Determination of the phytochemical screening, total polyphenols, flavonoids content, and antioxidant activity of soursop leaves (*Annona muricata* Linn.)

M T Nguyen1,2, V T Nguyen1,2, L V Minh3, L H Trieu3, M H Cang4, L B Bui2 and X.T. Le5, V T Danh6

1Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
2NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
3Research Center of Ginseng and Medicinal Materials, National Institute of Medicinal Materials, Ho Chi Minh City, Vietnam
4Chemical Engineering & Processing Department, Nong Lam University, Ho Chi Minh city, Vietnam
5Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam
6BKU Institute of Advanced Applied Science and Technology (BKIST), Ho Chi Minh City, Vietnam

* labasm2013@gmail.com, tiennguyenpharm@gmail.com

Abstract. *Annona muricata* Linn. (soursop) plant is a medicinal plant that has been utilized in folk medicine for treatment of infectious and inflammatory diseases. This study aimed to determine the phytochemicals content, total phenolic and flavonoid contents and antioxidant activities of *A. muricata* leaves. Antioxidant activity was performed via 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. The phytochemical was extracted separately with distilled water and 96% ethanol by maceration. The results show that a wide variety of pharmacologically active compounds such as alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, saponins were present in the leaves of *A. muricata*. The total phenolic and flavonoid content of ethanol extract in *A. muricata* leave achieved 609.08±5.82 mgGAE/g and 209.52±1.88 mgQE/g, respectively. The antioxidant activity of the ethanol extract of *A. muricata* was correlated with total phenolic and flavonoid content with values IC50 of 20.75±0.28 µg/ml, 12.84±0.21 µg/ml for DPPH and ABTS scavenging activity, respectively.

1. Introduction
In recent years, medicinal plants have been receiving a great deal of public attention in clinical and therapeutic potential due to their benefit [1-5]. *Annona muricata* (namely soursop) belong to the Annonaceae family which cultivated mainly in tropical and subtropical countries [6-7]. *A. muricata leave* plays a vital function in traditional medicine such as stomach pain, hypertension, and diabetes. [8-9]. Different pharmacological properties are shown in the *A. muricata* extract including antioxidant, antibacterial, anti-mutagenic, antiviral [5-13]. The main group of secondary metabolites in *A. muricata* were acetonegins, terpenoids, coumarins, alkaloids and flavonoids [14]. The previous
study shows that ethanolic leaf extract of *A. muricata* has significant antioxidant activities [15,16]. The ethanolic from leaves was also have nearly comparable antioxidant activity with butylated hydroxyanisole (BHA) and vitamin C [15]. Although this plant has different medicinal applied, relatively little is explored about its antioxidant effects. The present study was carried out to evaluate phytochemical screening, total polyphenol, flavonoids content, and antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-and-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) of the extract of *A. muricata*.

2. Materials and methods

2.1. Sample collection and preparation

The leaves of *A. muricata* were collected from district, Tien Giang province, Vietnam in January 2019. The leaves of *A. muricata* were washed with water and cut into small pieces, drying was done at room temperature, and the dried leaves were powdered.

2.2. Qualitative Phytochemical Analysis

About 25g dried powder of the leaves was extracted with different solvents: diethyl ether, ethanol 96% and water. Determination of compound groups in each extract is carried out using specific chemical reactions [17-20]. Plant extracts hexane, ethyl acetate, methanol, and methanol water were subjected to chemical tests for the presence of sterols, triterpenoids, carotenoids, tropolone, quinones, alkaloids, and flavonoids

2.3. Quantitative Phytochemical Analysis

2.3.1. Determination of total phenolic content (TPC)

First, the 1mL extract was pipetted into a test tube containing 1 mL Folin-Ciocalteu reagent 10% (v/v). After 5 minutes, 1 mL Na₂CO₃ 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 30 minutes in the dark. Finally, the absorbance was spectrophotometrically measured (UV/VIS - 1800 Shimadzu Spectrometer) at 765 nm, and the results were shown in mg of gallic acid equivalents per volume of sample (mg GAE/g) [21].

