

A STUDY ON ANTIBACTERIAL EFFECTS, POLYPHENOL CONTENTS AND ANTIOXIDANT ACTIVITIES OF LEAVES OF ASSAM APPLE PLANT (*DOCYNIA INDICA* (WALL.) DECNE)

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Abstract: This study was performed to investigate the antibacterial effects, polyphenol contents and antioxidant activities of leaf materials from Assam apple plants. Leaves were extracted with 4 solvents, including: ethanol, methanol, ethyl acetate and hexane, and were dissolved and diluted by dimethyl sulfoxide to evaluate antibacterial effects, polyphenol contents and antioxidant activities. The results showed that extracts from methanol and ethanol exerted the best antibacterial effects, exerting inhibition on both gram-positive and gram-negative bacteria, as well as had the highest polyphenol contents and the strongest antibacterial activities. However, methanol was the superior solvent than ethanol in all of 3 investigated properties. Therefore, we suggested that methanol should be applied in the extract of Assam apple plant leaves. Our results confirm the high potential of these leaves as herbs used for bacterial infections, and also as materials to enhance health through beneficial components, such as polyphenols and antioxidant ingredients. However, future researches are still necessary, such as *in vivo* experiments are needed to elucidate the effects and widen the uses of this material in practice.

Keywords: Assam apple plant leaf, *Docynia indica* (Wall.) Decne, extract, antibacterial effect, polyphenol, antioxidant activity.

1. Introduction

Antibiotics are substances that originally produced by microorganisms like fungi, bacteria, actinomycetes, and applied to inhibit the growth of or kill other microorganisms, primarily

bacteria. Nowadays, antibiotics are also made by synthetic or semi-synthetic processes. The overuse or misuse of antibiotics cause harmful effects on hosts' health, such as the inducement of negative influences on gut microbiota, or leading to the resistances, in which antibiotics become ineffective, creating the advantageous conditions for bacteria to grow, transmit from human to human, and finally giving

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rise to the emerge of “antibiotic-resistant superbugs”. In these recent decades, “super-resistant” bacteria have increased at a warning level and generated many serious problems. The emerge of infectious diseases with these resistant bacteria might lead to the death of patients, and therefore it is necessary to search for new drugs that can alternate the use of antibiotics and solve the problems of bacterial resistances.

According to Nguyen Tan Dat and Nguyen Ba Tiep (2016) [1], accompanying with the new trend of developing the “green” animal raising systems, medicinal plants are encouragingly applied as alternates to replace antibiotics in the prevention and treatment of diseases, so as to limit harmful effects resulted from the antibiotic uses. It is especially important in developing countries, where antibiotics have been usually abused and misused to increase the profits. The application of medicinal plants is considered as an effective solution to reduce the negative effects of antibiotic overuse. In addition, nature-derived herbal materials also have other advantageous aspects, such as the prices are cheaper than chemical synthetic drugs, less side-effects even in long application, decrease environmental pollution, less toxic residues, and therefore contribute to the producing of “clean” food that are safe for human consumption. In these recent years, the role of medicinal plants as a safe solution to alternate chemical synthetic drugs have been demonstrated to be more and more important for the pharmaceutical industry [2,3]. In addition, besides the antibacterial effects that make them a potential substitute for

antibiotics, herbal materials are also known for many other beneficial properties with various promising health-promoting functions, such as the rich polyphenol contents and antioxidant activities.

Assam apple plants (*Docynia indica* (Wall.) Decne) are common herbs that have been popularly used in the Northern mountainous areas, including Yen Bai, Lao Cao, Son La, Ha Giang and Lai Chau provinces. In addition to the effects such as to stimulate digestion, to tickle the taste buds, to enhance the cholesterol elimination, the plants are also known as a therapy for bacterial infections and many other health promoting functions [4,5]. Therefore, in this study, we investigated the antibacterial effects, polyphenol contents and antioxidant activities of the extracts obtained from leaves of this herb, so as to elucidate the properties and exploit their potentials in practice.

2. Materials and Methods

2.1. Herbal plants

Leaves of Assam apple plants (*Docynia indica* (Wall.) Decne) used in our study were purchased from Binh An Herbal company, Nghia Trai province, Hai Duong.

2.2. Research period and institute

Research time: From September, 2024 to May, 2025

Research institute: Laboratory of Pharmacology and Drug development, Centre of Research Excellence and Innovation, Vietnam National University of Agriculture.

