Egyptian Journal of Chemistry

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Effects of green extraction solvents on phenolic contents and bioactivities of *Persicaria odorata* (Lour.) Sojak

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Abstract

Persicaria odorata (Lour.) Sojak is one of the widely consumed herbs in Southeast Asian countries. It is a popular culinary ingredient in many Asian dishes in part due to its unique pungent flavor. This study aimed to determine phenolics, antioxidant, potential anti-inflammatory activities and inhibition of α-glucosidase of *P. odorata* extracts obtained with ethanol (ET), 80% ethanol (EW) and water (AQ). These extractants had influences on the presence of phytochemical groups (i.e., alkaloids, saponins, tannins and triterpenoids), phenolic contents and bioactivities in the extracts obtained. The 80% ethanol extract had the highest total contents of phenolics (207.59 ± 14.32 mg GAE/g) and flavonoids (126.00 ± 7.07 mg QE/g). All the phenolics examined were present in ET and EW while half of them were detected in AQ. The extracts prepared with ethanol and 80% ethanol 80% in general exerted higher antioxidant activity. In addition, ET and EW exhibited the stronger inhibitory effect on α-glucosidase (2.44 ± 0.12 and 2.20 ± 0.08 mmol ACAE/g, respectively). With respect to in vitro anti-inflammatory activity, ET showed an inhibitory effect against albumin denaturation comparable with that of the 200 μg/mL diclofenac solution. The study provides a better understanding of phytochemicals and potential health endorsing properties of *P. odorata*, which is helpful for design of new naturally occurring agents for disease prevention and treatment.

Keywords: Vietnamese coriander; phenolics; antioxidant; glucosidase; anti-inflammatory

1. Introduction

Persicaria odorata (Lour.) Sojak, belonging to the Polygonaceae family, is commonly known as Vietnamese coriander or rau răm. It can be eaten fresh in summer rolls, served with other fresh green vegetables in salads or added in some hot soups in Vietnamese cuisine. Besides, this herb is often used in laksa, a spicy soup popular in Malaysia and Singapore. Leaves of *P. odorata* contain numerous volatile organic compounds, including (Z)-3-hexenal, (Z)-3-hexenol, decanal, undecanal, and dodecanal, which are typical for the pungent flavor of the plant [1]. The leaves are also known for treatment of fever, acne, flatulency, diarrhea and inflammation [2]. Research has revealed that *P. odorata* possesses multiple bioactivities, such as anti-inflammatory, antiproliferative, antidiabetic, antihemolytic, antibacterial and antifungal activities [2-7]. It was shown that two glycosides, namely quercetin-3-rhamnoside and scutellarein-7-glucoside, detected in *P. odorata* could contribute to about 50% of the anti-inflammatory activity of ethanolic extract of the plant, by reducing of IL-6-secretion [2]. One study suggested that isorhamnetin, gallic acid and chlorogenic acid present in *P. odorata*, contributed to strong antioxidant activity and potential antidiabetic effects of the plant by inducing Mn-SOD, GPx-1, GRe and catalase synthesis and inhibiting α-glucosidase and α-amylase [7]. As previously studied, phenolics, a major class of phytochemicals commonly found in plants, are widely known to have capacity of terminating the formation of free radicals in oxidative stress-associated diseases [8]. Additionally, a large number of reports have demonstrated correlations between phenolics and anti-inflammatory as well as antimicrobial properties, leading to multiple applications of phenolic-rich plant extracts to food preservation and disease treatment [9].

Plant extracts are widely known to contain a huge number of plant materials, such as proteins, fats, carbohydrates, alkaloids, phenolics and pigments. These constituents can conventionally be recovered by solid-liquid extraction using effective solvents, such as methanol, ethyl acetate, hexane or chloroform. However, these chemicals have been shown to have drawbacks as they are harmful for users and the environment. Moreover, the resulting extracts are not

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Receive Date: 15 January 2023, **Revise Date:** 13 February 2023, **Accept Date:** 11 March 2023, **First Publish Date:** 12 March 2023
DOI: 10.21608/EJCHEM.2023.187525.7454

always safe as they may be composed of residual solvents. Green extraction in which the use of alternative solvents, such as ethanol or water, has proved to be able to limit petroleum-based constituents and guarantee safe extracts and quality [10].

