Phytochemical Screening, Antioxidant Activities, Total Phenolics and Flavonoids content of Leaves from *Persicaria odorata* Polygonaceae

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Abstract. *Persicaria odorata* Polygonaceae is a widely used herb in Asia. It contains several interesting bioactive components, and possesses properties that promote health. This is an important medicinal plant used to treat inflammation and swelling, diarrhea and excessive bleeding, and so on. Base on phytochemical analysis, the *Persicaria odorata* leaves contained lots compounds such as alkaloids, tannins, anthraquinone, flavonoids, terpenoids, coumarins, saponins and reducing compounds. The presence of those phytochemicals in this herbs indicated its potential medicinal effects, along with various vital biological and physiological properties. The leaves were extracted using ethanol and water to produce the respective extracts. The total phenolic and flavonoids content of each extract were determined by the Folin-Ciocalteu method and the aluminum chloride reagent, respectively. The ethanolic extract from leaves of *Persicaria odorata* showed higher total phenolic (58.56 ± 3.86 µGAE/mg) and flavonoid contents (70.65 ± 4.14 µQE/mg) than the aqueous extract. The percentage of radical scavenging activity was determined using radical scavenging assay with DPPH and ABTS. The ethanol extract of *Persicaria odorata* leaves had a high antioxidant activity with an *IC₅₀* of 311.26 ± 3.06 µg/mL DPPH and 167.66 ± 6.67 ABTS. The presence study showed that in ethanolic and aqueous extracts the medicinal potential of *Persicaria odorata* leaves and the positive relation between the total content of polyphenols and antioxidant activities.

1. Introduction
For a long time, the herbs were used in traditional medicines because of their inexpensiveness, availability and efficacy [1-3]. Several plant species are considered to have strong pharmacological effects, and more than 60% of all Western prescription medicines are extracted from natural compounds [4-7]. Moreover, major attention has been given to the role of phytochemicals in vegetables as a dangerous side effect of aerobic metabolism in the prevention of oxidative stress-induced diseases that trigger reactive oxygen species like singlet oxygen and different radicals. These radicals may be involved in a variety of diseases including cardiovascular dysfunctions, injury to the tissue, damage to DNA and tumor development [8-10].
The *Persicaria odorata* Polygonaceae leaves have been found to be used in traditional medicine, cuisine, pharmacy, and cosmetics [9]. The plant’s biological activities reported earlier include activities involving anticancer, antifungal, algaecide, antidiabetic, antibacterial, antioxidant [12][12]. *Persicaria odorata* has a range of beneficial properties and is a potential source of natural aliphatic aldehydes [14]. In 2009, research on Nanasombat has shown that *Persicaria odorata* extract had the highest antioxidant activity among all plant extracts with an EC_{50} of 315.4 mg extract / mg DPPH and phenolic compounds (52.0 mgGAE/mg dry extract) [7]. Moreover, research of Sasongko et al. was about analysis an essential oil of *Persicaria odorata* leaves [14]. The sample examined included alpha-caryophyllene (24.19%), decanal (11.79%), dodecanal (38.68%), caryophyllene (22.47%). Following that, the antibacterial study was conducted using a system of disk diffusion to examine the antibacterial activity against the *Staphylococcus aureus* and *Escherichia coli*. The essential oil from dry leaves has been noted to have the highest positive effect of antibacterial activity compared with wet leaves [14].

Liquid – liquid extraction is the classical method used to isolate compounds based on their relative solubility in two different immiscible liquids such as an organic solvent and water. In this method after extraction of sample with ethanol, solvent mixture containing water is used. These two solvents had been selected for their low toxicity, popularity and low cost. Phenolic compounds such as flavonoids, simple phenols and tannins which determine antioxidant activity are mostly insoluble in plants. Intended for use in the food and drug industry, herbal plants are often extracted in non-toxic and well-soluble solvents such as ethanol and water.

In present study, the leaves of *Persicaria odorata* were extracted using ethanol and water to produce the respective extracts. The present work investigated the phytochemical and antioxidant activities, total polyphenols and flavonoids content of ethanolic and aqueous extracts from *Persicaria odorata*.