2.3. Research materials and chemicals

a) **Bacteria:** We used 6 bacterial strains, including 3 gram-positive bacteria such as *Bacillus cereus* ATCC 11778; *Bacillus subtilis* ATCC 7953 and *Staphylococcus aureus* ATCC 259223, and 3 gram-negative bacteria such as *Escherchia coli* ATCC 25922, *Salmonela* ATCC 13311 và *Pseudomonas aeruginosa* ATCC 9027, which were purchased from the American Type Culture Collection (ATCC) and stored in the Department of Internal Medicine – Diagnose – Pharmacology and Toxicology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture.

b) **Solvents, reagents and culture media:** Solvents such as Ethanol, Methanol, Ethyl Acetate, Acetone, Hexane, Dimethyl Sulfoxide, and reagents and chemicals such as Folin Cio calteu, 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), acid chlorogenic, Vitamin E (VTME), and culture media, including Muller Hinton broth and agar, were purchased from Sigma Aldrich company (Merck, German).

2.4. Experimental design

Researches were performed with the *in vitro* experiments and observance.

2.5. Research methods

2.5.1. Research methodology

a) **Extraction:** Herbal materials were extracted at the solid-to-liquid ratio of 1:30, meaning that each 10g dried leaves were mixed with 300ml solvent. The mixtures were then stirred in 10 min before being left at room temperature in 24 h for diffusion. After that, solutions were transferred to Falcon tubes and centrifuged at the speed of 3000 rpm in 10 min before passing through filter papers to remove all of precipitations. Filtrates were then evaporated at low atmospheric pressure until all of the solvents were eliminated and dried extracts were obtained. These extracts were than dissolved and diluted until experimental concentrations with dimethyl sulfoxide (DMSO). DMSO was chosen because the solution had been demonstrated to induce no effects on the growth of bacteria [6].



Diagram 1. The description of extracting processes (Mixtures of plant materials and solvents were centrifuged, filtered, and the filtrates were evaporated at low atmospheric pressure to eliminate solvents and obtain dried extracts).

b) **The evaluation of antibacterial effects:** Agar well diffusion methods were applied to evaluate the inhibitory effects on bacteria, following the methods described by Gonelimali *et al.* (2018) [7], and with

some modifications to adjust with the conditions of applying plant extracts as experimental solutions [8]. Muller Hinton agar plates were inoculated with bacteria at a final concentration of 10^6 cfu/mL,

and holes with a diameter of 10 mm were punched aseptically with a cork borer. One hundred μL of extracts at tested concentrations were then added into the well. Agar plates were incubated at $37\text{ }^\circ\text{C}$ for 24 h. Inhibition zones were identified as the regions of petri plates surrounding the wells that did not have visible bacterial growth. Inhibitory diameters (excluding 10 mm of well diameters) were then measured to assess the magnitude of bacterial growth suppression. Experiments were performed in triplicate.

c) The measurements of polyphenol contents and antioxidant activities:

The measurement of polyphenol contents: Polyphenol contents were determined according to the method described by Suda et al. (2005) [9], applying Folin-Ciocalteu's Phenol Reagent and acid chlorogenic standard. 0.1 ml acid chlorogenic or extracts at tested concentrations were mixed with 0.5 ml Folin-Ciocalteu's Phenol Reagent. This mixture was allowed to stand at room temperature for 3 min. Then, 0.5 mL of 10 % Na_2CO_3 and 2.5 mL of distilled water were added. After 1 h of incubation, the absorbance was measured using a spectrophotometer (722 Ultra Violet – Visibility Spectrum, Jinghua company, China) at 750 nm. Acid chlorogenic (Merck, German) was applied to establish a standard calibration curve, and total polyphenol contents were expressed as mg acid chlorogenic equivalent per 10 mg weight of dried extract.

The measurement of antioxidant activities: Antioxidant activities were evaluated followed the method of Masuda et al. (2002) [10], applying DPPH Reagent and VTME standard. DPPH solution was made by mixing 0.1 g of DPPH powder with

50 mL of ethanol at 96%. Briefly, 50 μl of DPPH solution and 2.4 mL of DMSO were added to 0.1 mL of standards or extracts at tested concentration. The absorbance was determined using the spectrophotometer at a 515 nm wavelength with a blank containing only the sample and solvent. For the control absorbance measurement, 50 μl of DPPH solution was added to 2.45 mL of DMSO solvent. The result was expressed as a percentage of antioxidant activity, calculated using the equation:

$$\text{DPPH radical scavenging (AA \%)} = \frac{\text{Ac} - \text{As} - \text{Ab}}{\text{Ac}} \times 100$$

Where:

Ac (A control) is the absorbance of DMSO and DPPH solution

As (A sample) is the absorbance of extracts and DPPH solution

Ab (A blank) is the absorbance of extracts and DMSO.