In this study, phenolic compounds extracted with ethanol and/or water, antioxidant, in vitro anti-inflammatory and α-glucosidase inhibitory activities of *P. odorata* extracts were examined. This will hopefully provide a better understanding of how this group of phytochemicals contribute to potential health benefits this kind of herb possesses and open a new avenue for applications of *P. odorata* in food and nutraceutical industries.

2. Experimental

2.1. Chemicals

Phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rutin and quercetin were purchased from Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). Ethanol and acetone (99.5% ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Samples

Leaves of *P. odorata* (Figure 1) obtained from a vegetable farm in Ho Chi Minh city, Vietnam were carefully washed under a running tap water. Afterwards, the leaves were air-dried and kept in Ziploc bags in a refrigerator (4 °C, 65% relative humidity). The study was conducted during the period from December 2021 to May 2022.

2.3. Determination of chlorophyll contents

Chlorophyll in *P. odorata* leaves was determined using the methods previously published by Lichtenthaler and Wellburn (1983) [11]. The sample (1 g) was ground in a blender and extracted with acetone (10 mL) in a precleaned tube, followed by a vigorous shaking for 24 hours. The tube underwent a centrifugation step (5500 rpm, 15 min) using a centrifuge (EBA 20, Hettich, Germany) and the supernatant was obtained. Chlorophyll (μg/g) was determined via measuring absorbance of the extract at 645 and 662 nm in a spectrophotometer (ASV11D, AS ONE Corp., China) and calculated as follows:

Chlorophyll a = (11.24 × A662 – 2.04 × A645) × V/W

Chlorophyll b = (20.13 × A645 – 4.19 × A662) × V/W

where V represents the volume of the solvent (mL) and W is the amount of the sample (g).

2.4. Preparation of crude extracts

The sample was ground in a blender (Black & Decker, China) and then extracted with ethanol, ethanol/water (8:2, v/v) or water at a ratio of 1:10 (g/mL), followed by shaking on an orbital shaker (SK-L180 Pro, DLAB, China) for 24 hours and centrifugation at 5500 rpm for 15 min (EBA 20, Hettich, Germany). The crude extract was prepared by removing the solvent in a rotary evaporator (RE100S, DLAB, China) and the residue collected was used to screen for the presence of phytochemicals, determine total phenolic and flavonoid contents and evaluate bioactivities. The extraction yields for the ethanol, ethanol/water and water extracts obtained were 7.2, 5.9 and 2.9%, respectively.

2.5. Phytochemical screening

Phytochemicals, including saponins, anthraquinones, tannins and alkaloids, in the sample were qualitatively analyzed using the tests previously reported by Aly et al. (2019) [12].

2.6. Determination of phenolics

Total phenolic content (TPC) and flavonoid content (TFC) in the extracts of *P. odorata* were determined following previously published methods with minor modifications [13,14]. Individual phenolic compounds in the plant were identified and quantified on a Shimadzu LC-2030C HPLC system connected with diode-array detector and a VertiSep GES C18 reverse-phase column (250 × 4.6 mm, 5.0 μm particle size). The mobile phase comprised 100% methanol (solvent A) and 1% formic acid/water (solvent B) [15]. The oven was operated at 40 °C and wavelengths were set at 295 nm and 340 nm for detection of phenolic acids and flavonoids, respectively.

2.7. ABTS free radical scavenging assay

Preparation of a working ABTS solution was performed by combining 7 mM ABTS and 2.45 mM K2S2O8 in phosphate buffered saline (1:1, v/v) and then incubating for 12 hours at room temperature under a constant dark condition. The working solution (3 mL) was mixed with 100 μL of a diluted extract of *P. odorata* or reference standard (ascorbic acid solution). The absorbance measurement at 734 nm was carried out in a spectrophotometer (ASV11D, AS ONE Corp., China). The calibration curve plotted for the reference standard was $y = 0.006x – 0.0035$ ($R^2 = 0.99$). The results were shown as mg AAE/g (mg of ascorbic acid equivalents per g of extract) [16].

