

**OBTAINING THE EXTRACT CONTAINING RICH CONTENT OF  
PHENOLIC COMPOUNDS FROM DIEP HA CHAU (*PHYLLANTHUS  
AMARUS*) GROWN IN PHU YEN PROVINCE  
FOR MEDICATION PURPOSES**

**THU NHẬN DỊCH CHIẾT GIÀU CÁC HỢP CHẤT PHENOLIC CÓ TÁC  
DỤNG TRONG PHÒNG VÀ ĐIỀU TRỊ BỆNH TỪ CÂY DIỆP HẠ CHÂU  
ĐẰNG (*PHYLLANTHUS AMARUS*) TRỒNG TẠI TỈNH PHÚ YÊN**

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**ABSTRACT**

*Phyllanthus Amarus is a medicinal herb widely used in traditional medicine for liver related diseases. Phenolics and antioxidant activity are mainly responsible for the hepatoprotective potential of these plants and are subjects to investigate in this study. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The extraction method combining the use of different solvents and ultrasound treatment was employed to isolate highly antioxidant phenolic compounds. The total phenolic content (TPC) and antioxidant activity of Phyllanthus Amarus extract were evaluated using the Folin-Ciocalteu method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. Methanol was demonstrated as the most suitable solvent for the isolation of highly antioxidant and rich phenolic content extract from Phyllanthus Amarus plants. The incorporation of the optimal solvent condition and ultrasound technique is highlighted as a promising candidate for the extraction medicinal herbs.*

**Keywords:** *Phyllanthus amarus; phenolics; antioxidant activity; methanol; ultrasound.*

**TÓM TẮT**

*Phyllanthus Amarus là loại thảo dược được dân gian sử dụng rộng rãi trong điều trị các rối loạn chuyển hoá của gan. Phenolic và hoạt tính chống oxy hoá xem như là tiềm năng bảo vệ gan của loại thực vật này, được tiến hành khảo sát trong nghiên cứu. Hoạt tính chống oxy hoá của phenolic là do tính chất cho nhận điện tử, đóng vai trò trong việc hấp thụ và trung hoà các gốc tự do, hay phân ly các gốc peroxide. Phương pháp trích ly kết hợp sử dụng các dung môi khác nhau và sóng siêu âm được khảo sát với hàm mục tiêu là hàm lượng phenolic chống oxy hoá cao. Hàm lượng phenolic tổng và hoạt tính chống oxy hoá của dịch trích Phyllanthus Amarus được đánh giá qua phương pháp Folin-Ciocalteu và khả năng quét gốc tự do 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanol được minh chứng là dung môi phù hợp nhất cho quá trình trích ly phenolic và hoạt tính chống oxy hoá từ Phyllanthus Amarus. Sự kết hợp giữa các điều kiện tối ưu của dung môi trích ly và sóng siêu âm hứa hẹn nhiều hy vọng cho quá trình trích ly các loại thảo dược.*

**Từ khóa:** *Phyllanthus Amarus; hợp chất phenolic; hoạt tính chống oxy hoá; methanol; sóng siêu âm.*

## 1. INTRODUCTION

Hepatitis is a common disease in developed and developing countries. Despite, considerable progress in the treatment of liver diseases, search for newer drugs continues because of limitations in the existing synthetic drugs. Hence, many researchers of traditional medicines are attempting to develop new drugs for hepatitis.

The plants of the genus *Phyllanthus* (Euphorbiaceae) are widely distributed in most tropical and subtropical countries, and have long been used in folk medicine to treat kidney and urinary bladder disturbances, intestinal infections, diabetes and hepatitis B. In recent years, the interest in these plants has increased considerably. Substantial progress on their chemical and pharmacological properties, as well as a few clinical studies of some *Phyllanthus* species has been made. Several compounds including alkaloids, flavonoids, lignans, phenols and terpenes were isolated from these plants. The natural antioxidants have the capacity to improve food quality and stability and can also act as nutraceuticals to terminate free radical chain reactions in biological systems, and thus may provide additional health benefits to consumers [1].

