Agr. Nat. Resour. 58 (2024) 59–66

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Tyrosinase inhibitors from fruits of *Piper sarmentosum*: Isolation, characterization and structure-activity relationship study

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Article Info

Article history:
Received 25 September 2023
Revised 20 November 2023
Accepted 11 January 2024
Available online 14 February 2024

Keywords:
Phenylpropanoids,
Wild pepper,
Piper species,
Tyrosinase inhibition

Abstract

Importance of the work: *Piper sarmentosum* has long been used in Thai cuisine and traditional medicine. This was the first report on tyrosinase inhibition from the fruits of *P. sarmentosum*.

Objectives: To isolate and characterize the secondary compounds from the fruits of *P. sarmentosum* together with evaluation of their tyrosinase inhibitory activity.

Materials and Methods: The CH_2Cl_2 and acetone extracts were separated and purified using a variety of chromatographic techniques. The structures of isolated compounds were identified based on their spectroscopic data, particularly nuclear magnetic resonance. The tyrosinase inhibitory activity of the isolated compounds was evaluated using the colorimetric method with kojic acid as the positive control.

Results: In total, 21 compounds were isolated from the *P. sarmentosum* fruits, consisting of 6 phenylpropanoids, 9 phenylpropanamides, 5 alkyl amides and 1 lactone. Compounds 4, 9, and 18 had the most potent tyrosinase inhibitory activity with values for the concentration at 50% inhibition of 1.7 mM, 2.7 mM and 2.8 mM, respectively.

Main finding: Phenylpropanoids, phenylpropanamides and alkyl amides showed potent tyrosinase inhibition. This was the first report on tyrosinase inhibition in this plant. Therefore, the fruits of *P. sarmentosum* have potential for further research for their use in cosmetic and pharmaceutical applications.

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https://doi.org/10.34044/j.anres.2024.58.1.07

Introduction

Tyrosinase is a copper-containing enzyme that plays a crucial role in melanin synthesis, which is responsible for skin coloration (Chang, 2009). However, overproduction of melanin can lead to hyperpigmentation and other related skin disorders (Qian et al., 2020). Therefore, inhibition of tyrosinase activity has become an important strategy for the treatment of various skin conditions, including hyperpigmentation and skin aging. Numerous studies have demonstrated that natural compounds, such as kojic acid, arbutin, and azelaic acid, have potent tyrosinase inhibition (Chang, 2009; Couteau and Coiffard, 2016; Zolghadri et al., 2019; Qian et al., 2020). However, some of these compounds caused undesirable adverse effects, such as kojic acid manifesting as redness, irritation, itchiness and a rash (Couteau and Coiffard, 2016). Furthermore, arbutin and kojic acid are unstable (Pillaiyar et al., 2017). Several studies have been conducted on natural sources of tyrosinase inhibitors from various plants, including Thai medicinal plants (Chang, 2009; Zolghadri et al., 2019).

Piper sarmentosum is a climbing plant whose fruit has been used as a seasoning and traditional medicine (Singtongratana et al., 2013; Sun et al., 2020). Studies have identified antimicrobial, antiplatelet, α-glucosidase inhibition, antituberculosis and antiplasmodial activities associated with this plant (Rukachaisirikul et al., 2004; Li et al., 2007; Dumsud et al., 2013; Chanprapai and Chavasiri, 2017). The major components of *P. sarmentosum* are alkyl amides such as antituberculosis agents named pellitorine and guineensine (Rukachaisirikul et al., 2004) and phenylpropanoids such as α-glucosidase inhibitor named chaplupyrrolidone B (Dumsud et al., 2013). However, to date, no research has been conducted on the tyrosinase inhibitory activity of *P. sarmentosum*. Therefore, the current aimed to isolate secondary metabolites from the fruits of *P. sarmentosum* and evaluate their tyrosinase inhibitory activity.

Materials and Methods

General

The organic solvents (MeOH, CH_2Cl_2, EtOAc and *n*-hexane) used as mobile phases in column chromatography were commercial grade and utilized without further purification. Thin layer chromatography (TLC) and preparative TLC were performed on silica gel 60 F_{254} (Merck) and visualized using short-wave ultraviolet (UV) light and 5% anisaldehyde reagent. Column chromatography was carried out on silica gel (Merck, 70–30 mesh), and Sephadex LH-20. Medium-pressure liquid chromatography (MPLC) was carried out on a Biotage-Isolera unit. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL (500 MHz) JNM–ECZ500R/S1 NMR spectrometer.