2.8. DPPH free radical scavenging assay

The mixture consisting of a diluted extract of *P. odorata* or ascorbic acid solution (200 μL) and 40 μg/mL DPPH solution in 80% methanol in water (300 μL) was incubated at 37 °C under a constant dark condition. The calibration curve plotted for ascorbic acid was $y = 0.0456x + 0.0295$, with $R^2 = 0.98$. The absorbance measurement at 517 nm was performed in a spectrophotometer (ASV11D, AS ONE Corp., China) and the results were presented as mg AAE/g (mg ascorbic acid equivalents per g of extract) [17].

2.9. In vitro anti-inflammatory activity

In vitro anti-inflammatory effect of the plant was predicted by assessing inhibition of albumin denaturation. In this assay, the inhibitory effect of the

extracts was assessed and compared with that of diclofenac, a nonsteroidal anti-inflammatory drug [18]. In detail, an extract of *P. odorata* in 5% DMSO or diclofenac sodium solution (100 μL) was allowed to react with 100 μL of bovine serum albumin solution (0.16%) and 200 μL of sodium acetate buffer (25 mM, pH 5.5). The reaction mixture was kept at 37 °C for 45 min, and then heated to 67 °C for 3 min. After cooling down to room temperature, the mixture was spectrophotometrically measured for absorbance at 660 nm using a spectrophotometer (ASV11D, AS ONE Corp., China). The percentage inhibition of albumin denaturation was estimated as follows:

$$\%I = (Ab - As)/Ab \times 100\%$$

where, Ab and As are absorbance of the blank and sample/standard.

2.10. Inhibition of α-glucosidase

Fifty microliters of a *P. odorata* extract diluted in 5% DMSO was allowed to react with 40 μL of α-glucosidase (0.05 U) diluted in phosphate buffer (0.1 M, pH 6.8). After 20 min incubation at 37 °C, 4-nitrophenyl-β-D-glucopyranoside (p-NPG) (5 mM, 40 μL) was added in, and the mixture underwent 20 min incubation at 37 °C once more. To stop the reaction, sodium carbonate solution (130 μL, 0.2 M) was added, and the measurement of absorbance was carried out at 405 nm in a spectrophotometer (ASV11D, AS ONE Corp., China) [19]. The results were shown as milligram acarbose equivalents per gram of extract (mg ACAE/g).

2.11. Statistical analysis

All the experiments were carried out in triplicate. One-way ANOVA followed by Tukey's HSD test at $\alpha = 0.05$ was employed to evaluate statistically significant differences in means of TPC and TFC, phenolic concentrations and bioactivities. Principal component analysis was applied to emphasize similarities and dissimilarities in the phenolic data among the extracts. All the statistical analyses were implemented using XLSTAT software (version 2016, Addinsoft, Paris, France).

3. Results and discussion

3.1. Phytochemical screening

In this study, tannins, triterpenoids, saponins and alkaloids in the extracts of *P. odorata* were screened. The results showed that all these groups of compounds were found to be present in the ethanolic (ET) and ethanol/water (EW) extracts (Table 1). Prior research showed that methanolic extracts of *P. odorata* tested positive for alkaloids, saponins and tannins [3, 20]. In another study, only tannins and triterpenoids were found in ethanol 96% of the plant [21]. In addition to the above organic solvents used for extraction, water was examined for its ability to extract these examined phytochemicals. In the present study, none of the phytochemical classes were detected in the aqueous (AQ) extract, indicating a poor extractability of AQ on tannins, triterpenoids, saponins and alkaloids.

Table 1. Screening of phytochemicals in the *P. odorata* extracts

Phytochemicals	Test methods	Indicator		
		ET	EW	AQ
Tannins	Ferric chloride test	+	+	–
Triterpenoids	Liebermann-Burchard test	+	+	–
Saponins	Frothing test	+	+	–
Alkaloids	Wagner's test	+	+	–

+: present; –: absent

3.2. Chlorophyll

In this study, chlorophylls in fresh leaves of *P. odorata* were determined using acetone as an extraction solvent. The results of spectrophotometric analysis revealed that the plant consisted of 893.97 ± 3.16 μg of chlorophyll a and 274.57 ± 7.10 μg of chlorophyll b per gram of fresh leaf. Previously, these pigments in the plant were investigated as their contents were reportedly influenced by LED lights [22]. Other than that, limited information about these constituents in *P. odorata* is available. Chlorophyll is a widely recognized phytonutrient that can help improve functions of essential detoxification pathways, anti-inflammatory, antioxidant and chemoprotective agents [23].