*Phyllanthus amarus* although considered from the farm point of view as a weed, is a valuable medicinal plant used by herbalists [2]. The roots, leaves, fruits, and whole plants are used in medicinal preparations. The domestic consumption of this leafy plant is moderately high in Brazil, China, Vietnam and other South-East countries. Several pharmacological studies demonstrated that the oral administration of *P. amarus* extracts exhibited the anti-inflammatory [3], anti-viral [4], anti-hepatotoxic activity [5,6] and also suppress the liver tumour development induced by N-nitrosodiethylamine in rats [7]. Phytochemical investigation of *P. amarus* has resulted in the isolation of bioactives including lignans, flavonoids, triterpenes, alkaloids, and tannins [8]. The bioactivity of this plant proposed us to investigate its

chemical components. The pharmacological activity of *Phyllanthus* species is mainly a result of the presence of lignans and polyphenols. Polyphenols belong to a heterogeneous class of compounds possessing a variety of effects towards antioxidant behavior. In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few decades [9]. Among the various natural antioxidants, phenolic compounds are reported to have the character of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical [10]. Also, phenolic compounds of plant materials have been shown to neutralize free radicals in various model systems. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [11]. In the present study, investigation of the effect of conditions for extraction in the *Phyllanthus Amarus* mash treatment on the phenolic level and antioxidant activity in extract.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Diep Ha Chau (*Phyllanthus Amarus*) used in this study was originated from a garden in Phu Thanh ward, Tuy Hoa city, Phu Yen province (Vietnam). The fresh samples were crushed in a blender (Panasonic, Japan). The obtained mash was extracted with variety of organic solvents. Extraction of active substances was supported by ultrasonic waves with a frequency of 20 Hz.

### 2.2. Methods

#### 2.2.1. Impact of extraction conditions on the antioxidant level in *Phyllanthus Amarus* mash

This experimental section consisted of three series:

First series: The samples were extracted with four different polarized solvents,

including acetone, ethanol, methanol and water at the same concentration. The fresh mash was homogenized with the solvent at the ratio of 1:10 and subsequently sonicated at room temperature for 30 minutes.

Second series: The extraction time was varied from 10 to 80 min.

Third series: Different amounts of solvent were added into flasks of samples. The ratios of mash/solvent were 1/10, 1/20, 1/30, 1/40, 1/50 (g/mL). Samples subsequently were sonicated at room temperature for 30 minutes.

For all series, a control sample without solvent and ultrasonic treatment was carried out. After the period of extraction, the mash was filtered through a cloth. The obtained suspension was separated by a refrigerated centrifuge (Sartorius, Switzerland) and the supernatant was collected for further analysis.

### 2.2.2. Assessment of phenolic content and antioxidant activity of the extract under the optimal conditions

A variety of extract volume was evaluated for antioxidant ability ranging from 20  $\mu$ L to 100  $\mu$ L.

### 2.3. Analytical methods

Total phenolic content was evaluated by spectrophotometric method using Folin-Ciocalteu reagent [12]. The results were expressed as the equivalent to mg of gallic acid per litre of extract (mg GAE/L).

The antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) method [13]. The results were presented as equivalent to millimole of vitamin C per liter of extract ( $\mu$ mol CEAC/L).

## 3. RESULTS AND DISCUSSION

### 3.1. Impact of extraction conditions on the antioxidant level in *Phyllanthus Amarus* mash

#### 3.1.1. Impact of extract solvents on antioxidant level in *Phyllanthus Amarus* mash

From Fig.1, it can be seen that acetone, ethanol and methanol extracts of P.Amarus

gave significantly higher phenolic content than water ( $p < 0.05$ ). The phenolic content of the extracts in the 4 types of solvent acetone, ethanol, methanol and water were 521.6167; 502.1; 608.3867; 435.57 mg GAE/L respectively. The results showed that there was no significant difference in total phenolic content in the extracts with acetone and ethanol.

