Original article

Chemical composition and antioxidant activity of *Alphonsea monogyna*

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Abstract

Background: *Alphonsea monogyna* has been recorded in Vietnam and studied for its alkaloid constituents, essential oil composition and antibacterial activity. However, there is limited research on its other chemical constituents and antioxidant properties. This study aimed to determine the total phenolic, total flavonoid and total alkaloid contents, along with antioxidant activity of *A. monogyna*. **Materials and methods**: Whole plants of *A. monogyna* were collected in Hue City. The total phenolic and total flavonoid contents were determined using the Folin-Ciocalteu and $AlCl_3$ methods, respectively. The total alkaloid content was determined by the gravimetric method. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays were used to evaluate antioxidant activity. **Results:** The ethanol extract from the branches of *A. monogyna* exhibited a notable total phenolic content of 102.68 ± 1.25 mg GAE/g extract. The flavonoid content was found to be 2.13 ± 0.06 mg QE/g extract in the leaves and 1.29 ± 0.04 mg QE/g extract in the branches. The total alkaloid content in the leaves and branches of *A. monogyna* was 0.70 ± 0.02% and 0.28 ± 0.02%, respectively. In both DPPH and ABTS assays, the branch extract showed stronger antioxidant activity than the leaf extract, with IC_{50} values of 45.62 ± 0.64 µg/mL and 8.37 ± 0.28 µg/mL, respectively. In comparison, the positive control, ascorbic acid, demonstrated significantly lower IC_{50} values of 2.69 ± 0.09 µg/mL for DPPH and 1.27 ± 0.08 µg/mL for ABTS. **Conclusion**: This study provided valuable insights into the chemical composition of *Alphonsea monogyna*, detailing the total phenolic, flavonoid, and alkaloid contents, as well as its antioxidant activity.

Keywords: *Alphonsea monogyna, phenolic, alkaloid, DPPH, ABTS.*

1. BACKGROUND

Oxidation process occurs naturally in the body. However, when imbalanced, it can promote the formation of free radicals - molecules capable of causing cellular damage and contributing to aging and are linked to diseases like cancer, Alzheimer's, cataracts and heart disease [1]. Medicinal plants and wild herbs with significant antioxidant potential are rich in natural antioxidants, which can be classified into three main groups: vitamins, carotenoids and phenolic compounds [2]. Studying plant species rich in bioactive compounds not only opens potential applications in pharmaceuticals and functional foods but also plays an important role in the conservation and development of valuable natural medicinal resources.

The genus *Alphonsea* belongs to the Annonaceae family, comprising about 30 species distributed in China and Indo-Malayan region [3]. Preliminary studies indicated that this genus contained essential oils [4], alkaloids [4], and flavonoids [4 - 6], with notable biological activities such as antioxidant [4, 7 - 9], cytotoxic [4], antifungal and antibacterial properties [4, 5] and anti-inflammatory [4, 6]. These properties highlight its potential for further research and applications in various therapeutic fields.

In Vietnam, seven species belonging to the genus *Alphonsea* have been recorded, including *A. boniana*, *A. tonkinensis*, *A. gaudichaudiana*, *A. squamosa*, *A. monogyna*, *A. philastreana* and *A. sonlaensis* [10]. Among these species, *A. monogyna* has been studied for its alkaloids [4], essential oil composition and antibacterial activity [11]. A literature review shows that studies on the total phenolic, flavonoid and alkaloid contents and antioxidant activity of *A. monogyna* are still relatively limited. Therefore, this study aimed to quantify these bioactive compounds, as well as to evaluate the antioxidant activity of *Alphonsea monogyna*.

2. MATERIALS AND METHODS

2.1. Materials

The leaves and branches of *Alphonsea monogyna* belong to the Annonaceae family and were collected in Hue city, in November 2024. The scientific

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identification of the species was confirmed by Dr. Anh Tuan Le (Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, VAST, Vietnam). The collected medicinal materials were cleaned, damaged parts were removed, then dried at 55 °C, ground into powder and sieved through a 0.18 mm mesh to ensure uniform particle size. The powdered material was stored in a dry and ventilated place. Some images of *A. monogyna* were shown in Figure 1.

