

## Resveratrol oligomers from roots of *Ampelocissus martini* Planch.

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### ABSTRACT

Phytochemical investigation of roots of *Ampelocissus martini* Planch. (Vitaceae family) led to the isolation of five resveratrol oligomers comprising (–)-ampelopsin F (1), (+)-hopeaphenol (2), (–)-α-viniferin (3), (–)-wilsonol A (4) and (–)-stenophyllol B (5). The structure elucidation was performed using their spectroscopic data as well as comparison with those of literature data. All the isolated compounds were submitted to anti-HIV-1 activities employing the syncytium inhibition assay, using the  $\Delta^{\text{Tat/Rev}}$ MC99 virus and 1A2 cell line system, and the reverse transcriptase (RT) assay. The results revealed that the enzymatic inhibition of tested compounds except compound 1 exhibited the potential inhibition in anti-HIV 1-RT assay with IC<sub>50</sub> in the range of 1.68–5.85 μM better than the positive control, navirapin (IC<sub>50</sub> at 7.77 μM). Based on the results, their activities displayed the RT enzymatic inhibition better than the positive control. In addition, the chemical isolation of *A. martini* was studied for the first time herein. All known secondary metabolites except compound 2 were firstly reported from *Ampelocissus* genus. Furthermore, the chemotaxonomic significance of the isolates was discussed.

### 1. Subject and source

The genus *Ampelocissus* (Vitaceae family) consists of 115 species widespread in the tropical and subtropical regions (Wen et al., 2018). More than 12 species widely distribute in various parts of Thailand (Trias-Blasi et al., 2015). *Ampelocissus martini* Planch., synonym of *A. martinii* or *Vitis martinii* (Planch.) Ridl. (Govaerts et al., 2021), was locally known as wild-grape or Som-Kung in Thai. This plant has been usually found in deciduous forest with bamboo and dipterocarp plants. In general, its morphology is similar to cultivated grapes (*Vitis* genus) including stem and fruit (Jirum et al., 2013; Siripipatthana et al., 2021). Thai people, especially in northern and northeastern regions, consume its fruits and use its various parts as folk medicine. For example, its ground-roots are normally used for antipyretic. In Thai ethnobotanical utilities and traditional medicine, *A. martini* roots have been used as an active ingredient for mild laxative and cough remedy (Vittaya et al., 2019). To our knowledge, chemical constituents along with their biological activities on this plant, *A. martini*, especially its roots have never been previously studied. Thus, we investigate the chemical and

biological constituents of *A. martini* roots. In this study, *A. martini* was collected from Sang-Kha District, Surin Province, Thailand and identified by S.H. (RRU-SH-010).

### 2. Previous work

The crude extracts in different solvents from various parts of *Ampelocissus martini* such as seeds (Simchuer et al., 2018; Siripipatthana et al., 2020), fruits (Jirum et al., 2013), and roots (Srihanam et al., 2019; Siripipatthana et al., 2021) were studied for total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC), total triterpenoid content (TTC), total sterol content (TSC), as well as their biological activities. For example, the methanol extracts of stems and roots of *A. martini* collected from Chumphon Province, Thailand provided a good activity against radical scavenging and anti-microbial assays (Vittaya et al., 2019). From our best searching for the studies of phytochemicals and biological activities of *A. martini*, the isolation of secondary metabolites from various parts of *A. martini* has not been reported.