A similar trend is also noted for the antioxidant activity of the extract as shown in Fig.1. Accordingly, the extract solvents with acetone, ethanol, and methanol gave higher antioxidant activity than that with water. In particular, the antioxidant activity of the methanol extract was highest 4766.67  $\mu$ mol CEAC/L. From the results we chose methanol solvent for subsequent experiments. Phenolics are usually extracted from plant material with an acidified organic solvent, most commonly methanol. This solvent system destroys the cell membranes, simultaneously dissolves the phenolics, and stabilizes them (Naczka, M.; Shahidi, F., 2006). An example is described by Ross et al., where aglycone forms of glycoside flavonoids are obtained by acid hydrolysis of the bean extracts, using a methanol/water (85:15 v:v) system.

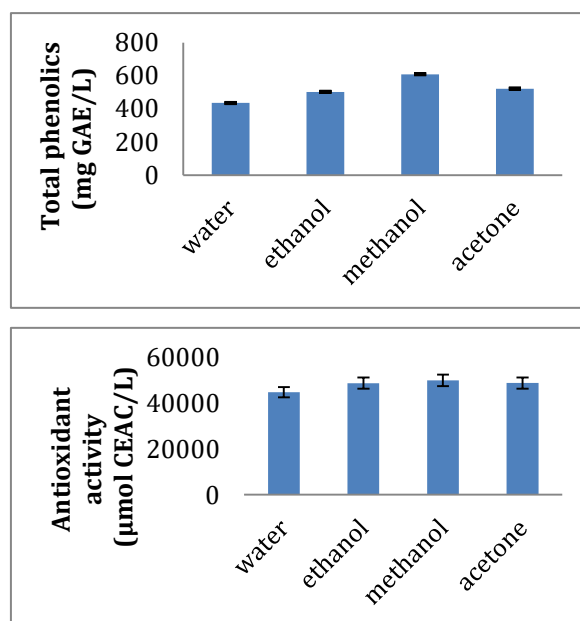
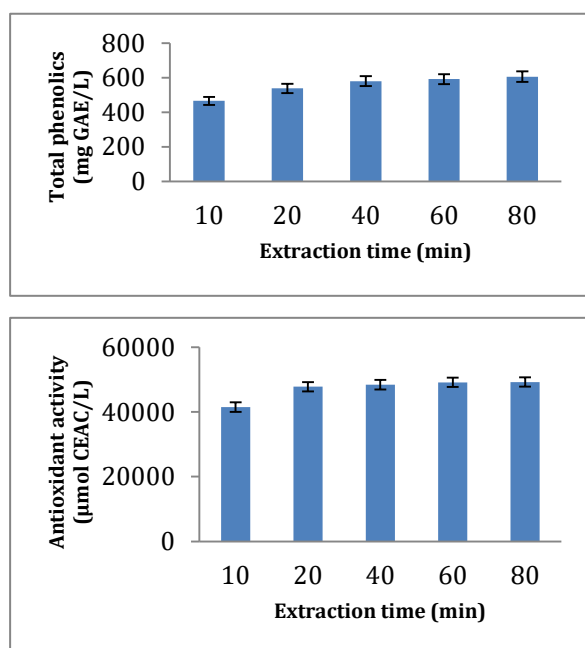


Figure 1. Effect of extract solvents on the level of total phenolics and antioxidant activity of P.Amarus mash

### 3.1.2. Impact of extraction time on antioxidant level of *Phyllanthus Amarus* mash



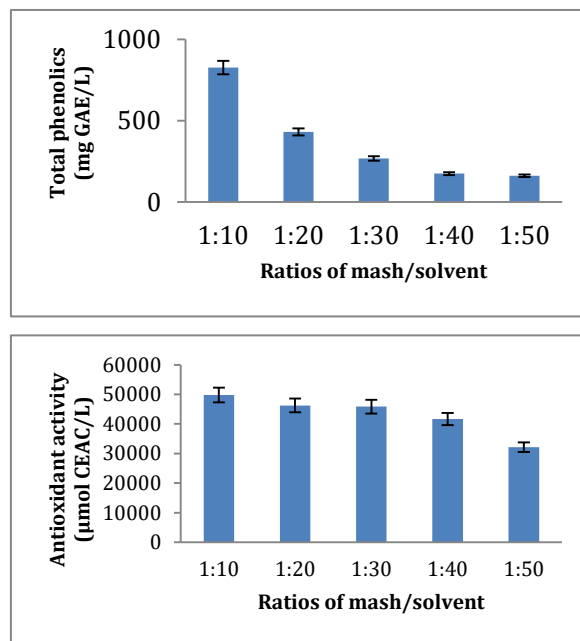
**Figure 2.** Effect of extraction time on the level of total phenolics and antioxidant activity of *P.Amarus* mash