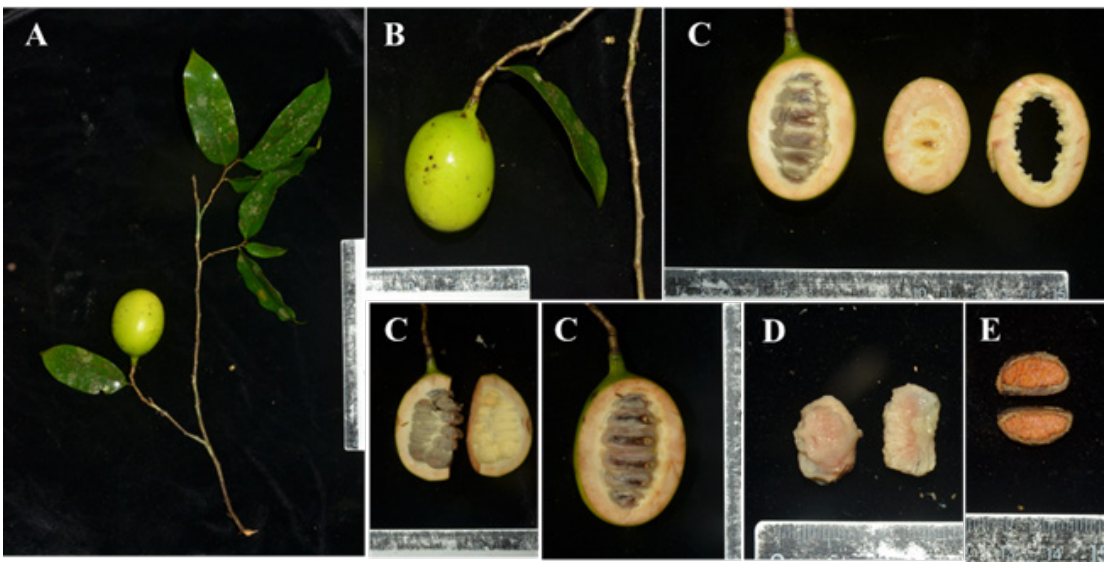


Figure 1. Some of the pictures of *Alphonsea monogyna*
A: Branches, leaves, fruit; B, C: Fruit; D, E: Seeds

2.2. Methods

2.2.1. Preparation of the crude extract

The powder of dried leaves and branches was weighed 10 g each sample, then soaked at room temperature with ethanol solvent (100 mL x 24 hours x 3 times), occasionally shaking and stirring. The extract was filtered through cotton, then concentrated by rotary evaporation (Buchi R-100, Switzerland) to recover the solvent under reduced pressure until obtaining the crude ethanol extracts.

2.2.2. Qualitative identification of phenolics, flavonoids and alkaloids

The ethanol extracts were preliminarily analyzed for the presence of phenolic, flavonoid and alkaloid groups using characteristic chemical reactions [12].

- Phenolic identification: About 20 mg of extract was weighed, 10 mL of distilled water was added, boiled and filtered through cotton. 1 mL of the filtrate was placed in each test tube, then a few drops of 5% $FeCl_3$ solution were added. The appearance of greenish-black, dark green or green precipitate indicated the presence of phenolic compounds.

- Flavonoid identification (Shinoda test): About 20 mg of extract was weighed, 10 mL of ethanol was added, heated and filtered through cotton. A small amount of metallic magnesium and a few drops of concentrated HCl were added. The appearance of pink, orange, or red turning to purple color indicated the presence of flavonoids.

- Alkaloid identification: About 20 mg of extract was weighed, 10 mL of 1% HCl solution was added, mixed well and filtered through cotton. Into each test tube, 1 mL of the filtrate was added followed by Dragendorff's, Mayer's and Bouchardat's reagents, respectively. The appearance of orange-red, creamy white and brown precipitates corresponding to each reagent indicated the presence of alkaloids.