2.3.2. Determination of total flavonoid content (TFC)

Base on the aluminum chloride colorimetric method, the total flavonoid content was determined. Mixing 0.5 mL the extract with 0.15 mL 5% NaNO₂. After 5 minutes, mixing with 0.3 mL 10% AlCl₃. Then, 1mL 1M NaOH and 2 mL distilled water was added and vigorously shaken. The absorbance was spectrophotometrically measured (UV/VIS - 1800 Shimadzu Spectrometer) at 510 nm [21].

2.3.3. Determination of antioxidant capacity

**DPPH**

The antioxidant activity of the individual essential oil was tested using 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay (Analytical chemistry laboratory - University Nguyen Tat Thanh). 600 µL DPPH (OD 517 nm = 0.0403 ± 0.013) into 500 µL solution sample. The sample solution with pre-concentration and the mixed the stable at room temperature in the dark within 37 min. The optical measurement of the mixture by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. Blank sample, but 500 µL solution replaced EtOH 99.7%. Standard sample: Ascorbic acid (Vitamin C) (0.1g ÷ 0.01) was dissolved EtOH 99.7% into volume flask 100mL, in the dark (C = 100 µL/mL). The percent DPPH scavenging effect was calculated by [21]

\[
%I = \frac{Ab - As}{Ab} \times 100
\]
In there: Ab - Absorbance of blank sample, As - Absorbance of sample, %I - Percent inhibition

**ABTS**

ABTS scavenging activity was used. First, adding 10 mL of 2.6 mM K$_2$S$_2$O$_8$ in 10 mL of 7.4 mM ABTS solution in 15 hours. Next, preparing the working solutions by putting 1 ml of stock solution into 60 mL of methanol to take the absorbance value of 1.1 ± 0.02 at 734 nm. Then, 0.5 mL of sample added with 1.5 mL of the working solution for 30 minutes RT. Using UV-VIS spectrophotometer measured the mixture at 734 nm. The percentage of ABTS decolorization of the sample was determined according to the equation [22]:

$$\%\text{decolorization} = \left[ 1 - \left( \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \right) \right] \times 100$$  \hspace{1cm} (2)

3. Results

3.1. Qualitative Phytochemical Analysis and percentage yields

Phytochemical screening of the extracts revealed the presence of alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, saponins. Table 1 shows the result of phytochemical constituents of *A. muricata* leaves in water and 96% ethanol. Evaluation of chemical components of *A. muricata* leaves revealed the presence of alkaloids, coumarin, tannin, cardiac glycosides, flavonoids, carbohydrates, phenols. The previous study demonstrates the phytochemistry of Annona muricata leaf including tannins, flavonoids, saponins, terpenoids [23]. Moreover, the literature survey showed that the high contents of alkaloids, flavonoids, and phenols in the methanolic fractions of the fruit and leaf of *A. muricata* [24]. Besides, the previous study also showed the phytochemistry of Annona muricata leaf including tannins (0.44±0.0013 mg/g), flavanoids (1.92±0.02 mg/g), phenolics (104.43±0.013 mg/g), carotenoids (0.302±0.001 mg/g), α-tocopherol (14.80±0.02 mg/g), reduced glutathione (7.4±0.01 mg/g), lycopene (0.34±0.01 mg/g) and Vit C (1.98±0.011 mg/g) [25].

3.2. Quantitative Phytochemical Analysis

**Total phenolic and flavonoid content**

Phenolic compound plays a vital role in plants due to their scavenging ability. Moreover, flavonoids play great importance because they help the human body to cure different diseases. TPC of ethanolic extract of *A. muricata* were measured by Folin–Ciocalteu reagent in terms of GAE and was performed as mg gallic acid equivalents per gram of dried sample (mg QE/g). TPC was 609.08±8.52 mg GAE/g (figure 1). The TFC was performed as mg quercetin equivalents per gram of dried sample (mg QE/g). Flavonoid contents was 209.52±1.88 mg QE/g (Figure 2). These values are slightly higher than TFC and TPC of the same family and other medicinal plants. The results greatly recommend that the phenolics are essential components of this plant. The literature survey showed that total phenolics in the water extract were computed to be (683.69−) µg/mL gallic acid equivalents (GAE) while it was (372.92) µg/mL GAE in the ethanolic extract [26]. The previous result illustrated that the total phenol content of the extract ranged from 50.51 ± 3.21 mg/100 g to 560.21 ± 6.22 mg/100 g (pericarp) while the total flavonoid contents ranged from 85.65 ± 7.63 mg/100 g to 275.45 ± 10.01 mg/100 g [27].