The antioxidant level in *P.Amarus* mash treated with different extraction times is shown in Fig.2. The total phenolic level as well as antioxidant activity in extract increased significantly during the first 60 min of the extraction. However, when extending the extraction time from 60 to 80 minutes, the content of total phenolics increased slightly and did not differ significantly ( $p>0.05$ ). With 60 min extraction, the phenolic level was 591.03 mg GAE/L. A similar trend is also noted for the antioxidant activity of the extract. Too long extraction time increased the amount of hydroxyl radical which negatively impacted on the antioxidant activity of the extract. 60 mins was therefore the appropriate time for antioxidant extraction in *P.Amarus*.

### 3.1.3. Impact of different amounts of the solvent (methanol) on antioxidant level of *Phyllanthus Amarus* mash

The effect of different amounts of methanol solvent on antioxidant level in *P.Amarus* mash is visualized in Fig.3.

Increase in amounts of the solvent reduced gradually the content of total phenolics and antioxidant activity in the extract. The ratio of mash/methanol 1/10 (g/mL) was suitable for extraction to obtain the highest phenolic content of 825.31 mg GAE/L and antioxidant activity of 49750 µmol CEAC/L.



**Figure 3.** Effect of ratios of mash/solvent on the level of total phenolics and antioxidant activity of *P.Amarus*

### 3.2. Assessment of phenolic content and antioxidant activity of the extract under the optimal conditions

Based on the results of section 3.1, solvent of methanol, extraction time of 60 min and a ratio of mash/solvent of 1/10 (g/mL) were selected as the optimal conditions of treatment for extraction of *P.Amarus* with high antioxidant levels.

Based on the results of section 3.1, solvent of methanol, extraction time of 60 min and a ratio of mash/solvent of 1/10 (g/mL) were selected as the optimal conditions of treatment for extraction of *P.Amarus* with high antioxidant levels.

Different volumes of extract were used to evaluate the content of total phenolics and antioxidant activity (table 1). Increase in volume from 20 µL to 100 µL enhanced

phenolic level of 35.91%, a similar trend was noted for the antioxidant activity of the extract increased by 23.40%. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing volume of the plant extract. The antioxidative effect of *Phyllanthus* extract is may be due to the phenolic components. Thus, the DPPH radical scavenging activity of *Phyllanthus* extracts may be mostly related to their phenolic hydroxyl group. The concentration of hydrogen peroxide in solvent may vary according to the phenolic compounds. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of  $H_2O_2-H_2O$  [14]. **Table 1.** Content of phenolics and antioxidant activity of the extract under the optimal conditions

Volume (μL)	Total phenolics (mg GAE/L)	Antioxidant activity (μmol CEAC/L)
20	674.07±1.98	39466.67±57.74
40	687.92±2.50	39683.33±56.38
60	725.52±2.50	44083.33±28.87
80	829.64±2.50	47950.00±50.00
100	916.41±5.00	48700.00±50.00

#### 4. CONCLUSION

The investigation on the solvent types, ultrasonic treatments as well as optimal experimental conditions for the extraction of *P. Amarus* mash significantly increased both phenolic level and antioxidant activity of the plant. Ultrasound can improve the extraction yield and thus enable new commercial extraction opportunities and processes. The main targets have been polyphenols and carotenoids and in both aqueous and solvent extraction systems. The ultrasound extraction trials have demonstrated improvements in extraction yield ranging from 6 to 35% [15]. Many studies have examined the stability of the analyses during ultrasound-assisted process. Herrero et al. evaluated the phenolic-compound decomposition when phenolics were subjected to solid-liquid, subcritical water or microwave-assisted extraction, and sonication was performed in order to assess the type of energy that provides a lower degradation of the analyses [16]. The method was applied to two types of strawberries in order to demonstrate the degradation than do others [17]. Incorporation of optimal conditions into ultrasound in mash treatment is therefore very promising.

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