2.2.3. Determination of phenolic and flavonoid contents

2.2.3.1. Determination of total phenolic content

Total phenolic content was measured using a modified Folin-Ciocalteu assay [13]. Briefly, 0.2 mL of sample was mixed with 0.8 mL water and 1.0 mL of 10% Folin-Ciocalteu reagent, shaken for 5 minutes, then 2.5 mL of 7.5% sodium carbonate was added. After 30 minutes incubation in the dark at room temperature, absorbance was read at 760 nm. Results were expressed as mg gallic acid equivalents per gram of extract based on a gallic acid calibration curve, according to the following formula:

$$\mathbf{TPC = (C1 \times V \times k)/m}$$

where TPC: the total phenolic content expressed in mg/g (as gallic acid equivalents, GAE), C1: the concentration of gallic acid obtained from the

calibration curve (mg/mL), V: the volume of the extract (mL), k: the dilution factor, m: the mass of the plant extract (g).

2.2.3.2. Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay. In brief, 2.0 mL samples were mixed with 2.0 mL of 2% $AlCl_3$ and incubated at room temperature for 10 minutes. Absorbance was measured at 440 nm [13]. Quercetin served as the standard and results were expressed as mg quercetin equivalents per gram of extract and based on the formula below:

TFC = (C1 x V x k)/m

where TFC: the total flavonoid content expressed in mg/g (as quercetin equivalents, QE), C1: the concentration of quercetin obtained from the calibration curve (mg/mL), V: the volume of the extract (mL), k: the dilution factor and m is the mass of the plant extract (g).

2.2.3.3. Determination of total alkaloid content

5 g of branches and leaves powder of the medicinal material was accurately weighed into a 100 mL conical flask with a ground-glass stopper, then the material was moistened with concentrated ammonia for 2 hours. The alkalized material was soaked with $CHCl_3$ solvent (50 mL x 12 hours x 3 times) at room temperature, occasionally stirring. The extract was filtered through cotton and the solvent was evaporated until a residue is obtained. The residue was dissolved with 2% HCl solution (10 mL x 3 times). The acid solution was filtered and washed with petroleum ether (5 mL x 3 times). The extract solution was alkalized with concentrated ammonia to pH = 9-10, then shaken with $CHCl_3$ (10 mL x 3 times). The $CHCl_3$ extracts were combined and filtered through cotton, then evaporated on a water bath to obtain residue, dried at 60 °C until a constant weight was achieved and weighed [14]. The total alkaloid content was calculated using the following formula:

% alkaloid = [(a-b) x 100/m(100-c)] x 100

where a: the weight of flask and alkaloid residue (g), b: the weight of flask (g), m: the weight of powdered medicinal material (g), c: the moisture percentage of the material.

2.2.4. Evaluation of antioxidant activity

2.2.4.1. DPPH assay

Antioxidant activity was assessed using a modified DPPH assay by mixing 2 mL of 0.135 mM DPPH solution with 2 mL of sample at various concentrations and incubating for 30 minutes at room temperature. Absorbance was measured at 517 nm [13]. Ascorbic acid was used as the standard, while methanol was used as the blank. DPPH free radical scavenging activity was calculated using the following formula:

DPPH scavenging capacity = [($A_{control}$ – A_{sample})/ $A_{control}$] x 100

where $A_{control}$: the absorbance of DPPH solution and methanol, A_{sample}: the absorbance of DPPH solution containing the tested samples.

The IC_{50} value is the concentration of extract required to scavenge 50% of DPPH radicals, was calculated using Microsoft Excel.