Plant material

Fruits of *P. sarmentosum* were collected during September–December 2017 at the CU Centenary Park, Chulalongkorn University, Bangkok, Thailand. The specimens (CENP-PP012-2018) were deposited in the herbarium at the Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Extraction and isolation

The dried fruits (900 g) of *P. sarmentosum* were separately extracted using MeOH (3×2.5 L) at room temperature for 2 d. After filtration, the filtrate was evaporated and suspended in H_2O and further partitioned with hexane, CH_2Cl_2 and acetone to produce extracts of hexane (50.0 g), CH_2Cl_2 (85.2 g) and acetone (6.30 g). The CH_2Cl_2 extract was separated using column chromatography over silica gel eluted with hexane, hexane-to-EtOAc ratios (5:95, 10:90, 15:85, 20:80, 30:70, 40:60, 50:50, 40:60, 20:80, 0:100) EtOAc-to-MeOH ratios (95:5, 90:10, 80:20, 60:40, 40:60, 20:80, 0:100) and MeOH to produce seven fractions (DS1–DS7).

The fraction DS2 was separated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (2A–2C). Compound 1 was obtained as a pure state in subfraction 2B. Subfraction 2C was purified using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) followed by silica gel column chromatography (CC) eluted with a hexane-to-CH_2Cl_2-to-EtOAc ratio of 13.0:0.2:0.2 to produce compounds 2, 3 and 4.

The fraction DS3 was separated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (3A–3C). Subfraction 3B was purified using C-18 reverse phase flash column chromatography (FCC) using a gradient solvent system with a MeOH-to-H_2O ratios of 30:70 through to 100:0 to produce compound 5. Compound 9 was obtained as a major component of subfraction 3C.

The fraction DS4 was separated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (4A–4C). The subfraction 4B was isolated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (4B1–4B3). Subfraction 4B2 was isolated using Sephadex LH-20 (1:9 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (B2A–B2C). The subfraction B2B was purified using silica gel CC with a ratio of hexane-to-CH_2Cl_2-to-EtOAc (3:1:1) to produce three subfractions (B1–B3). Subfraction B2 was purified using silica gel CC with a ratio of hexane-to-CH_2Cl_2-to-EtOAc (3.0:1.0:1.0) to produce two subfractions (21–22). Subfraction 22 was purified using silica gel CC with a ratio of hexane-to-CH_2Cl_2-to-EtOAc (3.0:1.0:1.0) to produce three subfractions (22A–22C). Compound 7 was obtained as a major component of subfraction 22A. Subfraction 22B was purified using silica gel CC with a ratio of hexane-to-CH_2Cl_2-to-EtOAc (3.0:1.0:1.0) to produce compounds 7 and 8. Subfraction 22C was purified using silica gel CC (9:1 CH_2Cl_2-to-EtOAc ratio) to produce compound 6. Subfraction 4C was purified using silica gel CC with a ratio of hexane-to-CH_2Cl_2-to-EtOAc (3.0:1.0:1.0) to produce four subfractions (C1–C4). Subfraction C2 was purified using silica gel CC with a ratio of acetone-to-CH_2Cl_2 (2:98) to produce compound 10. Subfraction C4 was obtained in pure state as compound 11.

The fraction DS5 was isolated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce four subfractions (5A–5D). Subfraction 5B was recrystallized to produce compound 12. Subfraction 5C was separated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (5C1–5C3). Subfraction 5C2 was purified using silica gel CC (1:1 EtOAc-CH_2Cl_2 ratio) to produce two subfractions (5C21–5C22). Subfraction 5C22 was purified using C-18 reverse phase FCC with a gradient solvent system with ratios of MeOH-to-H_2O (30:70 through to 100:0) to produce compound 13. Subfraction 5C3 was isolated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (5C3A–5C3C). Subfraction 5C3B was separated using silica gel CC with a step gradient of ratios of MeOH-to-CH_2Cl_2 (5:95, 10:90, 15:85, 20:80, 30:70, 40:60, 50:50) to produce two subfractions (B1–B2). Subfraction B2 was purified using C-18 reverse phase FCC with a gradient solvent system of ratios of MeOH-to-H_2O (30:70 through to 100:0) to produce compound 14. Subfraction 5D was isolated using Sephadex LH-20 (1:1 MeOH-CH_2Cl_2 ratio) to produce two subfractions (5D1–5D2). Subfraction 5D1 was separated using C-18 reverse phase FCC with a gradient solvent system of ratios of MeOH-to-H_2O (30:70 through to 100:0) to produce subfraction (D22A). Subfraction D22A was purified using silica gel CC with a ratio of CH_2Cl_2-to-MeOH-to-EtOAc (4.0:0.5:0.5) followed by further separation using C-18 reverse phase FCC with a gradient solvent system of ratios of MeOH-to-H_2O (30:70 through to 100:0) to produce compound 15. Subfraction 5D2 was isolated using Sephadex LH-20 with a ratio of MeOH-to-CH_2Cl_2 (1:1) followed by silica gel CC with a step gradient of ratios of MeOH-to-CH_2Cl_2 (0:100 through to 50:50) to produce compound 20**.**

The fraction DS6 was isolated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (6A–6C). Subfraction 6B was isolated using Sephadex LH-20 (1:1 MeOH-CH_2Cl_2 ratio) to produce three subfractions (6B1–6B3). Subfraction 6B2 was purified using silica gel CC with a step gradient of ratios of MeOH-to-CH_2Cl_2 (0:100 through to 50:50) to produce two subfractions (B21–B22). Subfraction B22 was separated using Sephadex LH-20 using a MeOH-to-CH_2Cl_2 ratio (1:1) to produce compound 16.

