from *Machilus japonica* stems and their inhibitory effects on LDL oxidation, *International Journal of Molecular Sciences*, 15(9), 16418-16429. **15**. Lou Y., Xu T., Cao H., Zhao Q., Zhang P., Shu P. (2022), Tyrosinase and Acetylcholinesterase Inhibitors from *Cercis glabra* Leaves, *Natural Antioxidants*, 27(24), 8667. **16**. Feliciano A. S., Medarde M., Lopez J. L., Miguel Del Corral J. M., Barrero A. F. (1986), Two new cinnamyl isovalerate derivatives from *Juniperus thurifera* leaves, *Journal of Natural Products*, 49(4), 677-679. **17**. Rodney A., Fernandes., Kumar P., Bhowmik A., Gorve D. A. (2022), Regioselective Disulfide-Catalyzed Photocatalytic Oxidative Cleavage of 1-Arylbutadienes to Cinnamaldehydes, *Organic Letters*, 24(19), 3435-3439. **18**. Gall D. L., Kim H., Lu F., Donohue T. J., Noguera D. R., Ralph J. (2014), Stereochemical features of glutathione-dependent enzymes in the *Sphingobium* sp. strain SYK-6 β-aryl etherase pathway, *Journal of Biological Chemistry*, 289(12), 8656-8667. **19**. Khatua M., Goswami B., Hans S., Kamal., Mazumder S., Samanta S. (2022), Hemilabile Amine-Functionalized Efficient Azo-Aromatic Cu-Catalysts Inspired by Galactose Oxidase: Impact of Amine Sidearm on Catalytic Aerobic Oxidation of Alcohols, *Inorganic Chemistry*, 61(44), 17777-17789. **20**. Degotte G., Pirotte B., Frédérich M., Francotte P. (2021), Polyhydroxybenzoic acid derivatives as potential new antimalarial agents, *Archiv der Pharmazie*, 354(11), 2100190. **21**. Acebey L., Jullian V., Sereno D., Chevalley S., Estevez Y., Moulis C., Beck S., Valentin A., Gimenez A., Sauvain M. (2009), Anti-leishmanial lindenane sesquiterpenes from *Hedyosmum angustifolium*, *Planta Medica*, 76(4), 365-368. **22.** Thao T. T. P., Ninh P. T., Loc T. V., Thanh N. T., Sung T. V. (2019), Chemical constituents, cytotoxic and α-glucosidase inhibitory activity of a compound isolated from *Litsea glutinosa*, *Chemistry of Natural Compounds*, 55, 186-188.

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ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *EMBELIA RIBES* BURM. F. FRUITS COLLECTED IN SOUTHERN VIETNAM AND ITS CHEMICAL COMPONENTS

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Summary

Acetylcholinesterase Inhibitory Activity of *Embelia ribes* Burm. f. Fruits Collected in Southern Vietnam and its Chemical Components

Extracts from *Embelia ribes* Burm. f. fruits in southern Vietnam showed good effects on acetylcholinesterase (AChE) inhibition, with IC_{50} values of 0.23-0.45 mg/ml. Among them, the dichloromethane fraction showed the potent inhibitory activity. The chemical investigation of this fraction resulted in the isolation of five compounds (**1-5**). Their chemical structures were identified as kaempferol (**1**), quercetin (**2**), hydroxymethylfurfural (**3**), 5-(8′-*Z*-heptadecenyl)resorcinol (**4**), and embelin (**5**) based on spectral data analysis and literature references. The *in vitro* AChE inhibitory activity shows embelin may be a principal compound in the most active dichloromethane fraction.