2.2.4.2. ABTS assay

The ABTS assay based on Arnao et al. was used to evaluate antioxidant activity with slight modifications. Equal volumes of 7 mM ABTS and 2.4 mM potassium persulfate were mixed and incubated in the dark for 14 hours. The solution was diluted with methanol to an absorbance of 0.82 ± 0.01 at 744 nm. The reaction mixture consisted of 2 mL of extract and 2 mL of ABTS working solution and absorbance was measured at 744 nm after 7 minutes [15, 16]. Ascorbic acid was used as the positive control, using methanol as the blank. ABTS free radical scavenging activity was calculated as below:

ABTS scavenging capacity = [($A_{control}$ – A_{sample})/ $A_{control}$] x 100

where $A_{control}$: the absorbance of ABTS solution and methanol, A_{sample}: the absorbance of ABTS solution containing the tested samples.

The IC_{50} value is the concentration of extract required to scavenge 50% of ABTS radicals, was calculated using Microsoft Excel.

2.2.5. Statistics analysis

All experiments were performed in triplicate and analyzed using Microsoft Excel. Results are presented as mean ± standard deviation (SD) from three independent replicates per sample.

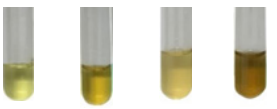
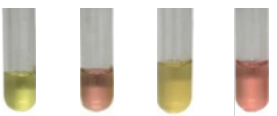
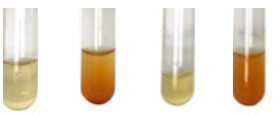
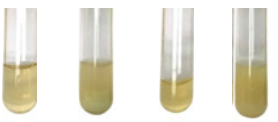
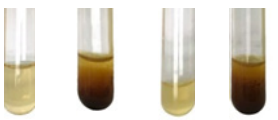
3. RESULTS

3.1. Chemical compositions

3.1.1. Qualitative identification of the presence of phenolic, flavonoid and alkaloid groups

The qualitative results of phenolic, flavonoid, and alkaloid groups in the leaves and branches of *A. monogyna* using characteristic chemical reactions were presented in Table 1.

Table 1. Qualitative results of phenolics, flavonoids and alkaloids in *Alphonsea monogyna*

No.	Class of phytochemicals	Test/Reagent	Results				Conclusions	
			Leaves		Branches		Leaves	Branches
			C	S	C	S		
1	Phenolics	5% $FeCl_3$					+	+++
2	Flavonoids	Shinoda					++	++
3	Alkaloids	Dragendorff					+++	+++
		Mayer					+++	+++
		Bouchardat					+++	+++

Notes: +: mildly positive, ++: moderately positive, +++: highly positive, C: control, S: sample

Data in Table 1 indicated that flavonoid, phenolic and alkaloid groups were present in both the ethanol leaf and branch extracts of *A. monogyna*. These results provided a basis for determining the total phenolic, total flavonoid and total alkaloid contents in this species.

3.1.2. Determination of total phenolic (TPC) and total flavonoid contents (TFC)

The total phenolic and flavonoid contents in *A. monogyna* were determined using spectrophotometric methods, based on standard calibration curves of gallic acid and quercetin. The results showed a strong linear correlation between the standard concentrations and absorbance values, with the following regression equations: $y = 0.0041x + 0.018$ ($R^2 = 0.9962$) for gallic acid and $y = 0.0501x - 0.0054$ ($R^2 = 0.9958$) for quercetin. Based on these calibration equations, the absorbance of the test samples was measured to calculate the total phenolic and flavonoid contents in each extract of *A. monogyna* and the results are presented in Table 2.

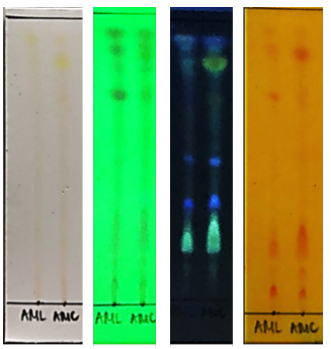
Table 2. Total phenolic and total flavonoid contents of *Alphonsea monogyna*

No.	Sample	TPC ± SD (mg GAE/g extract)	TFC ± SD (mg QE/g extract)
1	Leaves	55.65 ± 1.86	2.13 ± 0.06
2	Branches	102.68 ± 1.25	1.29 ± 0.04

The ethanolic extracts of *A. monogyna* leaves and branches exhibited total phenolic contents of 55.65 ± 1.86 mg GAE/g extract and 102.68 ± 1.25 mg GAE/g extract, respectively. In contrast, the total flavonoid content was found to be 2.13 ± 0.06 mg QE/g extract for the leaves and 1.29 ± 0.04 mg QE/g extract for the branches. These findings suggested that the branch extract possessed a higher concentration of phenolic compounds, whereas the leaf extract was richer in flavonoids.

