Chemical Constituents of *Persicaria sagittata* (L.) H.Gross: Antioxidant Activity and Chemotaxonomy Significance

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**Abstract**

**Background:** *Persicaria sagittata* (L.) H.Gross (Polygonaceae) has long been used in Indonesian traditional medicine to treat diarrhea, skin diseases, and internal problems. To date, the chemistry and biological activity of *P. sagittata* have not been investigated, requiring more research.

**Objectives:** This study aimed to investigate the chemical constituents of *P. sagittata* stems, evaluate their antioxidant activity, and discuss their chemotaxonomy values in genus *Persicaria*.

**Methods:** Compounds isolated from the plant stems were identified using physicochemical and spectroscopic measurements (1D and 2D NMR) and by comparison with literature data. The antioxidant activity was evaluated based on the qualitative and quantitative DPPH assays. The chemotaxonomy value was assessed based on extensive studies of the phytochemically investigated *Persicaria* species.

**Results:** We successfully isolated 10 compounds for the first time from *P. sagittata*, including arborinone (1), 25-hydroxycholesterol-5-en-3β,3β-lactate (2), β-sitosterol (3), methyl-4-hydroxy cinnamate (4), protocatechuic acid (5), gallic acid (6), methyl gallate (7), quercetin (8), vanicoside A (9), and vanicoside B (10). Of these, compound 6 (IC₅₀: 8.88 μM) exhibited the most significant antioxidant activity relative to ascorbic acid (IC₅₀: 30.49 μM), followed by compounds 7, 9, 8, 5, and 10 (IC₅₀: 15.37, 26.82, 29.18, 32.38, and 35.06 μM, respectively). Meanwhile, compounds 1-4 were inactive toward DPPH radicals (IC₅₀ > 400 μM). The chemistry of *P. sagittata* showed a relatively similar profile to other species profiles, implying a close chemotaxonomy relationship of *P. sagittata* with other species of genus *Persicaria*. Compounds 2 and 4 were first reported in genus *Persicaria*, which may serve as chemical markers for *P. sagittata*.

**Conclusions:** The chemical constituents of *P. sagittata* have potent antioxidant activities, in particular, phenolics, flavonoids, and sucrose esters. The presence of compounds 1-10 could enrich the chemotaxonomy value of *P. sagittata* in genus *Persicaria*.

**Keywords:** *Persicaria sagittata*, Polygonaceae, Chemical Constituent, Antioxidant, Chemotaxonomy Significance

1. **Background**

   Genus *Persicaria* belongs to the Polygonaceae family and comprises 150 species of annual or perennial herbs of worldwide distribution in the northern temperate to tropical and subtropical regions (1). *Persicaria sagittata* (L.) H.Gross (synonym: *Polygonum sagittatum* L., *Polygonum sieboldii* Meisn.), known as tearthumb, is a herb native to Asia, India, and North America that has been naturalized elsewhere (2). This species has been reported in Chinese traditional medicine (3) and has been used to treat diarrhea, skin diseases, and internal problems in Central Java, Indonesia.

   Based on our extensive search, the phytochemistry and biological activity of *P. sagittata* have not been studied thus far, requiring more research. Hence, the present study first reported the phytochemistry of *P. sagittata* and discussed the antioxidant activity and chemotaxonomy value of isolated compounds from genus *Persicaria*.

2. **Objectives**

   This study aimed to investigate the chemical constituents of stems of *P. sagittata*, evaluate their antioxidant
activity based on the DPPH assay, and discuss their chemotaxonomy values in genus *Persicaria*.

3. Methods

3.1. General

Kieselgel 60 PF$_{254}$ (aluminum plate), silica gel 60 G, and 60 PF$_{254}$ containing gypsum were purchased from Merck (Darmstadt, Germany). Diphenyl-picylhydrazyl (DPPH) and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Absorbance was measured using a Cary 100 Conc UV-Vis spectrophotometer (Varian, Palo Alto, CA, USA). Melting points were measured using a Melting Point M-565 apparatus (Buchi, Flawil, Switzerland). The NMR spectra (1D and 2D) were recorded using an ECP 500 MHz spectrometer (JEOL Ltd., Tokyo, Japan).

3.2. Plant Material

Stems of *P. sagittata* were collected from the Medicinal Plants and Traditional Medicine Research and Development Centre of the National Institute of Health Research and Development (NIHRD), Tawangmangu, Central Java, Indonesia, in July 2015 where the specimen was identified and deposited.

