Preliminary phytochemical screening and determination of total polyphenols and flavonoids content in the leaves of *Houttuynia cordata* Thunb

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Abstract: *Houttuynia cordata* Thunb (*H. Cordata*), an aromatic medicinal herb belonging to family *Saururaceae*, is a plant that grows in moist habitats, well known for its medicinal properties and widely used worldwide. *H. Cordata* is an aromatic medicinal herb belonging to family *Saururaceae* and is restricted to specialized moist habitats. Three extracts, successively obtained from the leaves of *H. cordata*, namely the diethyl ether leaves extract (DELE), the ethanolic leaves extract (ELE) and the aqueous leaves extract (ALE), were used to determine the polyphenol and flavonoid content in the leaves. Phytochemical screening of plant extracts showed the presence of flavonoid, saponin, alkaloid, tannin, and steroid compounds. Quantitative determination of total phenolics, total flavonoids of methanolic extract were carried out using colorimetric methods. The result of quantitative determination showed that total polyphenol content of the DELE, ELE and ALE were 32.18 ± 2.64, 97.98 ± 1.77, and 22.22 ± 2.00 mg of gallic acid equivalents per gram dry extract, respectively. Meanwhile total flavonoid content of the DELE, ELE and ALE achieved 31.65 ± 1.07, 35.72 ± 1.23, and 4.06 ± 0.54 mg of quercetin equivalents per gram dry extract, respectively.

1. Introduction

In recent years, traditional medicines use of natural products (such as plants, animals, microorganisms, and marine organisms), which are of great importance [1-5]. Human have tended to use many products derived from nature. Among all products, essential oils with many wonderful uses have been used in many fields of production and life such as antimicrobial compounds, medicine, food additives, and so on [6-8]. *Houttuynia cordata* Thunb (*H. cordata*) is well known for its medicinal properties and widely used worldwide. *H. cordata* belongs to the *Saururaceae* family, commonly known as Chinese lizard tail which cultivated mainly in Mediterranean countries such as America, Africa, and Asia [9]. The previous study shows that *H. cordata* possesses a number of pharmacological important activities such as hypoglycaemic [10], antileukemic [11], anticancer [12], antioxidant [13] and so on. Thus, the present
study aimed to identify the various phytochemical constituents present in the extract by using standard methods of phytochemical screening. Aside from population studies, previous studies have highlighted that the flavonoids act as the key ingredients responsible for the activity against reactive oxygen species [14-16]. Because flavonoids and polyphenols could not be synthesized in the human body, it simply intakes from plants. However, relatively little is explored about the better extract and separate polyphenols and flavonoids from the \textit{H. cordata}. The objective of this study was to carry out preliminary phytochemical screening and to determine the total phenolic, flavonoid, flavonol contents of \textit{H. cordata} collected from the Ben Tre Province, Vietnam.

2. Materials and methods

2.1. Chemicals and reagents
Ethanol, methanol, diethyl ether, sodium hydroxide, hydrochloride acid, potassium chloride, ferric chloride, aluminium chloride, gallic acid, quercetin and L-ascorbic acid were purchased from Sigma-Aldrich. Distilled and deionized water were obtained from Center of Excellence for Biochemistry and Natural Products (NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam).

2.2. Plant Material
Leaves of \textit{H. cordata} were collected from the farms in Ben Tre Province, Vietnam in January 2019. The leaves sample were washed, dried under shade at 40°C to remove the water content. The dry leaves samples were ground into the fine powders before extraction which was carried out by the procedures described in Figure 1. Briefly, the leaves powder (100 g) was extracted with 1L of diethyl ether at 25°C for 24 hr, and then concentrated via vacuum evaporation to obtain the diethyl ether leaves extract (DELE) of \textit{H. cordata}. The residue was further extracted with 1L of 99.5% ethanol and 1L of distilled water by using the same procedure as above to produce the ethanolic leaves extract (ELE) and the aqueous leaves extract (ALE).

\textbf{Figure 1}. The extraction scheme of the leaves extracts from \textit{H. cordata} (at temperature of 25°C)
2.3. Phytochemical screening
Phytochemical screening was carried out using standard methods to detect the plant secondary metabolites including alkaloid, tanin, flavonoid, saponin, steroid, phenolic compounds etc. [17].

2.4. Total polyphenol content (TPC)
Base on the previous method, total polyphenol content was determined [18]. Firstly, the 0.5 mL extract was pipetted into a test tube containing 2.5 mL Folin-Ciocalteu reagent 10% (v/v). After 5 min, 2 mL Na₂CO₃ 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 30 min in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in mg gallic acid equivalents per gram of dry extract (mg GAE/g).