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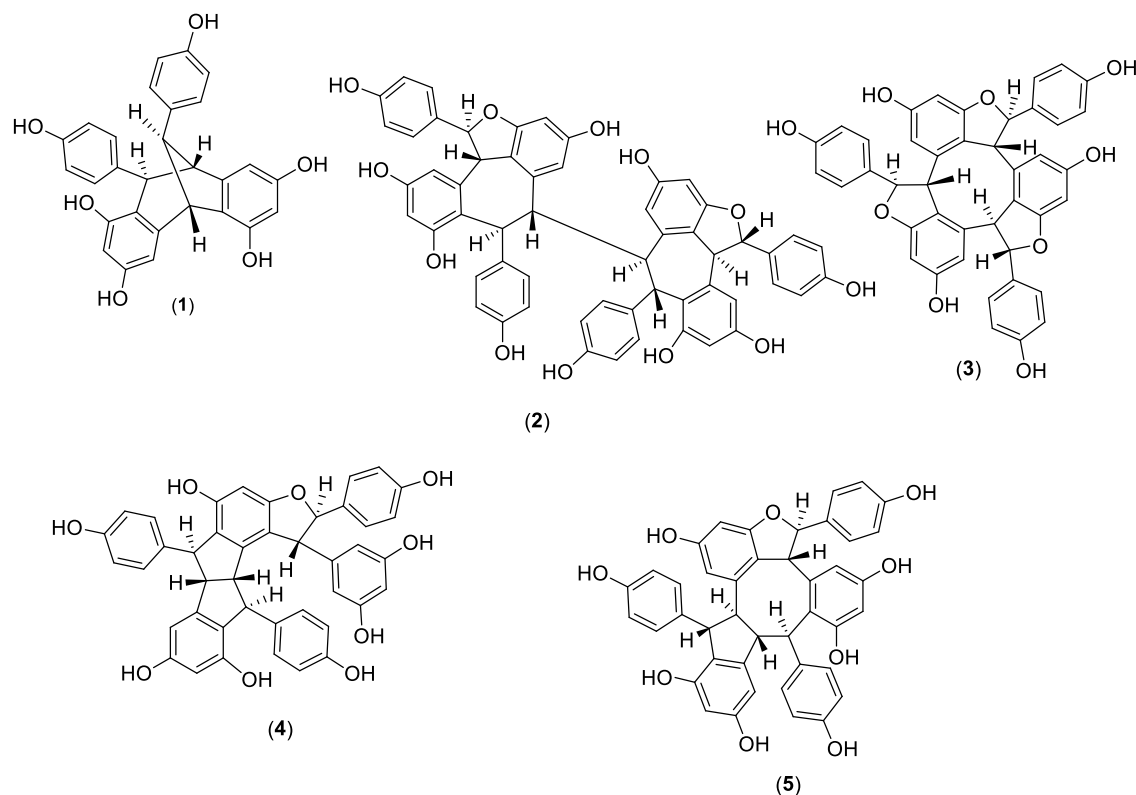


Fig. 1. Isolated compounds 1–5 from *A. martini* roots.

### 3. Present study

#### 3.1. General experimental procedures

Melting points (uncorrected) were measured in degree Celsius (°C) on a digital Electrothermal apparatus. Optical rotations were measured on a digital JASCO DIP 370 polarimeter, using a 50 mm microcell (1 mL). UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 365 spectrophotometer. FT-IR spectra were acquired on Bruker Alpha FT-IR spectrometer. 1D and 2D NMR spectra were recorded on Bruker Ascend™ 400 in deuterated solvent, using residual non-deuterated solvent peaks as an internal reference. HR-TOF-MS were determined on a Micromass VQ-TOF-2 model. Vacuum liquid column chromatography (VLC) and column chromatography (CC) were performed using silica gel (Si-gel) 60G (5–40 μm, E. Merck) and Si-gel 60H (63–200 μm, E. Merck), respectively. Gel filtration was conducted by Sephadex LH-20 (Amersham Biosciences). Pre-coated TLC aluminium sheets of silica gel 60 F<sub>254</sub> (TLC plates, 20 × 20 cm, E. Merck) were used for analytical purposes. Spots on TLC plates were visualized either by ultraviolet light or by anisaldehyde spraying reagent. All solvents used for extraction and isolation were distilled at their boiling point ranges prior to use.

#### 3.2. Extraction and isolation

Air dried and finely powdered roots of *Ampelocissus martini* (320 g) were extracted by maceration method with MeOH (1 L x 3 times) at room temperature. After filtration and removal of solvent under reduced pressure, the crude MeOH extract (19.04 g) was obtained. The crude MeOH extract was fractionated on silica gel by VLC technique, using hexanes, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc and MeOH as eluents to provide four fractions (A1–A4). The EtOAc fraction (5.62 g) was isolated on Sephadex LH-20 CC using MeOH as eluent to provide subfractions B1–B6. Subfraction B5 (877.7 mg) was subjected on Si-gel CC eluted by 40% acetone-hexanes to afford subfractions C1–C10. Subfraction C5 (174.3 mg) was purified

on Sephadex LH-20 CC using MeOH as eluent to provide compound 1 (60.2 mg). After recrystallization of subfraction C7 (282.3 mg), compound 2 (189.0 mg) was obtained. Subfraction B6 (2.39 g) was fractionated by Si-gel CC eluted by 60% EtOAc-hexanes to afford subfractions D1–D11. Subfraction D4 (736.9 mg) was separated by Sephadex LH-20 CC using 70% MeOH-CH<sub>2</sub>Cl<sub>2</sub> as eluent to yield compound 3 (528.2 mg). Subfraction D6 (404.2 mg) was isolated by Sephadex LH-20 CC using MeOH as eluent to provide subfractions E1–E5. Compounds 4 (96.9 mg) and 5 (104.1 mg) were obtained after purification of subfractions E2 (113.8 mg) and E4 (143.2 mg) on Si-gel CC eluted with MeOH:acetone:CH<sub>2</sub>Cl<sub>2</sub> (1:1.5:1.75), respectively.