The acetone extract was fractionated using a method similar to that for the fractionation of the dichloromethane extract, yielding eight fractions (ES1–ES8).

The fraction ES5 was separated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce three subfractions (5A–5C). Subfraction 5C was purified using Sephadex LH-20 with a ratio of MeOH-to-CH_2Cl_2 (1:1) to produce two subfractions (53A–53B). Subfraction 53B was separated using C-18 reverse phase FCC with a step gradient solvent system of ratios of MeOH-to-H_2O (20:80 through to 100:0) to produce compound 17.

The fraction ES7 was separated using Sephadex LH-20 with a ratio of MeOH-to-CH_2Cl_2 (1:1) to produce five subfractions (7A–7E). Subfraction 7B was isolated using Sephadex LH-20 (1:1 MeOH-to-CH_2Cl_2 ratio) to produce four subfractions (BA–BD). Subfraction BB was purified using silica gel CC with an MeOH-to-CH_2Cl_2 ratio (2:98) to produce three subfractions (BB1–BB3). Subfraction BB2 was separated using C-18 reverse phase FCC with a step gradient solvent system of ratios of MeOH-to-H_2O (20:80 through to 100:0) to produce compound 18. Subfraction BC was isolated using C-18 reverse phase FCC with a step gradient solvent system of ratios of MeOH-to-H_2O (20:80 through to 100:0) to produce compounds 18 and 19. Subfraction 7E was separated using Sephadex LH-20 with a ratio of MeOH-to-CH_2Cl_2 (1:1) to produce three subfractions (7E1–7E3). Finally, subfraction 7E2 was separated using C-18 reverse phase FCC with a step gradient solvent system of ratios of MeOH-to-H_2O (20:80 through to 100:0) to obtain compound 21.

Elemicin (1) brown gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.40 (*s*, 1H), 5.95 (*m*, 1H), 5.13 (*m*, 1H), 5.08 (*m*, 1H), 3.84 (*s*, 6H), 3.81 (*s*, 3H), and 3.33 (*d*, 6.7, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 153.3x2, 137.3, 135.9, 116.1, 105.6x3, 60.9, 56.2, 56.2, 40.6 (Miyazawa and Kohno, 2005).

1-Allyl-2-methoxy-4,5-methylenedioxybenzene (2) brown gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.38 (*brs*, 1H), 6.35 (*brs*, 1H), 5.94 (*m*, 1H), 5.91 (*s*, 2H), 5.10 (*m*, 1H), 5.08 (*m*, 1H), 3.88 (*s*, 3H) and 3.28 (*d*, 6.7, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 148.9, 143.5, 137.4, 134.5, 133.5, 115.7, 107.8, 102.6, 101.2, 56.5, 40.1 (Zheng et al., 1992).

N-(3-phenylpropanoyl) pyrrole (3) brown gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.19–7.30 (*m*, 5H), 7.19–7.30 (*m*, 2H), and 6.26 (*t*, 2.5, 2H) and 3.08–3.09 (*m*, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) 169.6, 140.1, 128.6, 126.4, 128.3, 118.9, 113.1, 36.3, 30.2 (Likuitwitayawuid et al., 1987).

3-(4-Methoxy-phenyl)-1-(1*H*-pyrrol-1-yl) propan-1-one (4) brown gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.29 (*brs*, 2H), 7.17 (*d*, 8.2, 1H), 6.83 (*d*, 8.2, 1H) 6.28 (*t*, 2.5, 2H), 3.79 (*s*, 3H), 3.10 (*m*, 2H), and 3.08 (*m*, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 169.9, 158.2, 132.1, 129.4, 118.9, 114.0, 113.1, 55.3, 36.7 and 29.5 (Maehara et al., 2012).

Pellitorine (5) yellow gum; ^{1}H and ^{13}C NMR ($CDCl_3$, 500 MHz) 7.16 (*dd*, 15.0, 10.4, 1H), 6.09 (*dd*, 15.0, 10.4, 1H), 6.02 (*m*, 1H), 5.78 (*d*, 15.0, 1H), 3.13 (*t*, 6.5, 2H), 2.11 (*q*, 7.1, 1H), 1.77 (*m*, 1H), 1.38 (*m*, 2H), 1.27 (*m*, 4H), 0.88 (*d*, 6.4, 6H), 0.86 (*t*, 7.0, 3H); HRMS m/z 246.1840 $[M+Na]^+$, molecular formula $C_{14}H_{25}NONa$ (Park et al., 2002).

