spectrophotometer, against the blank sample. The calibration curve was made by preparing quercetin (5 to 60 µg/mL) solution in methanol. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of dried extract).

RESULTS

The colour, consistency, and percentage yield of *Artocarpus altilis* extracts are tabulated in Table No.1. Water extract of leaf was non-sticky and semisolid in nature while, ethanol extract of leaf and both water and ethanol extracts of fruit were sticky and semisolid in nature. Water extract of leaf had highest percentage yield (16.42%) whereas the ethanol extract of fruit had lowest percentage yield (5.68 %).

The possible phytochemical constituents present in the leaves and fruits of *A. altilis* extracts are illustrated in Table No.2. Carbohydrates and glycosides, proteins and amino acids, phenolic compounds and tannins, flavonoids and terpenoids were present in all four extracts while alkaloids, sterols, fixed oils and fats, gums and mucilage were absent in the extracts.

Quantitative phytochemical screening of the ethanol and water extracts of *A. altilis* was carried out using UV-visible spectrophotometer to determine the total phenolic content, total tannin content and total flavonoid content and the results are illustrated in Table No.3.

Total phenolic content (TPC) of the extracts of *A. altilis* was determined according to the Folin-Ciocalteu method using gallic acid as the standard. The calibration curve was constructed and TPC was calculated using the standard curve equation, $y = 0.0071x + 1.0464$ ($R^2 = 0.9187$). Table No.3 shows the total phenolic content in extracts of *A. altilis* leaf and fruit. The high amount of TPC (gallic acid equivalents, mg/g of dried extract) was found in water extract of leaf (9.245mg) followed by ethanol extract of fruit (8.879mg) and ethanol extract of leaf (4.318mg) while lowest amount of TPC was found in water extract of fruit (1.284mg).

The total tannin content (TTC) in extracts of *A. altilis* was determined by Folin- Ciocalteu method in terms of Gallic acid equivalent (GAE) in mg/g of the dried extract. The total tannin content was calculated using standard curve equation, $y = 0.0388x + 0.3562$, where $R^2 = 0.9694$. As shown in Table No.3, values for TTC ranged from 0.239 to 4.499 mg/g GAE of dried extract. The highest TTC was present in ethanol extract of leaf (4.499 mg/g) followed by ethanol extract of fruit (1.798 mg/g) and water extract of fruit (0.5134 gm/g). While, the

lowest TTC was observed in water extract of leaf which was justified by 0.2392 mg/g.

The total flavonoid content (TFC) of the extracts of *A. altilis* was determined according to aluminium chloride colorimetric method using quercetin as standard. The TFC was calculated with the help of standard curve equation, $y = 0.0254x + 0.0638$ ($R^2 = 0.9935$). Table No.3 showed the TFC that was ranged from 0.143 to 10.828 mg/g (quercetin equivalents, mg/g of dried extract). The highest TFC was observed in ethanol extract of leaf (10.828 mg/g) followed by ethanol extract of fruit (6.311 mg/g) and water extract of leaf (0.623 gm/g) while the water extract of fruit showed the lowest TTC (0.1425 mg/g).

DISCUSSION

A. altilis leaf and fruit extracts yield was ranged from 5.68% to 16.42% (Table No.1). The water extracts of *A. altilis* marked highest percentage yield than ethanol extracts. This may be due to the secondary metabolites that are high polar in nature are greatly extracted from leaf and fruits in polar solvent[17]. Based on the results of preliminary phytochemical studies (Table No.2), the phytochemicals present in *A. altilis* leaf and fruit extracts are carbohydrates and glycosides, proteins and free amino acids, phenolic compounds and tannins, flavonoids, volatile oils and terpenoids.