In this experiment, VTME (Merck, German) was applied to establish a standard calibration curve, and the antioxidant activity of each 10 mg extract were expressed as mg VTME equivalent per 10 mg weight of dried extract.

2.5.2. Data processing and statistical Analysis

Results are expressed as means \pm standard deviation (SD), and analyzed with Statcel software (2023). Tukey's test after one-way analysis of variance (one-way ANOVA) was applied to compare values of inhibitory zone diameters, polyphenol contents and antioxidant activities. Unpaired *t*-test was applied to compare the inhibitory zone diameters induced by ethanol extracts with those of methanol

extracts, on the same bacteria and at the same tested concentrations. Significance was established when the probability level was equal to or less than 5%.

2.5.3. Research parameters

Values of inhibitory zone diameters (mm), polyphenol contents (mg acid chlorogenic equivalent/ 10 mg extract) and antioxidant activities (mg VTME equivalent/ 10 mg extract) were applied as criteria to identify that among tested solvents, including

ethanol, methanol, ethyl acetate and hexane, which is optimal for the extraction of leaf materials from Assam apple plants.

3. Results

3.1. Results on antibacterial effects of extracts

Results on the diameters of inhibitory zones induced by extracts from leaves of Assam apple plants are shown in Table 1 and Figure 1.

Table 1. Inhibitory zone diameters (mm) induced by extracts from Assam apple plant leaves

Bacteria		Extracting solvent	Concentration (mg/ml)			
			25	12.5	6.3	
Gram (+)	<i>Bacillus cereus</i> ATCC 11778	Ethanol	11.9 ^b ± 0.3	9.5 ^b ± 0.1	7.6 ± 0.1	-
		Methanol	14.3^a ± 0.2	11.5 ^a ± 0.4	10.6 [*] ± 0.2	-
		Ethyl acetate	7.9 ^c ± 0.1	5.9 ^c ± 0.1	-	-
	<i>Bacillus subtilis</i> ATCC 6633	Ethanol	10.0 ^b ± 0.2	8.5 ± 0.4	5.9 ± 0.2	-
		Methanol	12.8^a ± 0.3	9.1 [*] ± 0.2	6.4 [*] ± 0.1	-
		Ethyl acetate	7.0 ^c ± 0.2	-	-	-
	<i>Staphylococcus aureus</i> ATCC 25923	Ethanol	8.2 ^b ± 0.3	6.3 ± 0.1	-	-
		Methanol	9.5^a ± 0.4	7.2 [*] ± 0.1	5.8 ± 0.1	-
		Ethyl acetate	7.3 ^c ± 0.1	-	-	-
Gram (-)	<i>Escherichia coli</i> ATCC 25922	Ethanol	4.5 ± 0.3	3.1 ± 0.1	-	-
		Methanol	6.6[*] ± 0.4	5.0 [*] ± 0.1	-	-
	<i>Salmonella typhimurium</i> ATCC 13311	Ethanol	4.1 ± 0.2	3.0 ± 0.2	-	-
		Methanol	5.8[*] ± 0.2	4.5 [*] ± 0.1	-	-
	<i>Pseudomonas aeruginosa</i> ATCC 9027	Ethanol	3.0 ± 0.2	-	-	-
		Methanol	4.6[*] ± 0.1	3.1 ± 0.2	-	-

Values with different superscript letters (a, b, c) indicate significant differences (p < 0.05) compared with other values from different extracts of similar concentrations on the same bacterium, assessed by one-way ANOVA followed by post hoc Tukey's test. * indicates significant difference (p < 0.05) between the inhibitory zones induced by methanol extract vs. that of ethanol extract on the same bacterium and at similar concentrations, assessed by unpaired t-test. Bold letters indicate the largest values of inhibitory zones induced by different tested extracts on the same bacterium. (-) means no inhibition. Extracts that induced no inhibitory zones at all 4 tested concentrations are not shown in the Table.



Figure 1. Inhibitory zoned induced by the methanol extract from Assam apple plant leaves on *Bacillus subtilis* ATCC6633 at 4 tested concentrations (50; 25; 12,5 và 6,3 mg/ml).