(2*E*, 4*Z*-decadienoyl)pyrrolidine (6) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.62 (*dd*, 12.5, 11.8, 1H), 6.16 (*d*, 14.7, 1H), 6.12 (*t*, 11.5, 1H), 5.78 (*m*, 1H), 3.52–3.54 (*t*, 6.8, 4H), 2.30 (*q*, 7.4, 2H), 1.96 (*quint*, 7.1, 2H), 1.87 (*quint*, 7.1, 2H), 1.27 (*m*, 4H) and 0.87 (*t*, 7.0, 3H); HRMS m/z 246.1840 $[M+Na]^+$, molecular formula $C_{14}H_{25}NONa$ (Huang et al., 2010).

Guineensine (7) white solid; ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz) 7.21 (*dd*, 15.2, 10.8, 1H), 6.91 (*brs*, 1H), 6.77 (*m*, 2H), 6.31 (*d*, 15.0), 6.09 (*m*, 3H), 5.95 (*s*, 2H), 5.78 (*d*, 15.0, 1H), 3.19 (*t*, 6.36, 2H), 2.20 (*m*, 4H), 1.82 (*m*, 1H), 0.95 (*d*, 6.7, 6H), 1.44 (*m*, 8H); HRMS m/z 406.2358 $[M+Na]^+$, molecular formula $C_{24}H_{33}NO_3Na$ (Park et al., 2002).

Pipercide (8) white solid; ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz) 7.18 (*dd*, 15.2, 10.8, 1H), 6.91 (*brs*, 1H), 6.73 (*m*, 2H), 6.31 (*d*, 15.0, 1H), 6.11 (*m*, 1H), 6.09 (*m*, 1H), 6.06 (*m*, 1H), 5.92 (*s*, 2H), 5.75 (*d*, 15.0, 1H), 3.19 (*t*, 6.4, 2H), 2.20 (*m*, 4H), 1.80 (*m*, 1H), 0.91 (*d*, 6.7, 6H), 1.44 (*m*, 8H); HRMS m/z 356.2227 $[M+H]^+$, molecular formula $C_{22}H_{30}NO_3$ (Park et al., 2002).

Benzenepropanoic acid (9) brown gum; ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz) 7.18–7.31 (*m*, 5H), 2.53 (*t*, 7.7, 2H), 2.97 (*t*, 7.7, 2H) (Monguchi et al., 2011).

Cinnamopyrrolidide (10) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.70 (*d*, 15.5, 1H), 7.53 (*dd*, 8.1, 1.8, 2H), 7.36 (*m*, 2H), 6.73 (*d*, 15.5, 1H), 3.60–3.64 (*t*, 6.9, 4H) and 1.91–2.01 (*m*, 4H) (Huang et al., 2010).

Sarmentamide A (11) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.17 (*m*, 1H), 7.20 (*m*, 1H), 4.41 (*t*, 2.1, 1H), 3.30 (*t*, 7.8, 2H), 3.01 (*t*, 7.8, 2H), 7.29 (*m*, 5H) (Tuntiwachwuttikul et al., 2006).

3,4,5–trimethoxycinanamoyl pyrrolidine (12) white solid; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.61 (*d*, 15.4, 1H), 6.75 (*s*, 2H), 6.63 (*d*, 15.4, 1H), 3.90 (*s*, 6H), 3.87 (*s*, 3H), 3.65 (*t*, 6.9, 4H), 3.56 (*t*, 6.9, 4H), 2.01 (*quint*, 6.9, 2H), 1.91 (*quint*, 6.9, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 164.5, 153.2x2, 141.6139.3, 130.7, 117.9, 104.8x2, 60.7, 56.0x2, 46.5, 45.9, 29.5, 24.2 (Li et al., 2007).

Sarmentamide B (13) brown gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 3.87 (*dd*, 4.5, 12, 1H), 4.35 (*brd*, 4.5, 1H), 5.11 (*brd*, 4.5, 1H), 3.15 (*brd*, 13.3, 1H), 3.95 (*dd*, 4.5, 14.0, 1H), 4.07 (*dd*, 4.5, 14.0, 1H), 6.63 (*d*, 15.5 Hz, 1H), 7.67 (*d*, 15.5, 1H), 7.55 (*m*, 2H), 7.40 (*m*, 3H), 2.05 (*s*, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 170.5x2, 165.7, 143.0, 130.0, 128.8, 128.1, 134.8, 117.8, 73.5, 71.4, 50.3, 49.6, 21.1x2 (Tuntiwachwuttikul et al., 2006).

Deacetylsarmentamide B (14) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 3.66–3.70 (*m*, 2H), 4.17 (*brd*, 3.1, 1H), 4.11 (*brd*, 3.8, 1H), 3.58 (*d*, 13.3, 1H), 3.92 (*dd*, 4.1, 11.4, 1H), 6.92 (*d*, 15.5, 1H), 7.59 (*d*, 15.5 Hz, 1H), 7.62 (*m*, 2H), 7.38 (*m*, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) 166.4, 143.6, 134.8, 131.1, 129.9, 129.2, 119.4, 74.7, 73.5, 53.7, 53.1 (Dumsud et al., 2013).