Polyphenols such as phenolic compounds, tannins and flavonoids possess numerous therapeutic properties. These polyphenols have been identified qualitatively in the extracts of *A. altilis* and it may also be the responsible for the beneficial medicinal uses of the plant. Therefore, total phenolic content, total tannin content and total flavonoid content are quantitatively estimated in leaf and fruit extracts of *A. Altilis* (Table No.3). High concentrations of total tannin content, total phenolic content and total flavonoid content were found in ethanol extract than water extract of *A. altilis* which may be due to the ability of ethanol as a good solvent for extraction of secondary metabolites, mainly polyphenols[18]. Moreover the findings of the study shows that the highest concentration of total tannin content, total phenolic content and total flavonoid content were present in *A. altilis* leaves. Thus it may be suggested that the polyphenols are greatly present in leaves than fruits of this plant.

Polyphenols such as phenol and tannin present in plant extracts reacts with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by UV- visible spectrophotometer. In this reaction, the phenolate ion is oxidized and phosphotungstic-phosphomolybdic compounds are reduced[19]. This forms a blue chromophore composed of phosphotungstic- phosphomolybdenum complex, where the concentration of phenolic compounds and alkaline solution determines the maximum absorption of the chromophores[20]. Flavonoids were determined by Aluminium chloride ($AlCl_3$) reagent which forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids[21].

The consumption of fruits and vegetables that have phenolic compounds, tannins and flavonoids provides several health benefits to the humans due to their medicinal properties. It is evident from earlier studies that phenolic compounds, tannins and flavonoids extracted out from the medicinal plants possess antioxidant property[22]. In addition, tannins are shown to possess protective properties which are generally associated to their ability to bind with proteins. Tannins also exhibit other biological effects such as accelerating blood clotting, reduce blood pressure and decrease cholesterol level[23]. Medicinal plants containing high phenolic content have been commonly used as anthelmintics, antimicrobials and anti-inflammatory agents[24]. Whereas, flavonoids exhibit anticancer property due to their ability as a free radical scavenger (ROS)[25]. Hence, it is evidenced that polyphenols such as phenolic compounds, tannins and flavonoids play a vital role for the treatment of human diseases.

Table No.1: Colour, consistency and % yield of *Artocarpus altilis* extracts

S.No	Extracts	Colour	Consistency	% yield
1	Ethanol extract of Leaf	Reddish brown	Sticky semisolid	5.82
2	Water extract of Leaf	Reddish dark brown	Non-sticky semisolid	16.42
3	Ethanol extract of Fruit	Pale green	Sticky semisolid	5.68
4	Water extract of Fruit	Brownish yellow	Sticky semisolid	9.57

Table No.2: Preliminary phytoconstituent screening of *Artocarpus altilis* extracts

S.No	Phytoconstituents	Leaf		Fruit	
		Ethanol extract	Water extract	Ethanol extract	Water extract
1	Alkaloids	-	-	-	-
2	Carbohydrates and glycosides	+	+	+	+
3	Proteins and amino acids	+	+	+	+
4	Fixed oils and fats	-	-	-	-
5	Phenolic compounds and tannins	+	+	+	+
6	Flavonoids	+	+	+	+
7	Volatile oil	+	+	-	-
8	Terpenoids	+	+	+	+
9	Sterols	-	-	-	-
10	Gums and mucilages	-	-	-	-

+ denotes the presence; - denotes the absence

Table No.3: Quantitative phytochemicals of *Artocarpus altilis* extracts

S.No	Extracts	Total Phenolic content (mg/gm of dried extract)	Total Tannin content (mg/gm of dried extract)	Total Flavonoid content (mg/gm of dried extract)
1	Ethanol extract of Leaf	4.318	4.499	10.828
2	Water extract of Leaf	9.245	0.239	0.623
3	Ethanol extract of Fruit	8.879	1.798	6.331
4	Water extract of Fruit	1.284	0.513	0.143

CONCLUSION

The present study investigated the total phenolic content, total tannin content, and total flavonoid content present in *A. altilis* leaf and fruit. It may be a good resource to isolate these phytochemicals such as phenolic compounds, tannins, and flavonoids that may have many proclaimed pharmacological activities and may be prospective used as an alternative medicine for the treatment of numerous diseases. The findings indeed necessitates further research on isolation of phytoconstituents to develop the novel herbal drugs and their standardization.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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