2.5. Total flavonoid content (TFC)
Base on the aluminum chloride colorimetric method, the total flavonoid content was determined [19]. Mixing 0.5 mL of the extract with 0.1 mL 10% AlCl₃, then 0.1mL 1M CH₃COOK and 4.3 mL distilled water was added and vigorously shaken. The absorbance was spectrophotometrically measured at 415 nm and the results were shown in mg quercetin equivalents per gram of dry extract (mg QE/g).

2.6. Statistical Analyses
All experiments were carried out in triplicate and the results were expressed as mean values and standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS 15 (SPSS Inc. Chicago, U.S.A) and differences between samples were compared using Tukey’s test (P < 0.05).

3. Results and discussion

3.1. Phytochemical analysis
Preliminary phytochemicals screening (Table 1) showed that H. cordata leaves were found to be present oils, alkaloid, flavonoid, triterpenoid, saponin, tanin, polyphenol, and coumarin. As we know, the plant secondary metabolites are proved to possess interesting bioactivities such as anti-inflammatory, antidiabetic, antibacterial, antioxidant activities, wound healing properties, protection of the skin, health promotion, and disease prevention. Nowadays, phytochemicals are increasingly attracted to scientist for the isolation and structural determination because they are not only used directly as drugs for the treatment of disease but also as modeling compounds for the development of new drugs with less toxic and side effects to humans. Previous authors already reported that flavonoid and polyphenol exhibited many bioactivities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, α-glucosidase inhibition and anti-allergic activities [20, 21]. Saponin is another type of phytocomstituents which are responsible for tonic and antimicrobial activities of the plant [22].

| Phytochemical class | DELE | ELE | ALE |
|---------------------|------|-----|-----|
| Alkaloids           | +    | +   | -   |
| Oils                | +    | -   | -   |
| Tannins             | -    | +   | +   |
| Flavonoids          | +    | +   | -   |
| Terpenoids          | +    | +   | -   |
| Saponins            | -    | -   | +   |
| Polyphenols         | +    | +   | +   |
| Coumarins           | +    | +   | -   |

(+) indicates present, (-) indicates absence
3.2. Total polyphenol and total flavonoid contents in different fractions

In the plants, phenolic compounds are mainly secondary metabolites. They play an important role in antioxidant activity and stimulating the activity of these extracts [23]. Figure 2 illustrates the significant level of phenolic compounds in diethyl ether (DELE), ethanol (ELE) and aqueous (ALE) fractions of the leaves *H. cordata*. In ELE, the total phenolic content was highest (97.98 ± 1.77 mgGAE/g), followed by DELE (32.18 ± 2.64 mgGAE/g), and ALE (22.22 ± 2.00 mgGAE/g). These results indicated that the leaves of *H. cordata* contains abundant of polyphenol compounds and the influence of the extraction solvent on the total content of phenolic compounds extracted.

Flavonoids (vitamin P) is one of the secondary plant metabolites. These metabolites mainly appear in the plant to generate yellow pigments. In addition, the flavonoids are readily consume by humans and they play a major function in anti-allergic, anti-inflammatory, and anti-cancer activities [23]. The total flavonoid contents were highest in the ELE (35.72 ± 1.23 mgQE/g), followed by DELE (31.65 ± 1.07 mgQE/g) and ALE (4.06 ± 0.54 mgQE/g). The total polyphenol content and total flavonoid content in DELE showed no significant difference. This may be due to most of the polyphenol in the extract are flavonoid.

![Figure 2](image)

**Figure 2.** Total polyphenols content and total flavonoids content in *H. cordata* leaves extracts. Different letters indicate significant differences (p < 0.05)

The high solubility of phenolic compounds in polar solvents provides high concentrations of these substances in extracts using polarity solvents [24]. Therefore, the phenolic content obtained from ethanol solvent extraction is somewhat higher than that of diethyl ether in the study because the polarity of ethanol (0.654) is higher than that of diethyl ether (0.117) [25]. Some previous researches also show that the solubility of phenolic compounds in the extraction solvent depends on the polarity of the solvent. Moreover, ethanol is one of the most suitable solvents for separating phenolic from plants [26]. The research results of Phung et al. (2010) on the *H. cordata* leaf extract also show this [27]. Furthermore, the use of ethanol solvent will not be toxic, so it is safer than other solutions. To sum up, ethanol is a suitable solvent to extract phenolic compounds from *H. cordata* leaves.

4. Conclusions

In conclusion, preliminary phytochemical screening of this plant was found to give positive reactions for oil, flavonoid, saponin, alkaloid, tannin, and steroid compounds. The values of the phenolic compounds in the leaves of *H. cordata* were high. Three extracts successively collected from the leaves of *H. cordata*, namely the diethyl ether leaves extract (DELE), the ethanolic leaves extract (ELE) and the aqueous leaves extract (ALE). *H. cordata* could be a reasonable price and available sources of natural bioactive compounds which can be applied as a natural antioxidant for food industries.
Acknowledgments: This study was supported by grants from Nguyen Tat Thanh University, Vietnam.

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