(3*S*,4*R*)-3,4,5-Trihydroxypentanoic acid 1,4-lactone (15) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 2.36 (*dd*, 18.0, 2.5, 1H), 2.90 (*dd*, 18.0, 6.8, 1H), 4.41 (*dt*, 4.4, 2.3, 1H), 4.35 (*brq*, 3.5, 1H), 3.65–3.76 (*dd*, 12.4, 3.3, 2H): ^{13}C NMR ($CDCl_3$, 125 MHz) 89.4, 68.9, 61.2, 38.2 (Fernández et al., 1990).

Mixture of (1*E*,3*S*)-1-cinnamoyl-3-hydroxypyrrolidine (16) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) *trans* 3.57-3.71 (*m*, 2H), 3.57-3.71 (*m*, 2H), 4.45 (*m*, 1H), 2.00 (*m*, 2H), 3.72 (*m*, 2H), 6.91 (*d*, 15.5, 1H), 7.60 (*d*, 15.5, 1H), 7.62 (*m*, 2H), 7.38 (*m*, 3H): ^{13}C NMR ($CDCl_3$, 125 MHz) 167.3, 143.4, 136.6, 131.0, 129.9, 129.1, 119.6, 71.7, 55.9, 45.2, 33.5; *cis* 3.57-3.71 (*m*, 2H), 3.57–3.71 (*m*, 2H), 4.51 (*m*, 1H), 2.08 (*m*, 2H), 3.82 (*m*, 2H), 6.97 (*d*, 15.5, 1H), 7.60 (*d*, 15.5, 1H), 7.62 (*m*, 2H), 7.38 (*m*, 3H): ^{13}C NMR ($CDCl_3$, 125 MHz) 167.3, 143.4, 136.6, 131.0, 129.9, 129.1, 119.6, 70.1, 55.4, 45.9, 35.0; HREIMS *m/z* 240 $[M+Na]^+$ (calcd. for 240.1011 $C_{13}H_{15}NO_2Na$) (Shi et al., 2017).

Chaplupyrrolidone A (17) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.18 (*dd*, 6.1, 0.5, 1H), 7.29 (*dd*, 6.1, 0.5, 1H), 6.12 (*brs*, 1H), 3.25 (*m*, 2H), 2.97 (*t*, 7.7, 2H), 7.19–7.26 (*m*, 5H) (Dumsud et al., 2013).

Sarmentosine (18) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.12 (*td*, 15.3, 1.5, 1H), 6.92 (*td*, 15.3, 6.9, 1H), 2.34 (*m*, 4H), 6.92 (*td*, 15.3, 6.9, 1H), 6.02 (*td*, 15.7, 6.7, 1H), 6.31 (*td*, 15.7, 1.3, 1H), 6.86 (*d*, 1.5, 1H), 6.72 (*m*, 2H), 3.50 (*t*, 6.9, 2H), 1.94 (*m*, 2H), 1.84 (*m*, 2H), 3.48 (*t*, 6.9, 2H), 5.92 (*s*, 2H) (Likuitwitayawuid et al., 1987).

Piperlotine-A (19) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 3.62 (*t*, 6.8, 2H), 2.00 (*m*, 2H), 1.89 (*m*, 2H), 3.59 (*t*, 6.8, 2H), 6.60 (*d*, 15.5, 1H), 7.65 (*d*, 15.5, 1H), 7.48 (*d*, 8.8, 2H), 6.89 (*d*, 8.8, 2H), 3.83 (*s*, 3H) (Li et al., 2007).

N-trans-feruloyl tyramine (20) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 7.09 (*d*, 2.0, 1H), 6.77 (*d*, 8.2, 1H), 6.92 (*dd*, 2.0, 8.2, 1H), 7.40 (*d*, 15.7, 1H), 6.37 (*d*, 15.7, 1H), 7.02 (*d*, 8.3, 2H), 6.69 (*d*, 8.3, 2H), 2.76 (*t*, 7.0, 2H), 3.47 (*t*, 7.0, 2H) (King and Calhoun, 2005).

Coumaric acid (21) colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz) 6.45 (*d*, 15.5, 1H), 7.78 (*d,* 15.5, 1H), 7.40 (*m*, 2H), 7.55 (*m*, 3H) (Kalinowska et al., 2007).

Tyrosinase inhibition

Anti-tyrosinase activity was conducted as described by Ersoy et al. (2019) using L-DOPA as the substrate and kojic acid as a positive control. Briefly, 50 µL of tyrosinase (165 U/mL) was mixed with 20 µL of sample or kojic acid. All sample solutions were incubated at 37°C for 10 min and 300 µL of L-DOPA was added. The reactions were incubated at 37°C for 20 min. The absorbance was measured using spectrophotometry at 475 nm. A blank assay was conducted. The bioassay was performed in triplicate. The inhibition percentage of tyrosinase activity was calculated as: inhibition (%) = $(A_{control} - A_{sample}) / A_{control} \times 100$, where $A_{control}$ is the absorbance of the enzyme activity and A_{sample} is the absorbance of enzyme activity in the addition of the sample solution. The concentration at 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage and sample concentration.