Keywords: *Embelia ribes*, *Acetylcholinesterase, Embelin.*

1. Introduction

Embelia ribes Burm. f. (*E. ribes*) is a woody shrub that belongs to the family Myrsinaceae. The distribution area of this species is relatively wide, and the plant is present in many Southeast Asian and South Asian countries, such as Cambodia, Laos, Thailand, Vietnam, India, and China [1]. In Vietnam, *E. ribes* appears broadly from north to south. It has been used as a Vietnamese traditional medicine for the treatment of inflammatory, bacterial, dental, oral, and throat troubles, pain relief, skin diseases, and parasitic worms [2],[3]. Extracts and embelin isolated from *E. ribes* showed reversible contraceptive effects [3]. *E. ribes* and its main constituent, embelin, have attracted much attention for their effects, such as in the treatment of cancer [4], diabetes [5], and central nervous system diseases [6]. Some studies showed multiple effects of embelin, so it can be considered a potential drug candidate in modern medicine [7],[8]. Recent studies showed that *E. ribes* ethanolic extract and embelin had a significant *in vivo* neuroprotective effect. In particular, the effects of improving the learning ability of *E. ribes*, such as anti-depression and anti-memory decline, e.g., in Alzheimer's disease must be mentioned [9],[10]. More recent studies identified embelin from *E. ribes* as a multi-targeted anti-Alzheimer agent that plays a role in stopping the formation of amyloid-β oligomers and improving cholinergic transmission [11],[12].

Studies on chemical components showed that *E. ribes* fruits contain benzoquinones, benzofurans, resorcinol derivatives, and some

phenolic compounds [13],[14]. In Vietnam, there is little research on the chemical composition of *E. ribes*. In addition, the publication reported only on the chemical composition of its stems and leaves [15],[16]. Previous studies on *E. ribes* fruit extracts and embelin focused on their anthelmintic and birth control effects [2],[3]. Moreover, research on the AChE inhibitory effect of *E. ribes* and embelin in Vietnam has not yet received attention. The present study was planned to examine acetylcholinesterase (AChE) inhibitory activity of the 80% ethanol extract from the fruits of *E. ribes*, its fractions (*n*-hexane, dichloromethane, ethyl acetate, and aqueous), and its chemical constituents.

2. Materials and methods

2.1. Materials

Plant materials: The ripe fruits of *E. ribes* were collected in Dong Nai, Vietnam in July 2022. The sample was identified by MSc. Phan Van Truong, Centre of Medicinal Material Resources - CMMR (National Institute of Medicinal Materials). The voucher specimen (No. 82BD2023) was deposited at CMMR. The fruits were dried in the shade and then in the oven at the temperature ≤50°C before being stored in a vacuum bag at room temperature.

2.2. Chemicals: Potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4) for preparation of 0.1 M phosphate buffer-1 (pH 8.0) and 0.1 M phosphate buffer-2 (pH 7.0), tris(hydroxymethyl)aminomethane (Tris), Ellman's reagent, dithiobisnitrobenzoic (DTNB), acetylcholinesterase (AChE), tacrine, and acetylthiocholine iodide (ATCI) were purchased from Sigma. Dimethyl sulfoxide (DMSO) was purchased from Prolab.

2.3. General experimental procedures.

Silica gel (70-230 or 230-400 mesh, Merck), RP-18 (30-50 μm, FuJisilisa Chemical Ltd) and Sephadex™ LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Electrospray-ionization mass spectrometry (ESI-MS) was performed on an Agilent 1100 series LC-MSD ion trap spectrometer. ^{1}H-NMR and ^{13}C-NMR spectrometry were conducted on a 600 MHz Bruker Avance NEO spectrometer using deuterated solvents and tetramethylsilane as an internal standard. The chemical shifts were reported in δ (ppm). Thin-layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV radiation (245 nm and/or 365 nm) or at normal light by spraying with 10% H_2SO_4, followed by heating at 105°C.

2.4. In vitro acetylcholinesterase inhibition assay

An *in vitro* AChE inhibition assay was conducted according to the method developed by Ellman *et al.* [17]. Enzyme inhibition percentages were calculated by comparing the absorbance of samples (assay containing extract + enzyme + substrate + DTNB) with enzyme control (assay containing buffer + enzyme + substrate + DTNB). The values corresponding to enzyme control absorbance provided the benchmark for maximum activity of the employed enzyme in experiments, i.e. the maximum enzyme capacity for product formation from substrates, being considered an enzyme activity of 100%. In this assay, samples were dissolved in DMSO with a concentration of 10 mg/ml and further diluted with 0.1 M phosphate buffer pH 8.0 (0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL). AChE activity was determined in a 25 μl AChE (0.25 U/ml) solution in a 96-well flat-bottom microplate. The reaction was started by adding 125 μl of 3 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 25 μl of 15 mM acetylthiocholine iodide (ATCI or substrate), and 50 μl of 0.1 M phosphate buffer (pH 8.0). The spectrophotometric absorption at 412 nm was measured using a microplate reader after being incubated for 15 min at 25°C. The percentage of AChE inhibitory activity (% inhibition) was calculated by using the following equation:

$$(\%\ \text{inhibition}) = \frac{(A_{\text{Control}} - A_{\text{Blank control}}) - (A_{\text{Sample}} - A_{\text{Blank sample}})}{(A_{\text{Control}} - A_{\text{Blank control}})} \times 100$$

Whereas:

$A_{Control}$: Absorbance of the Control (with enzyme)

$A_{Blankcontrol}$: Absorbance of the Blank control (without enzyme)

A_{Sample}: Absorbance of the Sample (with enzyme)

$A_{Blanksample}$: Absorbance of the Blank sample (without enzyme)

All treatments were performed in triplicate.

Tacrin was used as a positive control. Results were expressed as the mean ± S.E.M. of all experiments. The IC_{50} was determined by plotting percentage inhibition against the concentration of the extract.

2.5. Extraction and isolation

The crushed dried fruits of *E. ribes* (5.0 kg) were extracted at room temperature with 80% ethanol (25 L) for 3 times (3 days a time, with mixing). The liquid extracts were collected, combined, and concentrated under reduced pressure at 70°C and then in *vacuo* to yield an ethanol extract (1.010 g). The extract was suspended in a suitable amount of water to form a suspension. The suspension was successively fractionated with *n*-hexane, dichloromethane (DCM), and ethyl acetate (EtOAc) to have the corresponding fractions. The solvents were separately removed by a rotary evaporator under reduced pressure, and the obtained extracts were dried in a *vacuo* oven at 60°C to obtain *n*-hexane (HCN, 70.5 g), dichloromethane (DCM, 65.1 g), and ethyl acetate (EtCN, 50.0 g) fractions. The remaining suspension was evaporated at 60°C in the water bath and then dried in a *vacuo* oven to obtain water extract (WCN, 814.4 g).

The DCM fraction (60 g) was chromatographed on a *silica gel* column, eluting with gradient solvents of *n*-hexane/EtOAc/methanol (20/1/1-1/1/1, v/v/v) and MeOH to give 6 fractions (D1-D6). The D1 fraction (4.5 g) was separated on a *silica gel* column, eluting with gradient solvents of *n*-hexane/acetone (15/1 - 2/1, v/v) to afford fractions D1.1 (2.5 g), D1.2 (800 mg), and D1.3 (150 mg). The D1.1 fraction was separated on a *silica gel* column with an eluent of DCM/MeOH (15/1, v/v) to afford compound **4** (6 mg). From the D1.2 fraction, compounds **1** (9 mg) and **3** (18 mg) were isolated by column chromatography on *silica gel* using DCM/MeOH (15/1 - 10/1, v/v) as eluents. Compound **2** (8 mg) was yielded from the D1.3 fraction using an RP-18 column with an eluent of MeOH/H_2O (3/1, v/v). The D2 fraction (6.5 g) was washed with *n*-hexane ten times before being recrystallized in 300 ml DCM/MeOH (2/1, v/v) to obtain compound **5** (20 mg).

*2.6. Physical constants and spectroscopic data of **1-5***

Compound **1** (yellow powder); ESI-MS *m/z*: 287.1 $[M+H]^+$, $C_{15}H_{10}O_6$. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 6.20 (1H, d, J = 2.0 Hz, H-6), 6.41 (1H, d, J = 2.0 Hz, H-8), 8.09 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.92 (2H, d, J = 8.5 Hz, H-3′, H-5′). ^{13}C-NMR (150 Hz, CD_3OD) δ_C (ppm): 148.1 (C-2), 137.1 (C-3), 177.4 (C-4), 162.5 (C-5), 99.3 (C-6), 165.6 (C-7), 94.5 (C-8), 158.2 (C-9), 104.6 (C-10), 123.7 (C-1′), 130.7 (C-2′, C-6′), 116.3 (C-3′, C-5′), 160.5 (C-4′).