3.3. Extraction and Isolation of Compounds

The dried powder of *P. sagittata* stems (230 - 270 mesh, 7 kg) underwent extraction with acetone (3 x 20 L, 24 h each time) at room temperature to yield a dried acetone extract (270 g). This extract was subjected to silica gel Vacuum Liquid Chromatography (VLC, 10 cm × 5 cm), eluted with a gradient mixture of n-hexane-CHCl$_3$MeOH (from 8:2 to 0:10) and subsequently with pure MeOH to yield five fractions (F1 - F5). Fracions F2 - F5 were subjected to further purification. Fraction F2 (14 g) was separated over silica gel VLC (10 cm × 5 cm), eluted with n-hexane-CHCl$_3$MeOH (from 80:10 to 100% MeOH) and MeOH to yield five subfractions (F21 - F25). Subfraction F23 (1.0 g) was further purified over silica gel Radial Chromatography (RC), eluted with CHCl$_3$MeOH (95:5) to give pure compound 1 (60 mg). Similarly, subfraction F24 (2.8 g) was chromatographed over silica gel RC, with CHCl$_3$MeOH (90:10) as a solvent to yield pure compound 2 (80 mg). Furthermore, the main fraction F3 (11.3 g) was separated over silica gel VLC (10 cm × 5 cm), eluted with a gradient mixture of n-hexane-CHCl$_3$MeOH (from 70:30 to 0:100% MeOH) and subsequently with pure MeOH to yield four subfractions (F31 - F34). Subfraction F32 (1.0 g) was later purified over silica gel RC, with n-hexane-CHCl$_3$MeOH (85:15) as a solvent to yield pure compound 3 (100 mg). Subfraction F33 (1.0 g) was also separated over silica gel RC with n-hexane-CHCl$_3$MeOH (75:25) to yield pure compound 4 (100 mg). The same method was applied to subfraction F34 and successfully purified compound 5 (200 mg). The main fraction F4 (12.6 g) was further separated over silica gel VLC (10 cm × 5 cm), eluted with n-hexane-CHCl$_3$MeOH from 6:4 to 10:0) and pure MeOH, to yield five subfractions (F41 - F45). Subfractions F42 (1.3 g), F43 (1.2 g), and F44 (2.2 g) were purified separately over silica gel RC with n-hexane-CHCl$_3$MeOH (5:4:1) as eluent to yield pure compounds 6 (20 mg), 7 (40 mg), and 8 (30 mg), respectively. Moreover, fraction F5 (18.4 g) was chromatographed over silica gel VLC (10 cm × 5 cm) to give five subfractions (F51 - F55). The purification of subfraction F53 over silica gel RC with the elution of CHCl$_3$MeOH (9:1) yielded pure compound 9 (40 mg). Subfraction F54 was also purified using the same method to yield pure compound 10 (30 mg). All isolated compounds were measured for their melting point values and the chemical structures were elucidated using NMR spectra (500 MHz for $^1$H and 125 MHz for $^{13}$C). The physicochemical properties and NMR data, as well as $^1$H and $^{13}$C NMR spectra of the isolated compounds (1-10), can be seen in Appendix 1 in Supplementary File.

3.4. Antioxidant Assay

The DPPH scavenging activity of the isolated compounds was assessed qualitatively using the TLC autographic spray method (4) and quantitatively using the Bios method (5) with slight modifications. For qualitative analysis, the isolated compounds (0.1 - 100 μg) were applied separately on the TLC plate, developed, and dried at room temperature. The plate was further sprayed with DPPH solution (0.2% in MeOH) and incubated for 30 min in dark at room temperature. The activity was determined by the observation of a yellow spot of the compound against the purple background on the plate. Meanwhile, for quantitative analysis, triplicate 1 mL aliquots of samples were mixed with 1 mL of DPPH solution (500 μM) and incubated for 30 min in dark at room temperature. Absorbances were then measured at 517 nm against methanol as a blank. Ascorbic acid was used as a positive control. The concentration of the sample (in mg/mL) at which the absorbance (at 517 nm) reached half of the initial value was determined as the IC$_{50}$ value. The experiment was repeated three times.