3.3. Phenolic contents

The results revealed that TPC of EW was significantly higher than those of ET and AQ ($p < 0.05$). As seen in Table 2, EW (207.59 ± 14.32 mg GAE/g) contained about 70% higher amount of phenolics compared to ET and AQ. In prior research, aqueous ethanolic extracts of *P. odorata* were reported to have TPC of 216.74 – 228.9 mg GAE/g [7,24]. These are shown to be highly comparable with the findings of the present study. With respect to ET and AQ, no statistically significant differences were noted for their TPC. Some recent investigations indicated that aqueous extracts of the plant were composed of 27.97 – 37.60 mg GAE per gram of extract [6,21]. These values are much lower in comparison with our study, and this discrepancy could be due to extraction methods, geographic and/or seasonal variations in sample collection. The results also indicated that EW was the richest in flavonoid among the extracts examined, with its TFC 5 – 12 times as high as those of the others (Table 2). Prior research reported ethanol/water extracts of *P. odorata* contained lower amounts of flavonoid [7,21] while aqueous extract of the plant had a similar TFC [6] in comparison with the present study.

To take a closer look at phenolic content, individual phenolic constituents were identified and quantified using HPLC-DAD as stated earlier. Eight phenolic acids and flavonoids were examined, and the results were shown in Table 2. All the compounds were quantitatively identified in ET and EW, while only half of them were present in AQ. Gallic acid was found in the two ethanolic extracts (ET and EW), with its concentration being 40% higher in the latter than in the former extract. Chlorogenic acid was detected at the levels following the order: ET > EQ > AQ. The results also showed ET comprised about six times as much chlorogenic acid as EW. Similarly, caffeic acid, p-coumaric acid, cinnamic acid and quercetin were present mostly in ET. Regarding AQ, the very low concentrations of the phenolic acids detected and none of the flavonoids found in this extract could be due to low extractive ability of water on these constituents. Previously, more than 30 phenolics in extracts of *P. odorata* fresh and dried leaves were identified using mass spectrometry [25]. Of these, chlorogenic acid, which was quantified using HPLC-DAD signals and the external standard, had the concentrations in fresh leaves ranging from 0.10 – 0.27 μg/g. One recent study has shown leaf crude extracts obtained with hot water are rich in flavonoids (396 μg of rutin and 75 μg of quercetin per gram of crude extract) [26]. The considerable variation in the results among the studies may be attributed to sampling location, extraction methods (extractants, extraction time, sample-solvent ratio) and HPLC method.

Principal component analysis was used to highlight the differences in phenolics among the extracts. As seen in Figure 2, the first principal component (F1) accounted for 74.67% while the second principal component (F2) explained 24.05% of the total variability. The biplot illustrated that ET was mostly composed of chlorogenic acid, p-coumaric acid and caffeic acid whereas EW was richer in gallic acid and rutin. It also indicated the AQ samples, which were poor in phenolics, were located on the far left half of the plot.

Table 2. Phenolic contents in the extracts of *P. odorata*

Phenolics		ET	EW	AQ
Gallic acid	μg/g FW	374.21 ± 22.73 b	523.95 ± 21.09 a	nd
Chlorogenic acid		1611.49 ± 215.43 a	250.36 ± 8.94 b	10.45 ± 0.51 c
Caffeic acid		93.01 ± 18.28 a	16.94 ± 1.72 b	nd
p-Coumaric acid		345.67 ± 35.65 a	16.30 ± 1.47 b	6.70 ± 0.47 b
Ferulic acid		154.85 ± 7.43 a	179.03.± 17.68 a	3.21 ± 0.13 b
Cinnamic acid		334.33 ± 24.22 a	271.77 ± 15.94 b	13.44 ± 0.62 c
Rutin		1441.72 ± 23.50 b	2279.95 ± 127.39 a	nd
Quercetin		265.73 ± 34.04 a	163.83 ± 13.33 b	nd
TPC	(mg GAE/g)	122.21 ± 7.15 b	207.59 ± 14.32 a	126.31 ± 22.01 b
TFC	(mg QE/g)	19.00 ± 2.83 b	126.00 ± 7.07 a	9.50 ± 2.12 c

Data are presented as mean ± standard deviation. Different lowercased letters for the same phenolic compound indicated significant differences among the extracts ($p < 0.05$).