Results and Discussion

The fruits of *P. sarmentosum* were sequentially extracted using organic solvents to produce hexane, dichloromethane, methanol and acetone extracts. The extraction procedure is summarized in Fig. 1. The chemical components of each extract were preliminarily screened using TLC. According to the TLC profiles, the CH_2Cl_2 and acetone extracts produced a series of spots, belonging to phenylpropanoids and alkylamides, the representative metabolites of the genus *Piper* (Rukachaisirikul et al., 2004). The dichloromethane and acetone extracts were independently fractionated and isolated using a series of chromatographic techniques until pure compounds were obtained. Details of isolation are summarized in Figs. 2–4.

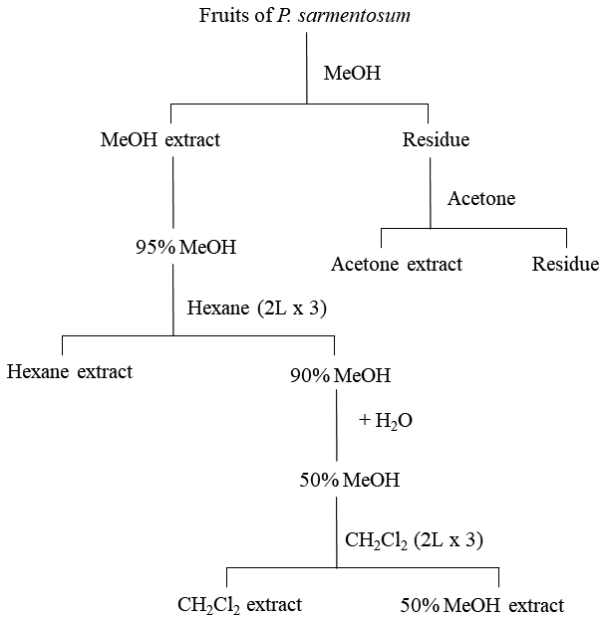

Fig. 1 Extraction scheme of *Piper sarmentosum* fruits

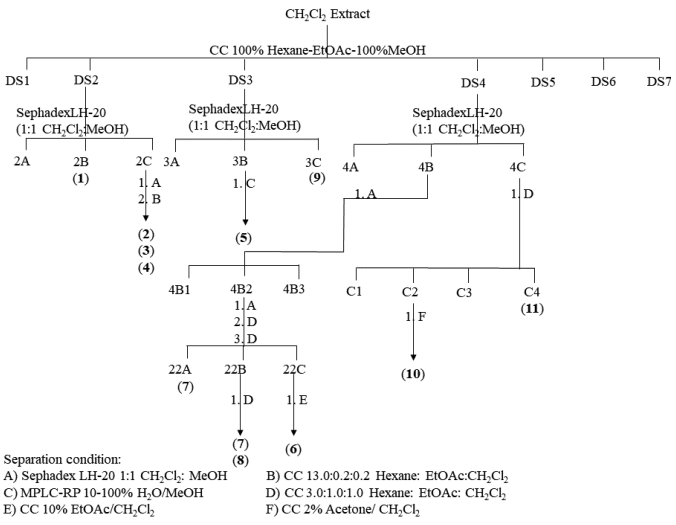

Fig. 2 Chromatographic separation of CH_2Cl_2 extract focusing on fractions DS2, DS3 and DS4, where MPLC-RP = medium-pressure liquid chromatography-reverse phase and CC = column chromatography

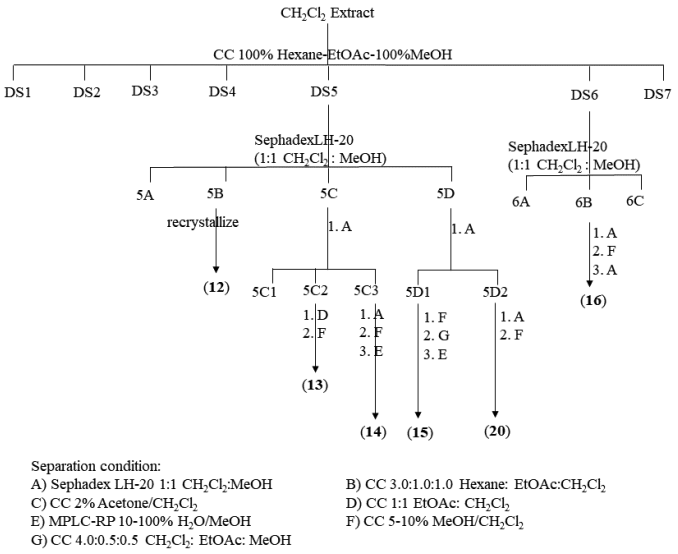

Fig. 3 Chromatographic separation of CH_2Cl_2 extract focusing on fractions DS5 and DS6, where MPLC-RP = medium-pressure liquid chromatography-reverse phase and CC = column chromatography