3.5. Chemotaxonomy Study

The chemotaxonomy value was assessed by an extensive systematic study of chemical compounds among species of genus *Persicaria*. The study included chemical compounds both from preliminary identification using analytical methods and from isolation and identification of pure compounds.
3.6. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 software (La Jolla, CA, USA). Data were analyzed by one-way ANOVA, followed by Tukey post hoc test. A probability of $P < 0.05$ represented a significance difference. The IC$_{50}$ values were presented as means ± standard deviation (SD) obtained from three repetitions.

4. Results and Discussion

4.1. Characterization of Isolated Compounds

Separation and purification of the acetone extract of dried powder of *P. sagittata* stems using silica gel vacuum liquid and radial chromatography techniques led to the identification of 10 compounds, including one triterpenoid (1), two steroids (2-3), one phenylpropanoid (4), three simple phenolics (5-7), one flavonoid (8), and two sucrose esters (9-10). The structures of the isolated compounds were determined based on their physicochemical properties and spectroscopic data (1D and 2D NMR), as well as by comparison with literature data (Appendix 1 in Supplementary File). These compounds were identified as arborinone (1) (6), 25-hydroxycholest-5-en-3β-yl acetate (2) (7), β-sitosterol (3) (8), methyl-4-hydroxycinnamate (4) (9), protocatechuic acid (5) (10), gallic acid (6) (11), methyl gallate (7) (12), quercetin (8) (13), vanicoside A (9), and vanicoside B (10) (14). The compound structures are displayed in Figure 1.

4.2. Antioxidant Activity

The acetone extract of *P. sagittata* stems showed qualitatively weak (+++) free radical scavenging activity with an IC$_{50}$ value of 94.21 µg/mL as compared to ascorbic acid as a positive control (IC$_{50}$ 5.37 µg/mL). The isolated compounds (1-10) from the acetone extract of *P. sagittata* stems were found to exhibit dose-dependent free radical scavenging activity when assayed using qualitative and quantitative DPPH methods (Table 1). The results of the qualitative analysis revealed that compounds 5-8 performed pronounced free radical scavenging activity as indicated by dominant light yellow spots on the TLC plate with intensities comparable to ascorbic acid. Strong scavenging activity was also exhibited by compounds 9-10. On the other hand, compound 4 showed weak scavenging activity, while compounds 1-3 were unable to scavenge DPPH free radicals. The lowest IC$_{50}$ value was achieved by compound 6 (IC$_{50}$ 8.88 µM), followed by its derivative, compound 7 (IC$_{50}$ 15.37 µM), suggesting that the replacement of the hydroxyl group (OH) with the methoxyl group (OCH$_3$) may reduce the scavenging activity. In addition, the absence of the OH group on the benzene ring of the hydroxylated benzoic acid derivative affected the scavenging activity of compound 5 with a high IC$_{50}$ value of 32.38 µM as compared to compound 6. Interestingly, compounds 9-10, classified as cinnamic acid sucrose esters, performed significant free radical scavenging activity (IC$_{50}$ 26.82 and 35.06 µM, respectively) when compared to the cinnamic acid derivative, compound 4 (IC$_{50}$ 456.83 µM), suggesting the important role of the sucrose core in the structures of compounds 9-10 to enhance the scavenging activity. Moreover, the presence of an acetox group (OCOCH$_3$) in compound 9 seemed to increase its scavenging activity compared to compound 10. Meanwhile, compound 8, a common flavonol found in genus *Persicaria* according to our search, was found to perform a consistent free radical scavenging activity in both qualitative and quantitative analyses. It is known that phenolics and flavonoids are major determinants of antioxidant activity in plants (15). The present study suggested that simple phenolics (5-7), a flavonoid (8), and sucrose esters (9-10) played significant roles in performing the free radical scavenging activity of the acetone extract of the investigated plant.

4.3. Chemotaxonomic Significance

Several species of genus *Persicaria* have been originally classified in genus *Polygonum*. The Plant List (2013) recorded 66 accepted species in genus *Persicaria* with low and medium confidence levels (16). Of these, about 25 species have been phytochemically investigated according to our literature search, covering more than 250 compounds. The dominant classes of compounds including phenolics, flavonoids, phenylpropanoids, and sesquiterpenoids have been highlighted among the species of genus *Persicaria* (17-19). Here, we discuss the isolation and identification of 10 compounds from the stems of *P. sagittata*, including one triterpenoid (1), two steroids (2-3), three simple phenolics (5-7), one phenylpropanoid (4), one flavonoid (8), and two sucrose esters (9-10) (Figure 1). These compounds were isolated from *P. sagittata* for the first time.