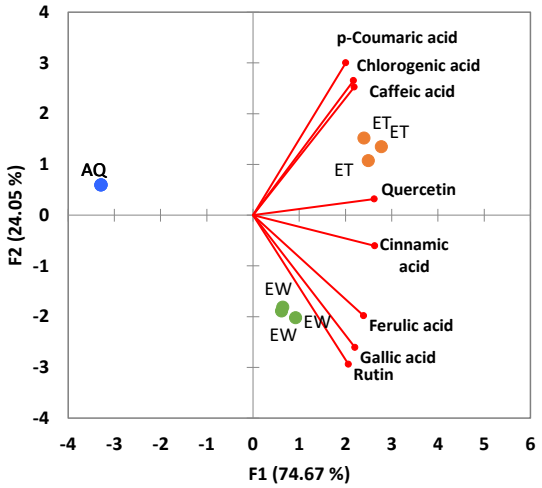

Fig 1. Principal component analysis performed on the phenolic concentrations of the *P. odorata* extracts. The figure displays a biplot achieved from the data matrix of phenolics and extracts.

3.4. Antioxidant activity

In the present study, DPPH and ABTS assays were employed to predict antioxidant activity of the P. odorata extracts and the results were presented in Table 3. The significantly higher ABTS antioxidant activity was noted for EW (67.03 ± 1.84 mg AAE/g), followed by AQ and ET. However, the trend for the antioxidant capacity of the extracts determined by the DPPH assay was different, following the order: ET > EW > AQ. Several studies have reported antioxidant activity of the plant extracts using ABTS and DPPH radical-scavenging assays. One study showed that methanolic extracts of the plant generally exhibited higher inhibitory effect on ABTS and DPPH radical production compared to its aqueous extracts at the corresponding concentrations [6].

3.5. Inhibition of α-glucosidase

Inhibition effect on α-glucosidase contributes to the maintenance of circulating glucose levels by lowering

the rate of blood sugar absorption. In the present study, the extracts of *P. odorata* were tested for inhibition of α-glucosidase. The results yielded evidence that ET and EW (4000 μg/mL) exerted stronger inhibitory activity (2.44 ± 0.12 and 2.20 ± 0.08 mmol ACAE/g, respectively). The aqueous extract (AQ) possessed the least potent effect compared the two others. Prior research reported the plant was among the commonly consumed herbs that displayed strong inhibition of α-glucosidase [27]. In a recent article, it has been shown that aqueous ethanolic extracts of the plant inhibited α-glucosidase approximately 30 times stronger than that of acarbose, an antidiabetic drug [7]. Flavonoids are considered as promising modulators of α-glucosidase activity and could be used for the development of inhibitors of this enzyme fighting metabolic disorders [28]. Previously, the use of in vitro and in silico experiments to investigate the potential of flavonoids to inhibit α-glucosidase demonstrated that quercetin was the most effective compound [29]. As stated earlier, ET and EW contained high amount of quercetin which could contribute to the inhibitory effect of these extracts on α-glucosidase.