From Table 1, we observed that the inhibitions on bacterial growth of leaf extracts were significantly dependent on the applied solvents. Specifically, only ethanol, methanol and ethyl acetate extracts induced effects, while hexane extract exerted no inhibition on all tested bacteria. In addition, methanol and ethanol extracts had better antibacterial properties, as shown by the significant effects on both gram-positive and gram-negative bacteria, while the extract with ethyl acetate only induced effects on gram-positive ones. Furthermore, methanol was superior than ethanol in the extraction. Specifically, inhibitory zone diameters of methanol extract were always larger than those of ethanol one, on all bacteria, including 3 gram-positive bacteria such as *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923; and also 3 gram-negative bacteria, including *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC

13311 và *Pseudomonas aeruginosa* ATCC 9027 (Table 1 and Figure 1).

3.2. Results on polyphenol contents and antioxidant activities of extracts

3.2.1. Results on polyphenol contents

In these measurements, we used acid chlorogenic as standards to calculate the polyphenol contents of extracts. The relationship between the concentrations of acid chlorogenic (mg/ml) and the increments in OD (Optical Density) values were established and shown in Figure 1.

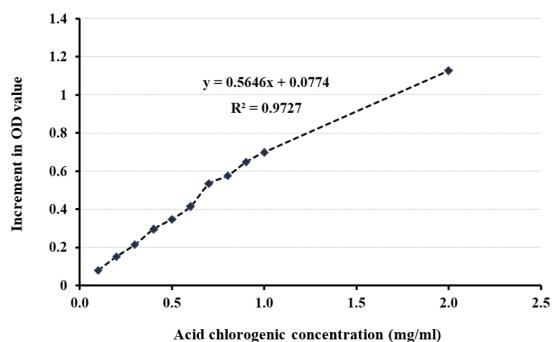


Figure 2. Relationship between the concentrations of acid chlorogenic (mg/ml) and the increments of OD values.

From Figure 2 we observed that there was a significant correlation between the concentrations of acid chlorogenic and the increments of OD values, expressed with the linear regression fomular: , in which was the increment in OD values and x was the concentration of acid chlorogenic. Because the relationship was statistically significant (Correlation Coefficient $R^2 = 0.9727$, $p < 0,001$), this fomular was applied to convert polyphenol contents in extracts to the mg acid chlorogenic equivalent per 10 mg extract, and the results are shown in Figure 3.

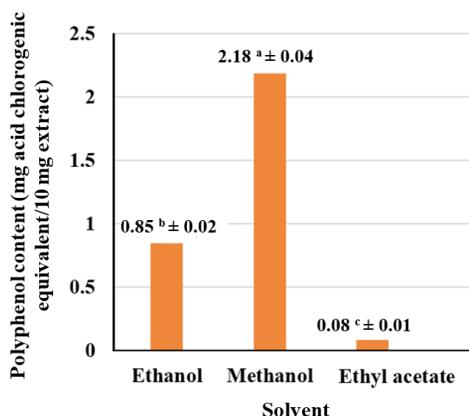


Figure 3. Polyphenol content (mg acid chlorogenic equivalent/10 mg extract) of Assam apple tree leaves extracted with different solvents.

Values with different superscript letters (a, b, c) indicate significant differences ($p < 0.05$) in polyphenol contents compared with other values from extracts with different solvents, assessed by one-way ANOVA followed by post hoc Tukey’s test. The polyphenol content of hexane extract was smaller than the lowest threshold detected with DPPH method, and therefore are not shown in the Figure.

From Figure 3 we observed that polyphenol contents in Addam apple plant leaves were various and significantly dependent on the extracting solvents, and ranged in the order of methanol > ethanol > ethyl acetate, correspondingly with the values of 2.18 ± 0.04 ; 0.85 ± 0.02 and 0.08 ± 0.01 (mg acid chlorogenic equivalent/10 mg extract). However, the extract from hexane had the polyphenol content that was smaller than the lowest threshold detected with DPPH methods.

3.2.2. Results on antioxidant activities

In these measurements, we used VTME (α tocopherol) as standards to calculate the antioxidant activities of extracts. The relationship between the concentrations of VTME (mg/ml) and the DPPH scavenging activity (AA%) were established and shown in Figure 4.