3.1.3. Determination of total alkaloid content

The total alkaloid content in the leaves and branches of *A. monogyna* was determined using the gravimetric method. After obtaining the alkaloid-rich extract, thin-layer chromatography (TLC) was performed on normal-phase silica gel using a solvent system of $CHCl_3$: MeOH = 6 : 1 saturated with NH_3. Detection with Dragendorff's reagent revealed the presence of alkaloid compounds, indicated by the appearance of orange-red spots. The chromatographic profile of alkaloid samples was presented in Figure 2.



(A) (B) (C) (D)

Note:
AML: Alkaloid extract from the leaves
AMC: Alkaloid extract from the branches
A: Visible light
B: UV 254 nm
C: UV 365 nm
D: Dragendorff reagent

Figure 2. TLC profile of alkaloid extracts from *Alphonsea monogyna*

The total alkaloid content of *A. monogyna* was displayed in Table 3. The leaf and branch powders of *A. monogyna* contained total alkaloid levels of 0.70 ± 0.02% and 0.28 ± 0.02%, respectively. These results indicated that the leaves contained a higher alkaloid content compared to the branches.

Table 3. The total alkaloid content in *Alphonsea monogyna*

No.	Sample	Total alkaloid content (%)
1	Leaves	0.70 ± 0.02
2	Branches	0.28 ± 0.02

3.2. Evaluation of antioxidant activity

The antioxidant activity of the ethanol extracts from the leaves and branches of *A. monogyna* was demonstrated in Table 4.

Table 4. Antioxidant activity of *Alphonsea monogyna*

No.	Sample	IC_{50} ± SD (µg/mL)	
		DPPH	ABTS
1	Leaves	160.90 ± 5.56	19.60 ± 0.41
2	Branches	45.62 ± 0.64	8.37 ± 0.28
	Ascorbic acid	2.69 ± 0.09	1.27 ± 0.08

The results showed that the ethanol leaf extract of *A. monogyna* exhibited DPPH and ABTS radical scavenging activities with IC_{50} values of 160.90 ± 5.56 µg/mL and 19.60 ± 0.41 µg/mL, respectively. The ethanol branch extract revealed stronger scavenging effects with IC_{50} values of 45.62 ± 0.64 µg/mL for DPPH and 8.37 ± 0.28 µg/mL for ABTS radicals. In both assays, the positive control (ascorbic acid) showed the highest radical scavenging ability with IC_{50} values of 2.69 ± 0.09 µg/mL and 1.27 ± 0.08 µg/mL for DPPH and ABTS, respectively. These findings indicated that the branch extract possessed a stronger free radical scavenging capacity than the leaf extract, although both extracts exhibited weaker activity compared to ascorbic acid.

4. DISCUSSION

Extraction was performed using ethanol as the solvent. Ethanol is a versatile and efficient solvent for extracting phenolic compounds and flavonoids from plants due to its ability to dissolve both polar and non-polar substances and to form hydrogen bonds. Moreover, it is favored for its low toxicity, safety, renewability and suitability for use in food

and pharmaceutical applications [17].

To determine the total phenolic, flavonoid and alkaloid contents in *A. monogyna*, the initial step was the preliminary identification of phenolic, flavonoid, and alkaloid groups through characteristic chemical reactions. The results showed that these compound groups were presented in the ethanol extracts of both the leaves and branches of *A. monogyna*. Thus, these findings helped to guide the subsequent quantitative analysis of total phenolic, flavonoid and alkaloid contents in this species.