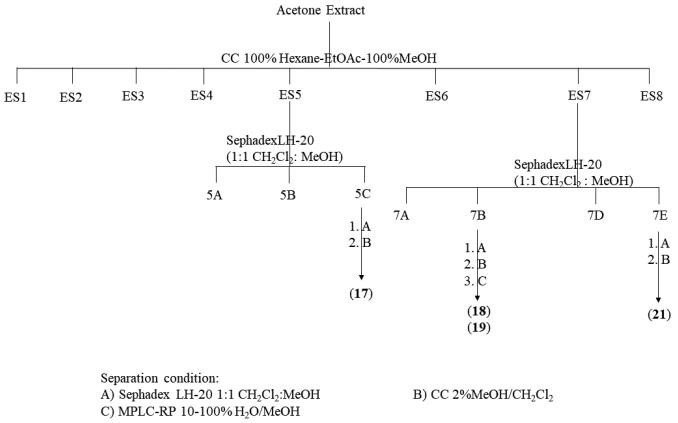

Fig. 4 Chromatographic separation of acetone extract, where MPLC-RP = medium-pressure liquid chromatography-reverse phase and CC = column chromatography

In total, 21 compounds were isolated from the CH_2Cl_2 and acetone extracts of the fruits of *P. sarmentosum*. Based on comparisons with their NMR and MS data with other reports, the compounds were identified as: elemicin (1) (Miyazawa and Kohno, 2005); 1-allyl-2-methoxy-4, 5-methylenedioxybenzene (2) (Zheng et al., 1992); *N*-(3-phenylpropanoyl) pyrrole (3) (Likuitwitayawuid et al., 1987); 3-(4-methoxy-phenyl)-1-(1*H*-pyrrol-1-yl) propan-1-one (4) (Maehara et al., 2012); pellitorine (5) (Park et al., 2002); (2*E*, 4*Z*-decadienoyl)pyrrolidine (6) (Huang et al., 2010); guineensine (7) and pipercide (8) (Park et al., 2002); benzenepropanoic acid (9) (Monguchi et al., 2011); cinnamopyrrolidide (10) (Huang et al., 2010);, sarmentamide A (11) (Tuntiwachwuttikul et al., 2006); 3,4,5-trimethoxycinanamoyl pyrrolidine (12) (Li et al., 2007); sarmentamide B (13) (Tuntiwachwuttikul et al., 2006); deacetylsarmentamide B (14) (Dumsud et al., 2013); (3*S*, 4*R*) -3, 4,5-trihydroxypentanoic acid 1, 4-lactone (15) (Fernández et al., 1990); (1*E*, 3*S*)-1-cinnamoyl-3-hydroxypyrrolidine (16) (Shi et al., 2017); chaplupyrrolidone A (17) (Dumsud et al., 2013); sarmentosine (18) (Likuitwitayawuid et al., 1987); piperlotine-A (19) (Li et al., 2007); *N*-trans-feruloyl tyramine (20) (King and Calhoun, 2005); and cinnamic acid (21) (Kalinowska et al., 2007). The chemical structures of all isolated compound are shown in Fig 5.

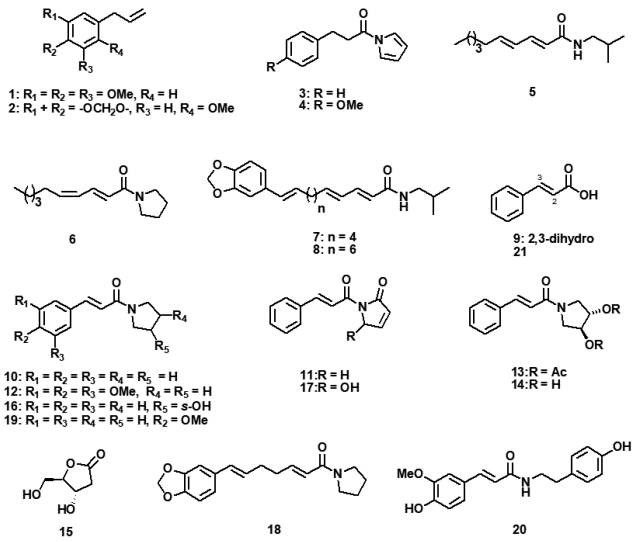

Fig. 5 Chemical structures of compounds 1–20

All isolated compounds were evaluated for tyrosinase inhibition (Table 1) except compounds 6 and 10 due to their limited available amounts.