Chemical structures of the isolated compounds were elucidated on the basis of their spectroscopic data (NMR, MS, UV, IR and OR) as well as comparison with those of previous literature as (–)-ampelopsin F (1) (Luo et al., 2001; Ito et al., 2013; Indriani et al., 2017), (+)-hopeaphenol (2) (Guebailia et al., 2006; Sasikumar et al., 2016), (–)-α-viniferin (3) (Mattivi et al., 2011; Rohaiza et al., 2012; Sasikumar et al., 2019), (–)-wilsonol A (4) (Jiang et al., 2012) and (–)-stenophyllol B (5) (Ohyama et al., 1998). Additionally, the NOESY experiment and optical rotation were operated to support their relative configurations. Their chemical structures are displayed in Fig. 1 (for <sup>1</sup>H, <sup>13</sup>C NMR and spectroscopic data were summarized and shown in Supplementary data).

#### 3.3. Anti-HIV-1 assay

Some natural resveratrol oligomers have been shown to have anti-viral activities. For example, two resveratrol oligomers, dibalanocarpol and balanocarpol, isolated from *Hopea malibato* Foxw. exhibited HIV-inhibitory activity of EC<sub>50</sub> 46 and 20 μg/mL, respectively (Dai et al., 1998). In addition, (–)-hopeaphenol and shoreaketone showed antiviral activity against influenza A virus as well as herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (Ito et al., 2018). Interestingly, (–)-hopeaphenol was claimed to be a potent HIV inhibitor without concomitant cytotoxicity (Tietjen et al., 2019). In this study, all isolated compounds

**Table 1**  
Anti-HIV-1 activities of isolated compounds 1–5.

Compound	Cytotoxic and Syncytium Reduction Assays <sup>a</sup>				Reverse Transcriptase Assay <sup>b</sup>		
	IC <sub>50</sub> (μM)	EC <sub>50</sub> (μM)	SI	Activity	% inhibition at 200 μg/mL	Activity	IC <sub>50</sub> (μM)
1	47.36	63.85	0.74	I	96.61	VA	33.27
2	8.87	7.83	1.13	A	100.00	VA	4.34
3	<5.75	<5.75	–	T	100.00	VA	4.19
4	55.43	25.71	2.16	A	97.37	VA	5.85
5	18.21	22.12	0.82	I	100.00	VA	1.68
AZT	–	6.02 × 10 <sup>-9</sup>	–	–	–	–	–
Navirapine	–	–	–	–	–	–	7.77

AZT, azidothymidine, and Navirapine were positive control for syncytium reduction and reverse transcriptase assays, respectively.

<sup>a</sup> Syncytium reduction assay: EC<sub>50</sub> = dose of compound reducing 50% syncytium formation by  $\Delta^{Tat/Rev}$ MC99 virus in 1A2 cells. IC<sub>50</sub> = dose of compound inhibiting 50% metabolic activity of uninfected 1A2 cells. SI, selective index: IC<sub>50</sub>/EC<sub>50</sub>. Activity: A, active (SI > 1); I, inactive; T, Toxic (IC<sub>50</sub> is less than the lowest concentration tested).

<sup>b</sup> RT assay: compounds were prescreened at 200 μg/mL. Activity; VA = very active (>70% inhibition).

were submitted for anti-HIV-1 activities employing the syncytium inhibition assay using the  $\Delta^{Tat/Rev}$ MC99 virus (Nara et al., 1987; Kiser et al., 1996) and 1A2 cell line system, as well as the reverse transcriptase (RT) assay (Tan et al., 1991). Table 1 shows that compounds 2 and 4 were active in the syncytium reduction assay, while compound 3 was toxic to  $\Delta^{Tat/Rev}$ MC99 virus and 1A2 cell line. All the tested compounds exhibited significant inhibition activity in the HIV-1 RT assay (>90% at 200 μg/mL). All the compounds except compound 1 had activity with IC<sub>50</sub> in the range of 1.68–5.85 μM compared to the positive control, nevirapine (IC<sub>50</sub> 7.77 μM). Compound 5 was the most active with an IC<sub>50</sub> of 1.68 μM.