Species in genus *Persicaria* have been reported to produce oleanane- and arborane-type triterpenoids. These include the rhizomes of *P. bistorta* (20) and the whole plants of *P. capitata* (18) and *P. nepalensis* (21). Arborinone (1) together with other arborane-type triterpenoids was found in the roots of *P. bistorta* (22), suggesting a close relationship between *P. bistorta* and *P. sagittata*. Meanwhile, steroids with stigmastane skeleton seem to accumulate in the aerial parts of *P. barbata* (23), *P. ferruginea* (24), and *P. glabra* (25), stems of *P. chinensis* (26) and *P. pulchra* (27), rhizomes of *P. bistorta* (20, 22), and the whole plants of *P. capi-
Chemical structures of compounds 1-10.

**Figure 1.** Chemical structures of compounds 1-10.

tata (28), P. nepalensis (21), and P. stagnina (29). β-sitosterol (3) is a common steroid found in plants and it has been reported in the rhizome of P. bistorta (20) and the whole plants of P. nepalensis (21) and P. stagnina (29). On the other hand, 25-hydroxycholest-5-en-3β-yl acetate (2) and methyl-4-hydroxy cinnamate (4) were described for the first time in genus *Persicaria* that herein may enrich the chemotaxonomy aspect of this genus (Table 2). These compounds may also be chemotaxonomically significant to differentiate *P. sagittata* from other species in the genus. The presence of cholestene-type steroids was previously reported in *P. amphibia, P. pensylvanica*, and *P. punctata* detected using gas-liquid chromatography and mass spectral analysis (30). Meanwhile, cinnamic acid derivatives are distributed in the aerial parts of *P. amphibia, P. bistorta, P. capitata, P. chinensis, P. glabra, P. lapathifolia, P. maculosa, P. mitis, P. stagnina, P. tinctoria* (25, 31-35) and rhizomes of *P. amplexicaulis* (36, 37). Sugar esters containing cinnamic acid have been distributed in several families of plants, covering more than 330 compounds (38). Vanicosides A (9) and B (10) were first reported from the leaves, stems, and roots of *P. pensylvanica* (14) and later were found in the whole plant of *P.
Table 1. DPPH Radical Scavenging Activity of Compounds 1-10. a, b

| Structure Number | Compound Name                          | DPPH Radical Scavenging Activity | TLC  | IC50 (µM)  |
|-----------------|----------------------------------------|----------------------------------|------|------------|
| 1               | Arborinone                             | +                                | +    | 609.46 ± 3.22 |
| 2               | 25-Hydroxycholest-5-en-3β-yl acetate   | +                                | +    | 587.22 ± 3.57 |
| 3               | β-Sitosterol                            | +                                | +    | 690.90 ± 4.53 |
| 4               | Methyl-4-hydroxy cinnamate             | ++                               | +    | 456.83 ± 6.59 |
| 5               | Protocatechuic acid                    | +++                              | +    | 32.38 ± 1.35  |
| 6               | Gallic acid                            | +++                              | ++   | 8.88 ± 0.43   |
| 7               | Methyl gallate                         | +++                              | +++  | 15.37 ± 0.44  |
| 8               | Quercetin                              | +++                              | +++  | 29.18 ± 0.77  |
| 9               | Vanicoside A                           | +++                              | +++  | 26.82 ± 0.93  |
| 10              | Vanicoside B                           | +++                              | +++  | 35.06 ± 0.68  |
|                 | Ascorbic acid (positive control)       | +++                              | +++  | 30.49 ± 1.40  |

a++ = very weak; ++ = weak; +++ = strong; ++++ = very strong
bIC50 values are given in mean ± SD (n = 3).

hydro Piper (39). Compound 10 was also found in the aerial part of P. lapathifolia (40). The presence of sucrose esters in P. capitata, P. hydropiper, P. lapathifolia, and P. pensylvanica is rare and they have not been found so far in other families with a history of sugar esters such as Polygalaceae and Smilacaceae (38). Hence, these compounds could be used as chemical markers for the species of genus Persicaria. Furthermore, protocatechuic acid (5) and gallic acid (6) were distributed in P. amphibia (35, 41), P. bistorta (42), P. capitata (28, 34), and P. lapathifolia (43). Meanwhile, methyl gallate (7) was found in the aerial part of P. lapathifolia (44). Quercetin (8) is a flavonol accumulating remarkably in different parts of 12 species of genus Persicaria (Table 2).