3.6. In vitro anti-inflammatory activity

The presence of heat, environmental stress or chemicals may cause proteins to be denatured, leading to loss of their physiological functions. The denaturation of tissue proteins may therefore be documented as a marker for inflammation [30]. In the present study, we evaluated in vitro anti-inflammatory potential of the *P. odorata* extracts by determining their inhibitory effects on albumin denaturation. Figure 3 displays the percentage inhibition of the extracts (4000 μg/mL) compared to those of the positive control (diclofenac sodium solutions at 100 and 200 μg/mL). Among the samples, EW and AQ had insignificantly different capacity to suppress denaturation of albumin, with the percentage inhibition reaching 95.07 and 90.58%. ET exerted a slightly weaker activity, showing that more water in the extractants appeared to improve the suppression of albumin denaturation. Additionally, ET was found to be on a par with the 200 μg/mL solution of diclofenac sodium (86.95%). The results also showed that all the extracts exhibited a stronger protective effect on albumin than that of the 100 μg/mL diclofenac solution. Based on the results obtained, it is suggested that the extracts of *P. odorata* may exert a greater potential to protect albumin from heat denaturation than those of the diclofenac solutions. The mechanism behind the ability of the extracts to prevent heat-induced denaturation of albumin is not well understood. However, one theory is that the phenolics in the extracts interact with aliphatic regions surrounding the lysine residues located on the albumin molecules [31].

Previously, several works were conducted to give understandings of in vitro anti-inflammatory properties of *P. odorata* extracts. For example, one study evinced the crude ethanolic extract of the plant and its fractions significantly suppressed in TNF-α (tumor necrosis factor alpha) and IL-6 secretions in lipopolysaccharide-stimulated RAW 264.7 macrophage cells [2]. In another study, the ability of the plant to reduce nitric oxide production and levels of PGE2, IL-6 and TNF-α in RAW 264.7 macrophage induced by lipopolysaccharide was reported [6,32]. Although limited information about effects against inflammation in vivo is available, the results from these studies altogether indicated that the plant could be used for development of naturally occurring anti-inflammatory drugs.

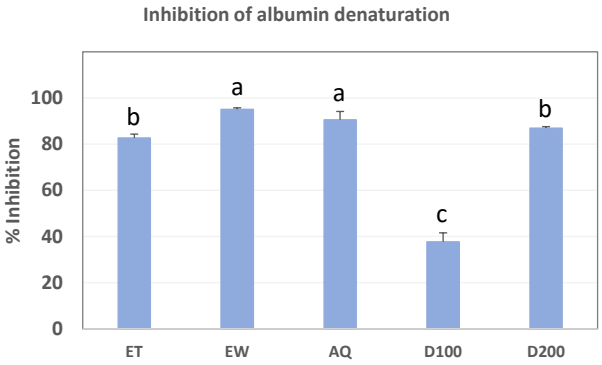

Fig 2. Percentage inhibition of the *P. odorata* extracts (4000 μg/mL) and diclofenac sodium solutions on albumin denaturation. D100 and D200 stand for the diclofenac sodium solutions at 100 and 200 μg/mL, respectively.

Table 3. Antioxidant activity and α-glucosidase inhibitory effects of the *P. odorata* extracts

	ET	EW	AQ
ABTS (mg AAE/g)	46.58 ± 0.76 c	67.03 ± 1.84 a	57.14 ± 1.27 b
DPPH (mg AAE/g)	31.37 ± 1.24 a	24.24 ± 1.06 b	1.50 ± 0.20 c
Inhibition of α-glucosidase (mmol ACAE/g)	2.44 ± 0.12 a	2.20 ± 0.08 a	1.92 ± 0.09 b

Data are presented as mean ± standard deviation. Different lowercased letters for the same phenolic compound indicated significant differences among the extracts ($p < 0.05$).

4. Conclusion

The use of green solvents (ethanol and/or water) had influences on phenolics, antioxidant activity, albumin denaturation and α-glucosidase inhibitory activities of *P. odorata*. Besides, the extraction solvents affected the presence of different phytochemical classes in the extracts obtained. Ethanol was much more effective in extracting phenolic constituents in the plant compared to water. Statistically significant differences were observed for the levels of all the compounds among the extracts. Extracts obtained with ethanol or ethanol 80% in general exhibited stronger bioactivities. Through principal component analysis, a visible differentiation in phenolic composition among the extracts was achieved. This study gives a better understanding of phytochemicals and potential health endorsing properties of *P. odorata*, which are important for the development of naturally occurring agents for prevention and treatment of diseases. It also helps explore new applications of the plant in food and nutraceutical industries. Future studies need to be focused on bioassay-directed fractionation to assess potential health benefits of the phytochemicals in *P. odorata*.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors would like to thank Van Lang university for financially supporting the research.

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