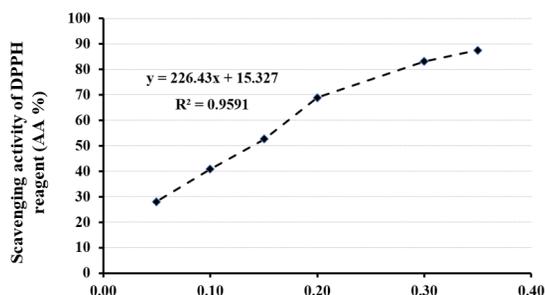


Figure 4. Relationship between the concentrations of VTME (mg/ml) and the DPPH scavenging activity (AA%)

From Figure 4 we observed that there was a significant correlation between the concentrations of VTME and the DPPH scavenging activities, expressed with the linear regression fomular: $y = 226.43x + 15.327$, in which y was the DPPH scavenging activity (AA %) and x was the concentration of VTME. Because the relationship was statistically significant (Correlation Coefficient $R^2 = 0.9591$, $p < 0,001$), this fomular was applied to convert antioxidant activities in extracts to the mg VTME equivalent per 10 mg extract, and the results are shown in Figure 5.

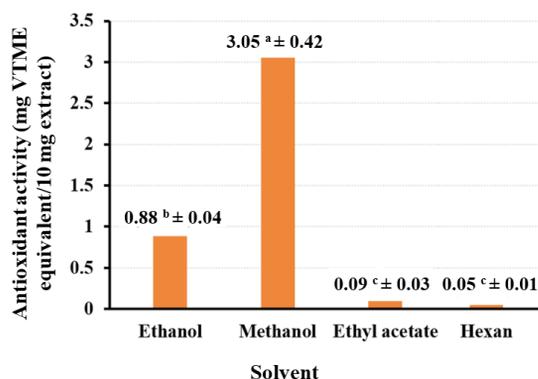


Figure 5. Antioxidant activities (mg VTME equivalent/10 mg extract) of Assam apple tree leaves extracted with different solvents.

Values with different superscript letters (a, b, c) indicate significant differences ($p < 0.05$) in antioxidant activities compared with other values from extracts with different solvents, assessed by one-way ANOVA followed by post hoc Tukey's test..

From Figure 5 we observed that antioxidant activities in Assam apple plant leaves were various and significantly dependent on the extracting solvents, and ranged in the order of methanol > ethanol > ethyl acetate > hexane, correspondingly with the values of 3.05 ± 0.42 ; 0.88 ± 0.04 ; 0.09 ± 0.03 ; 0.05 ± 0.01 (mg VTME equivalent/10 mg extract).

4. Discussion

Results on polyphenol contents and antioxidant activities of extracts from leaves of Assam apple indicate that solvents play important roles in the extraction of bioactive components in these materials. In addition, we also observed that extracts of high antibacterial effects were also rich in polyphenol contents and strong in antioxidant activities. Specifically,

methanol extract exerted the best inhibitory effects on bacteria, while also had the highest values in both of polyphenol and antioxidant properties. Similar relationships were also observed in cases of ethanol and ethyl acetate extracts. In contrast, the extract with hexane showed the weakest antibacterial effects and were also had the lowest values in both polyphenol contents and antioxidant activities. Therefore, we suggest that methanol and ethanol are suitable for the extraction of Assam apple leaves. However, methanol was optimal, as the extract with this solvent was superior in all of 3 investigated values.

In overall, our research confirm that Assam apple leaves are herbal materials with antibacterial properties and many other beneficial effects, probably due to the availability of polyphenol and antioxidant compounds, those are usually known to produce various health-promoting functions. Our results were similar to several previous reports about pharmacological effects of Assam apple plants (Nguyen Thi Minh Thu, 2012; Shende *et al.*, 2016) [5,11], and therefore further confirm the potentials of these herbal trees. In the fact, only fruits are considered as the main parts of Assam apple plants, which produce the values in all of economy, food and pharmacological aspects, while leaves are usually leaf un-used or thrown away, especially in the periods of pruning activities. However, our results have demonstrated that these by-products not only have good effects on bacteria but also contain many other beneficial bioactive components. Therefore, it is necessary to

research more and search for suitable ways to use these leaf materials as medicine, because such kinds of application for the by-product could enhance profits for both of agriculture and pharmaceutical fields. Similar to our investigation, our previous study of Nguyen, H.C et al. (2024) [12] about mangoes also indicated that the plant parts different from fruits, which were usually considered as having no values, especially during the pruning or harvesting activities, such as leaf materials, should also be applied for their potentious properties, rather than just being thrown away.

5. Conclusion

Assam apple plant leaves have not only antibacterial effects, but also polyphenol contents and antioxidant activities. Methanol is the optimal solvent for this herbal material, as it produces extracts of high biological values. However, it is still necessary to perform follow-up studies, including *in vivo* experiments, so as to elucidate the effects and widen the application of this plant parts as herbal medicine.

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