**DPPH**

There are different techniques for estimating the antioxidant activity of both synthetic compounds and natural. The DPPH scavenging assay is broadly applied to assess the free radical scavenging of plant extracts thanks to its sensitive, simple, rapid. The DPPH assay was a rapid and low-cost method, which usually used for evaluation of the antioxidative potential of different natural stocks. Reduction capacity of DPPH radical is obtained by decrease in its absorbance at 515 nm. Ethanol extract (IC$_{50}$ = 20.75±0.28 µg/ml) showed potent antioxidant activity. This activity might be due to the presence of phenolic and flavonoids compounds. The IC$_{50}$ value of standard ascorbic acid was 2.66±0.02 µg/ml (Figure 3). The previous study demonstrated that extract of the pericarp had the highest DPPH radical
scavenging ability (EC50 = 0.87 ± 0.01 mg/mL), when compared to the pulp (EC50 = 2.24 ± 0.07 mg/mL) and seed (EC50 = 5.44 ± 0.04 mg/mL) extracts [27].

Table 1. Phytochemical constituents of A. muricata leaves in different solvent extracts.

| No | Compounds            | Leaves |
|----|----------------------|--------|
|    |                      | Water  | Ethanol |
| 1  | Alkaloids            | ++     | ++      |
| 2  | Saponins             | +      | +       |
| 3  | Coumarins            | ++     | ++      |
| 4  | Flavonoids           | ++     | ++      |
| 5  | Carbohydrates        | +      | +       |
| 6  | Cardiac Glycosides   | -      | -       |
| 7  | Phlobatannins        | -      | -       |
| 8  | Terpenoids           | ++     | ++      |
| 9  | Phenols              | ++     | ++      |
| 10 | Tannins              | ++     | +       |

+ + = Strong positive test, + = Weak positive test, - = Negative test

ABTS
Proton radical scavenging is a fundamental function of antioxidants. ABTS is a protonated radical, which has an absorption maximum at 734 nm. ABTS plays a vital role in determining the antioxidant capacity. Table 2 illustrates the ABTS scavenging ability of A. muricata with value IC50 of 12.84 ±0.21 µg/ml, and ascorbic acid with an IC50 value of 2.66 ±0.02 µg/ml. The scavenging effect of ABTS has direct radio with concentration. The ethanolic extract from the leaves of A. muricata was quick and effective scavengers of the ABTS radical. The previous study showed that the pericarp of A. muricata extract (34.9 ± 2.1 mmol TEAC/100 g) had the highest scavenging ability while the seed (8.3 ± 2.6 mmol TEAC/100 g) had the least [27].
Table 2. ABTS scavenging activity of ethanolic extract of A. muricata and ascorbic acid.

| Concentration (µg/ml) | ABTS% A. muricata | Concentration (µg/ml) | ABTS% ascorbic acid |
|-----------------------|-------------------|-----------------------|---------------------|
| 5                     | 18.07 ± 0.31      | 1                     | 19.42 ± 0.24        |
| 10                    | 39.71 ± 0.29      | 2                     | 36.59 ± 0.26        |
| 15                    | 56.86 ± 0.33      | 3                     | 55.72 ± 0.31        |
| 20                    | 82.84 ± 0.39      | 4                     | 74.54 ± 0.46        |
| 25                    | 94.99 ± 0.41      | 5                     | 96.68 ± 0.34        |

4. Conclusion
The results show that a vast difference of pharmacologically active compounds such as alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, saponins was present in the leaves of A. muricata. This study also revealed the total polyphenol, flavonoids content, and antioxidant activity of the leaves of A. muricata. The phenolic content was found 609.08±5.82 µg GAE/mg extract. Flavonoid content was 209.52±1.88 µg QE/mg dry weight ethanol extract in A. muricata leaves. The antioxidant activity of the ethanol extract of A. muricata was correlated with total phenolic and flavonoid content with values IC₅₀ of 20.75±0.28 µg/ml, 12.84 ±0.21 µg/ml for DPPH and ABTS scavenging activity, respectively. This research illustrates that A. muricata extraction has a higher value of the antioxidant activity compare with different medicinal plants. Therefore, the A. muricata could be applied as a source of antioxidant.