2. Materials and methods

2.1. Materials and chemicals
Fresh plants of *Persicaria odorata* were purchased from local markets in Ho Chi Minh City, Vietnam during January–February 2020. Leaves of *Persicaria odorata* have been washed and sliced into tiny parts before drying in a 50 °C heated air-blowing oven. Samples had less than 15% water content after drying. It was ground in a mechanical blender to a fine powder and kept in place at room temperature before extraction. The leaves powder was extracted using ethanol and water to produce the respective extracts. Approximately 250 g of dry leaf powder was collected using various solvents with growing polarities of 96% ethanol and water. The extracts were used for the analysis of total phenolics, total flavonoid compounds and antioxidant activity. Both chemicals and solvents used in this analysis, including gallic acid standards, quercetin, vitamin C, ethanol, etc., were purchased from Sigma.

2.2. Evaluation of chemical components
The classification of compounds in each extract was calculated using different chemical reactions, such as ethanol, methanol, diethyl ether, sodium hydroxide, hydrochloride acid, aluminium chloride, gallic acid, quercetin and L-ascorbic acid and so on [15][14].

2.3. TPC
The total phenolic content of the extracts was measured using a modified Folin-Ciocalteu system [17]. The procedure consisted of 10 per cent combining 0.5 mL of test solution with 2.5 mL Folin-Ciocalteu reagent. After 5 minutes, the sample was raised to 1 mL Na_{2}CO_{3} by 20% (w/v). The mixture was then shaken vigorously, and incubated in the dark for 60 minutes. The absorbance was eventually calculated at 765 nm, and the findings were seen in µg of gallic acid equivalents per sample dry weight (µgGAE/mg).
2.4. TFC
The total content of flavonoids was calculated using the aluminum chloride colorimetric test [18]. The extraction of total flavonoids was performed according to Ebrahimzadeh et al. (2018) with a slight modification [19]. First, 0.5 mL of the extract and 0.1 mL of AlCl₃ 10% were mixed, vigorously shaken with 0.1 mL of CH₃COOK 1M. After this the solvent was balanced with purified water to a concentration of 5 mL. The mixture was incubated for 30 minutes in the dark. The measured absorbance was 415 nm. The TFC findings were shown in µg of quercetin equivalents per sample dry weight (µgQE /mg).

2.5. The antioxidant activity
The antioxidant activity of *Persicaria odorata* leaves was determined by DPPH and ABTS assays as described by Islam et al. 2019 [20]. The working solution of reagent was prepared to have absorbance value 1.1 ± 0.02. Optical UV / VIS combination test-1800 Shimadzu Spectrometer at 517 and 734 nm, respectively. After pre-concentration, 1 mL sample was mixed with 3 mL working solution of reagent. The mixer was incubated in the dark within 30 minutes at room temperature. Vitamin C was used as the positive reference. Calculation of the DPPH scavenging effect using the following equation: DPPH scavenging effect (%) or inhibition proportion (%I).

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%I = \frac{Ab - As}{Ab} \times 100
\]  
(Eq. 1)

Abbreviations: Ab - Absorbance of blank, As - Absorbance of sample, %I - Inhibition rate.

IC50 values are negatively proportional to antioxidant activity, the lower IC50 value indicates the maximum antioxidant activity of the sample being evaluated.

2.6. Statistical analysis
Both tests were performed in triplicates. The findings were used to measure the mean values and standard deviations (S.D.) One method Variance Analysis (ANOVA) was used to analyze the mean values. P values below 0.05 were considered significant. Test variations were measured using Tukey's SPSS (version 23, IBM, USA).

3. Results and Discussion

3.1. Evaluation of chemical components
Base on phytochemical analysis, *Persicaria odorata* leaves contained lots compounds such as alkaloids, anthraquinon, coumarin, flavonoids, reducing compounds, saponin tannins, and triterpenoid (Table 1). In research of Gouri Kumar Dash et al. (2016), preliminary phytochemical screening of various extracts revealed the presence of terpenoids, steroids, tannins, flavonoids, mucilages, carbohydrates, proteins and amino acids [12][7]. These phytochemicals, also known as plant secondary metabolites or non-nutrient plant compounds, are reported to have pharmacologically active agents that give protective effect against diseases [22].