The chemistry of P. sagittata reported in the present study showed a relatively similar profile to other species of genus Persicaria, which implied a close chemotaxonomy relationship of P. sagittata with other species of this genus. Compounds 2 and 4 are first reported in genus Persicaria, which may serve as chemical markers for P. sagittata to probably differentiate this plant from other species in the genus. Since we successfully isolated only 10 compounds from the plant, further research is highly required to explore more about the chemistry, biological activity, and chemotaxonomy value of genus Persicaria.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors’ Contribution: Idin Sahidin was the project leader responsible for the research design, as well as drafting and revising of the manuscript; Andi Bahrun was responsible for the investigation of traditional uses of the plant, as well as plant collection and authentication; Muhammad Taufik contributed to the research design, especially bioassay; Agung W. Mahatva Yodha performed chromatographic analyses and data interpretation; Carla
| Compound Name                           | Species (Part)                                                                 | Reference(s) |
|----------------------------------------|-------------------------------------------------------------------------------|--------------|
| Arborinone (1)                         | *P. bistorta* (L.) Samp. (root)                                               | (22)         |
| 25-Hydroxycholest-5-en-3,β-yl acetate (2)| -                                                                             | -            |
| β-sitosterol (3)                       | *P. bistorta* (root)                                                          | (20)         |
|                                       | *P. nepalensis* (Meisn.) Miyabe (whole plant)                                 | (21)         |
|                                       | *P. stagnina* (Buch. Ham.Ex Meisn.) Qaiser (whole plant)                      | (29)         |
| Methyl-4-hydroxy cinnamate (4)         | -                                                                             | -            |
| Protocatechuic acid (5)                | *P. amphibia* (L.) Delarbre (aerial part)                                     | (35, 43)     |
|                                       | *P. bistorta* (rhizome)                                                       | (45)         |
|                                       | *P. capitata* (Buch. Ham.Ex D.Don) H.Gross (whole plant, aerial part)         | (28, 34)     |
|                                       | *P. lapathifolia* (L.) Delarbre, *P. maculosa* Gray, *P. mitis* (Schrank) Holub (aerial part) | (35, 43)     |
| Gallic acid (6)                        | *P. amphibia* (whole plant, aerial part, leaf)                                | (41, 43, 46) |
|                                       | *P. capitata* (whole plant, aerial part)                                      | (28, 34)     |
|                                       | *P. lapathifolia* (aerial part)                                               | (43)         |
|                                       | *P. chinensis* (L.) H. Gross, *P. maculosa*, *P. nepalensis* (not mentioned)  | (42)         |
| Methyl gallate (7)                     | *P. lapathifolia* (aerial part)                                               | (44)         |
| Quercetin (8)                          | *P. amphibia* (whole plant, flower, leaf, stem)                               | (41)         |
|                                       | *P. amplexicaulis* (D.Don) Ronse Decq. (rhizome)                              | (36, 37)     |
|                                       | *P. bistorta* (aerial part)                                                   | (47)         |
|                                       | *P. capitata* (whole plant, aerial part)                                      | (18, 34)     |
|                                       | *P. chinensis* (not mentioned)                                                | (42)         |
|                                       | *P. decipiens* (R.Bt.) K.L. Wilson (aerial part)                              | (48)         |
|                                       | *P. glabra* (Willd.) M.Gómez (leaf)                                           | (49)         |
|                                       | *P. lapathifolia* (whole plant, aerial part, leaf, stem)                      | (47, 50, 51) |
|                                       | *P. maculosa* (leaf)                                                          | (52)         |
|                                       | *P. mitis* (aerial part)                                                      | (7)          |
|                                       | *P. semiglauca* (Meisn.) Sojak (aerial part)                                  | (48)         |
|                                       | *P. tinctora* (Aiton)H.Gross (leaf, stem)                                    | (32)         |
| Vanicoside A (9)                       | *P. pensylvanica* (L.) M. Gómez (leaf, stem, root)                            | (14)         |
|                                       | *P. hydropiper* (L.) Delarbre (whole plant)                                  | (39)         |
| Vanicoside B (10)                      | *P. pensylvanica* (leaf, stem, root)                                         | (14)         |
|                                       | *P. hydropiper* (whole plant)                                                 | (39)         |
|                                       | *P. lapathifolia* (aerial part)                                               | (40)         |
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