References
[1] Pham T T, Nguyen T H, Vo T T, Nguyen T T, Le T D, Vo D M H, Nguyen D H, Nguyen C K, Nguyen D C, Nguyen T T and Bach L G 2019 Polymers 11 177
[2] Nguyen T D-H, Nguyen T H, Nguyen V T N, Tran P L P, Vo D M H, Nguyen C K, Bach L G, Nguyen D H 2019 J. Appl. Polymer Sci. 136 47544
[3] Nguyen N T, Nguyen N N T, Tran N T N, Le P N, Nguyen T B T, Nguyen N H, Bach L G, Doan V N, Tran L B H, Le V T and Tran N Q 2018 Molecules 23 3347
[4] Nguyen P M, Nguyen H N, Nguyen T T, Bach L G 2018 Orient. J. Chem 4 2969-2976
[5] Nguyen P M, Nguyen H A, Pham T L P, Bach L G 2018 Res. Crops 19 730-735
[6] Formaggio A S, Vieira M C, Volobuff C R, Silva M S, Matos A I, Cardoso C A, Foglio M A and Carvalho J E 2015 Braz. J. Med. Biol. Res. 48 308–315
[7] Dragano N R, de Venancio V P, Paula F B, della Lucia F, Fonseca M J and Azevedo L 2010 Plant Foods Hum. Nutr. 65 319–325
[8] Florence N T, Benoit M Z, Jonas K, Alexandra T, Desire D D, Pierre K and Theophile D 2014 J. Ethnopharmacol 151 784–790
[9] Adewole S O and Ojewole J A 2008 Afr. J. Tradit. Complement Altern. Med. 6 30–41
[10] Feng P C, Haynes J L, Magnus K E, Plimmer J R and Sherratt H S 1962 J Pharm Pharmacol 14 556–561
[11] Padma P, Pramod N P, Thyagarajan S P and Khosa R L 1998 J Ethnopharmacol 61 81–83
[12] Takahashi J A, Pereira C R, Pimenta L P, Boaventura M A and Silva L G 2006 Nat. Prod. Res. 20 21–26
[13] Gouemo P N, Koudogbo B, Tchividoua H P, Nguema C A and Etoua M M 1997 Phytother Res 11 243–245
[14] Moghadamtousi S Z, Fadaeinasab M, Nikzad S, Mohan G, Ali H M and Kadir H A 2015 Int. J. Mol. Sci. 16 15625–15658
[15] Baskar R, Rajeswari V and Kumar T S 2007 IJEB 45 480-485
[16] Daud N N M, Rosdi M N M, Ya’akob H and Musa N F 2015 Jurnal Teknologi 77 27-37
[17] Harborne J B 1973 Phytochemical Methods Chapman and Hall Ltd 13
[18] Evans W 2002 Trease and Evans' Pharmacognosy New York Elsevier Health Sciences 21
[19] Godghate A, Sawant R, Sutar A 2012 J Chem 5 456-915
[20] Jaradat N, Hussen F, Al A A 2015 J Mater Environ Sci 6 1771 -1778
[21] Vuong Q V, Hirun S, Roach P D, Bowyer M C, Phillips P A and Scarlett C J 2013 J. Herbal Med 3 104–111
[22] Thaipong K, Boonprakob U, Crosby K, Cisneros-zevallos L and Byrne D H 2006 J Food Compos Anal 19 669–675
[23] Kingskey C, Paulinus N 2017 Food Sci. Nutrition 5(5) 1029-1036
[24] Kingsley C. Agua, Ngozi P Okelie, Ikechi Eze 2017 Egyptian J. Haematology 42 36-44
[25] Santhoshkumar, Nrinnda D 2015 European J. Exp. Biology 5(3):39-45
[26] Gavamukulya Y, Elella A 2014 Asian Pac J Trop Med
[27] Stephen A, Sunday I, Ganiyu O. 2015 Biochemistry Research International 15 347673.