The determination of total phenolic and total flavonoid contents in *A. monogyna* revealed differences between the two plant parts. The ethanol extract from the branches exhibited a greater total phenolic content than that from the leaves. Conversely, the ethanol leaf extract had a higher total flavonoid content compared to the branches. Studies on the total phenolic and flavonoid contents of other species within the genus *Alphonsea* have also been reported. For example, the methanolic leaf extract of *A. sclerocarpa* showed TPC and TFC values of 115.18 ± 1.87 mg GAE/g extract and 28.37 ± 2.05 mg QE/g extract, respectively [7]. In this study, the branches of *A. monogyna* exhibited a relatively high total phenolic content with TPC value of 102.68 ± 1.25 mg GAE/g extract. These results suggest that this plant part could be considered as a rich source of phenolic compounds.

Some studies on the alkaloid content of *Alphonsea* species have also been reported. For *A. sclerocarpa*, the bark contained 0.126% alkaloids, while the leaves contained 0.135% alkaloids [18]. In current study, significant levels of total alkaloids were detected in the branches and leaves of *A. monogyna*, particularly in the leaves (0.7%). These results contributed to the understanding that the genus *Alphonsea* is a valuable source of alkaloid compounds. Alkaloids played a crucial role in human medicine and serve as vital components of an organism's natural defense mechanisms [19]. Therefore, the high alkaloid content in *A. monogyna*, particularly in its leaves, highlights its potential as a promising candidate for further pharmacological and phytochemical investigations.

The results indicated that the ethanol extract from the branches of *A. monogyna* exhibited stronger DPPH and ABTS radical scavenging activities compared to the leaf extract, with IC_{50} values of 45.62 ± 0.64 μg/mL and 8.37 ± 0.28 μg/mL, respectively. This is consistent with the strong correlation observed with the total phenolic content, as phenolic compounds are typically the main contributors to antioxidant activity. Notably, both ethanol extracts showed stronger scavenging ability against ABTS radicals than DPPH radicals. This may be due to the presence of water-soluble or highly polar antioxidants in the samples, since the ABTS assay is based on the generation of the blue/green $ABTS^{+}$ radical cation and is applicable to both hydrophilic and lipophilic antioxidant systems. In contrast, the DPPH assay uses a radical soluble in organic media and is therefore more suitable for lipophilic antioxidant systems [20]. The methanol fraction extract from the leaves of *A. sclerocarpa* showed DPPH scavenging activity with an IC_{50} value of 30.45 μg/mL [7]. For *A. tonkinensis*, the methanol extract of stems and leaves demonstrated DPPH radical scavenging activity with an IC_{50} value of 249.1 ± 0.6 μg/mL [8]. Alkaloid compounds isolated from the bark of *A. cylindrica* also exhibited DPPH scavenging activities with IC_{50} values ranging from 44.51 to 144.15 μg/mL [9]. As a result, it could be seen that the branches of *A. monogyna* have potential in free radical scavenging activity.

Phenolic compounds, flavonoids and alkaloids are major classes of secondary metabolites known for their wide range of biological activities, particularly their antioxidant potential [2], [21]. The findings from this study provide scientific evidence for the utilization of *A. monogyna* as a natural source of antioxidants and valuable phytochemicals for pharmaceutical and nutraceutical applications.

5. CONCLUSION

Alphonsea monogyna was found to contain phenolic, flavonoid and alkaloid compounds in both leaves and branches. The branches exhibited higher total phenolic content and stronger antioxidant activity, while the leaves contained greater amounts of flavonoids and alkaloids. The correlation between phenolic content and radical scavenging capacity suggested that the branch of *A. monogyna* might serve as a potential natural antioxidant source. This is the first report on the total phenolic, flavonoid and alkaloid contents, along with the antioxidant properties of *A. monogyna*. Further studies need to be carried out to isolate and characterize the bioactive constituents responsible for these effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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