#### 4. Chemotaxonomic significance

Resveratrol oligomers are major phytochemical constituents in the Vitaceae family. Nine genera (*Ampelocissus*, *Ampelopsis*, *Cayratia*, *Cissus*, *Cyphostemma*, *Muscadinia*, *Parthenocissus*, *Rhoicissus* and *Vitis*) of the 16 genera in Vitaceae family have been reported to contain resveratrol derivatives (Riviere et al., 2012; Keylor et al., 2015; Wen et al., 2018). Thus, resveratrol oligomers were claimed to be chemotaxonomic significance in plants from Vitaceae family. Based on the searching for chemical constituents, no isolation and structure identification of chemical components from *Ampelocissus martini* were reported. After systematic isolation of the MeOH extract of *A. martini* roots by using various chromatographic techniques, five resveratrol oligomers 1–5 were obtained. (–)-Ampelopsin F (1) was isolated from plants in various families comprising *Caragana sinica* (Buc'hoz) Rehder in Fabaceae family (Luo et al., 2001); *Dipterocarpus grandiflorus* Blanco, *Vateria indica* L., *Shorea cordifolia* (Thw.) P. S. Ashton (Ito et al., 2004, 2010, 2013), *Vatica umbonata* Burck (Atun et al., 2004), *V. mangachapoi* Blanco (Wu et al., 2019) and *Dryobalanops oblongifolia* Dyer (Indriani et al., 2017) in Dipterocarpaceae family. In contrary, (+)-ampelopsin F, an enantiomer of 1, was found in *Ampelopsis* genus: *A. brevipedunculata* var. *hancei* (Planch.) Rehder (Oshima et al., 1993), and in *Vitis* genus: *V. amurensis* Rupr. (Ha et al., 2009) and *V. coignetiae* Pulliat ex Planchon (Oshima et al., 1995). Furthermore, (–)-α-viniferin (3) was found in *Vitis vinifera* L. (Mattivi et al., 2011; Giovannelli et al., 2014) and reported in several species of Dipterocarpaceae family consisting of *Shorea seminis* (De Vriese) v. Slooten (Aminah et al., 2002), *S. gibbosa* Brandis (Saryobudiono et al., 2008), *S. faguetiana* Heim. (Rohaiza et al., 2012), *Dipterocarpus hasseltii* Bl. (Muhtadi et al., 2006) and *Hopea ponga* (Dennst.) D. J. Mabberley (Sasikumar et al., 2019), whereas (+)-α-viniferin, an enantiomer of 3, was separated from various genus in Vitaceae family such as *Vitis riparia* Michx. (Langcake., 1981), *V. heyneana* Roem. & Schult (Ha et al., 2018), *Cayratia trifolia* (L.) Domin (Roat et al., 2008) and *Parthenocissus tricuspidata* (Siebold & Zucc.) Planchon (Kim et al., 2005). (–)-Wilsonol A (4) and (–)-stenophyllol B (5) were previously isolated from *Vitis wilsonae* Veitch (Jiang et al., 2012) and

*V. thunbergii* var. *taiwaniana* Lu (Hu et al., 2016), respectively. (+)-Hopeaphenol (2) has been reported in plant of *Ampelocissus* genus: *Ampelocissus indica* (L.) Planch. (Sasikumar et al., 2016). In addition, it was reported on the related genera in Vitaceae plants including *Vitis* genus: *V. amurensis* (Huang et al., 2001), *V. thunbergii* var. *taiwaniana* (Lin et al., 2015) and *V. chunganensis* Hu (He et al., 2009) as well as *Ampelopsis* genus: *A. brevipedunculata* var. *hancei* (Su et al., 2015; Chang et al., 2017). Therefore, (+)-hopeaphenol (2) was regarded to be the chemotaxonomic marker from *Ampelocissus* plants and all isolated compounds except compound 1 were remarkable as chemotaxonomic significance for Vitaceae family. Interestingly, this is the first report for (–)-α-viniferin (3), (–)-wilsonol A (4) and (–)-stenophyllol B (5) in *Ampelocissus* genus and (–)-ampelopsin F (1) in Vitaceae family. Data from this study on *A. martini* shows that it contains resveratrol oligomers that have anti-viral activity which could be research further.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bse.2022.104393>.

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