Table 1 Tyrosinase inhibition of isolated compounds

Compound	IC_{50} ± SD (mM)	Compound	IC_{50} ± SD (mM)
1	4.06±0.01	12	3.44±0.11
2	3.64±0.13	13	NA
3	5.37±0.07	14	6.56±0.55
4	2.65±0.14	15	NA
5	5.55±0.21	16	4.65±0.17
6	ND	17	NA
7	NA	18	2.77±0.31
8	NA	19	NA
9	1.68±0.04	20	NA
10	ND	21	3.19±0.29
11	13.35+0.47	Kojic acid	0.07±0.01

IC_{50} = concentration at 50% inhibition; ND = not determined; NA = not active

All isolated compounds could be classified into three types: phenylpropanoid (C_6-C_3), phenylpropanamide (C_6-C_3-N), and alkyl amide (R-CO-N). Generally, the phenylpropanoids 1, 2, 9 and 21 had the greatest tyrosinase inhibitory activity with IC_{50} values of 4.1 mM, 3.6 mM, 1.7 mM and 3.1 mM, respectively. The presence of propanoic acid in compounds 9 and 21 was likely to enhance inhibition more than the occurrence of allyl groups in compounds 1 and 2. Although the structures of compounds 9 and 21 were closely related, the inhibitory effect of compound 9 was twice that of compound 21. The presence of saturation in compound 9 played an important role in its inhibiting tyrosinase function. Hydroxy cinnamic acids are well known to inhibit tyrosinase function along with an antioxidant property (Takahashi and Miyazawa, 2010). Due to the greatest abundance of compound 9 in the extracts of *P. sarmentosum*, it could be expected that compound 9 is a major active contributor against tyrosinase. Elemicin (1) has been reported to exhibit a wide range of pharmacological effects, encompassing antimicrobial anti-acetylcholinesterase, antiviral and antioxidant properties (Wang et al., 2019).

Generally, the phenylpropanamides were equipotent inhibitors to phenylpropanoids with IC_{50} values in range 2.7–13.3 mM. However, some of them (compounds 13, 17, 19 and 21) were not active against tyrosinase. Although the isolated phenylpropanamides considerably varied in substituted groups and inhibition, some major correlations among them were detected. For example, 3,4,5-trimethoxycinanamoyl pyrrolidine (compound 12) showed tyrosinase inhibition with an IC_{50} value of 3.4 mM, whereas piperlotine-A (compound 19), whose structure comprised one methoxy group, exhibited no tyrosinase inhibition. This result suggested that the increase in methoxy groups on an aromatic ring improved tyrosinase inhibition. This conclusion was also consistent with the more potent inhibition of compound 4 (IC_{50} of 2.7 mM) than of compound 3 (IC_{50} of 5.4 mM). In addition to the methoxy group, the presence of an hydroxy group affected inhibition against tyrosinase depending on its location on the heterocyclic ring. In pyrrolidine-containing phenylpropanamides, the hydroxy groups enhanced the inhibitory effects of compounds 14 and 16 with IC_{50} values of 6.6 mM and 4.7 mM, respectively, whereas the phenylpropanamide 13 with no hydroxy was not active. On the other hand, the presence of a hydroxy group in Δ^3-2-pyrrolidone containing phenylpropanamides leads to a decrease in tyrosinase inhibition. This observation was clearly exemplified by sarmentamide A (compound 11), which had an IC_{50} value of 13.3 mM, while its dehydroxy congener (compound 17) was not active. 3,4,5-Trimethoxycinanamoyl pyrrolidine (compound 12), a major component from this plant, showed potent antiplatelet aggregation activity (Li et al., 2007).

Generally, the alkyl amides showed no tyrosinase inhibitory activity except for pellitorine (compound 5) and sarmentosine (compound 18), which exhibited inhibitory activity with IC_{50} values of 5.6 mM and 2.8 mM, respectively. Noticeably, the alkyl amides 5 and 18 comprised relatively shorter alkyl chain than the others, suggesting that less hydrophobicity improved the inhibitory effect against tyrosinase. Pellitorine (compound 5) and guineensine (compound 7), major compounds isolated from this plant, exhibited cytotoxicity (Muharini et al., 2015; Ratwatthananon et al., 2020).

In conclusion, the fruits of *P. sarmentosum* yielded 21 compounds, consisting of 6 phenylpropanoids (compounds 1–4, 9 and 21), 9 phenylpropamides (compounds 10–14, 16–17 and 19–20), 5 alkyl amides (compounds 5–8 and 18) and 1 lactone (compound 15). Of the isolated compounds, the phenylpropanoids displayed the most potent tyrosinase inhibitory activity, followed by the phenylpropanamides and alkyl amides. The lack of an unsaturated phenylpropanoid core structure enhanced tyrosinase inhibition, which was exemplified by benzenepropanoic acid (compound 9). Although there have been several reports of phenylpropanamides and alkyl amides as tyrosinase inhibitors, this was the first comprehensive report on tyrosinase inhibitory metabolites from *P. sarmentosum*. Further studies are needed to elucidate their potential as tyrosinase inhibitors in cosmetic and pharmaceutical applications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was supported by the Center of Excellence in Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University, through the Ratchadapisek Sompoch Endowment Fund. TM received support from the Ratchadapisek Somphot Fund for Postdoctoral Fellowship, Chulalongkorn University.

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