Large amount of important bioactive constituent that have been found in herbs include flavonoids, steroids, alkaloids, phenols, tannins and saponins [23]. In research of Ooi Phaik et al in 2019, the phytochemical screening test of the this herbs indicated there were presence of saponins, tannins, total phenols, flavonoids and alkaloids [24]. Phytochemical profile of *P. odorata* essential oil has been investigated using GC analysis [25][26], including mainly decanal and dodecanal aldehydes and analogical alcohols, as well as their sulfanyl derivatives, monoterpenoids and sesquiterpenoids.
Table 2. *Persicaria odorata* leaves the phytochemical constituents in various solvent extracts.

| Compounds          | Ethanol 96% | Aqueous | Conclusion |
|--------------------|-------------|---------|------------|
| Alkaloids          | –           | +       | +          |
| Antraquinons       | +           | //      | +          |
| Coumarins          | +           | –       | +          |
| Flavonoids         | +           | –       | +          |
| Reducing compounds | +           | –       | +          |
| Saponin            | –           | +       | +          |
| Tannins            | +           | +       | +          |
| Triterpenoids      | +           | +       | +          |

*Abbreviations: (–): Absent; (+): Present; (//): There is no presence of this compound in the extract*

3.2. Total phenolic and flavonoid content

Several studies have reported that the plant polyphenols exhibited potential antioxidant activities which are particularly active in preventing oxidative stress-related diseases [22]. The ethanolic extract from leaves of *Persicaria odorata* showed higher total phenolic (58.56 ± 3.86 µgGAE/mg) and flavonoid contents (70.65 ± 4.14 µgQE/mg) than the aqueous extract. In research of Chasiw et al. (2019), methanol, dichloromethane, and water were used to dry and extract leaves and stems. Methanol leaf extract displayed the highest antioxidant activity with maximum concentration of phenolic compounds (52.59 ± 0.58 mg equivalent gallic acid / g extract) and flavonoid (19.97 ± 0.11 mg equivalent quercetin / g extract) [22]. According to Pawłowska et al. (2020) research about the changes in the phenolic contents and composition of *Persicaria odorata* fresh and dried leaves. Total contents of phenolic compounds were established to be in the range of 7.13 - 32.17 µg/g.

![Figure 1. Total phenolics and flavonoids contents of *Persicaria odorata* leaves](image)
3.3. The antioxidant activity

Free radicals are able to actively destroy several biomolecules, causing numerous chronic diseases [22]. Antioxidants such as flavonoids, phenolic acids and tannins are present in several natural plant extracts can counteract such harmful effects, thus preventing this pathogenesis [27]. The ethanol extract of Persicaria odorata leaves had a high antioxidant activity with an IC$_{50}$ of 311.26 ± 3.06 µg/mL DPPH and 167.66 ± 6.67 ABTS.

Figure 2. The antioxidant potential of ethanol extract by DPPH and ABTS radical scavenging activity

The scavenging activity of P. odorata against DPPH showed the highest percentage yield with 12.96% [30]. The result in research of Somananda et al indicated that P. odoratum ethanolic extract exhibited high scavenging activity. The plant has a TFC of 4.92 ± 0.629 mg/g and TPC of 13.03 ± 0.61 GAE/mg/g. IC50 value of the plant has been determined as 190.19 ± 0.424 µg/ml [31]. Overall, due to the presence various phenolic compounds, P. odorata has prominent antioxidant activity which can be a potential source of natural antioxidants.

4. Conclusion

Results obtained from present study indicated that Persicaria odorata contain desirable amount of nutrients. The presence of phytochemicals (alkaloids, anthraquinone, coumarins, flavonoids, reducing compounds, saponins tannins, and triterpenoids) in this herbs indicated its potential medicinal effects, along with various vital biological and physiological properties, which could use to improve human health. Furthermore, the total phenolic (58.56 ± 3.86 µgGAE/mg) and flavonoid contents (70.65 ± 4.14 µgQE/mg), as well as its antioxidant activity (IC$_{50}$ of 311.26 ± 3.06 µg/mL DPPH and 167.66 ± 6.67 ABTS) were comparatively high. The findings suggest that Persicaria odorata has promising antioxidant activity and demonstrated the existence of numerous phenolic compounds that can be harnessed to treat free radical induced diseases as an effective source of natural antioxidants.

Acknowledgment:
This research was funded by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
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