Compound **2** (yellow powder); ESI-MS *m/z*: 302.9 $[M+H]^+$, $C_{15}H_{10}O_7$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 6.18 (1H, d, J = 2.4 Hz, H-6), 6.40 (1H, d, J = 1.8 Hz, H-8), 7.67 (1H, d, J = 2.4 Hz, H-2′), 6.88 (1H, d, J = 8.4 Hz, H-5′), 7.54 (1H, dd, J = 1.8, 8.4 Hz, H-6′); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 146.8 (C-2), 135.7 (C-3), 175.8 (C-4), 160.7 (C-5), 98.1 (C-6), 163.8 (C-7), 93.3 (C-8), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1′), 115.0 (C-2′), 145.0 (C-3′), 147.7 (C-4′), 115.7 (C-5′), 119.9 (C-6′).

Compound **3** (light yellow crystal); ESI-MS *m/z*: 127.2 $[M+H]^+$, $C_6H_6O_3$. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 9.61 (1H, s, -CHO), 7.22 (1H, d, J = 3.0 Hz, H-3), 6.52 (1H, d, J = 3.0 Hz, H-4), 9.61 (1H, s, H-6), 4.73 (2H, s, H-7). ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 152.5 (C-2), 110.0 (C-4), 160.4 (C-5), 177.6 (C-6), 57.7 (C-7).

Compound **4** (yellow oil); ESI-MS *m/z*: 345.2 $[M-H]^-$, $C_{23}H_{38}O_2$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 6.23 (2H, d, J = 1.8 Hz, H-4, H-6), 6.17 (1H, br s, H-2), 5.32-5.37 (2H, m, H-8′, H9′), 4.80-5.00 (2H, br s, OH) 2.48 (2H, t, J = 7.8 Hz, H-1′), 2.00-2.06 (4H, m, H-7′, H-10′), 1.26-1.36 (20H, overlap, 10 CH_2, (H-2′-H-6′ and H-11′-H-15’), 1.28 (2H, overlap, H-16′), 0.88 (3H, t, J = 6.0 Hz, H-17′); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 156.7 (C-1, C-3), 100.2 (C-2), 108.0 (C-4, C-6), 146.2 (C-5), 35.8 (C-1′), 29.0-32.0 (C-2′-C-6′, C-11′-C-15′), 27.3, 27.2 (C-7′, C-10′), 128.3 (C-8′), 129.9 (C-9′), 22.7 (C-16′), 14.1 (C-17′).

Compound **5** (twinkling orange lamellar crystals); ESI-MS *m/z*: 293.6 $[M-H]^-$, $C_{17}H_{26}O_4$; IR (KBr) υ_{max}: 3309 cm^{-1} (OH), 2921 and 2849 cm^{-1} (C-H), 1615 cm^{-1} (C=O), 1462 cm^{-1} (C=C), 982 cm^{-1} (C-O). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 7.69 (s, 2H, 2-OH) 6.00 (1H, *s*, H-6), 2.45 (2H, t, J = 7.2 Hz, H-1′), 1.28 (2H, overlap, H-10′), 1.26-1.31 (16H, overlap, H-2′-H-9′), 0.88 (3H, t, J = 6.6 Hz, H-11′); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 117.0 (C-3), 102.1 (C-6), 31.8 (C-9′), 29.5-29.2 (C-2′ - C-8′), 22.55 (C-1′), 22.46 (C-10′), 13.9 (C-11′).

3. Results and discussion

3.1. AChE inhibition assay of extract and fractions

AChE inhibition assay was performed on Tacrin (standard), 80% ethanol extract, and fractions (*n*-hexane, DCM, EtOAc, and aqueous)

of *E. ribes* fruits. The results showed that the DCM fraction gave the best inhibitory effect with an IC_{50} of 0.23 mg/ml, followed by the *n*-hexane fraction with an IC_{50} of 0.29 mg/ml. The aqueous fraction, EtOAc fraction, and 80% EtOH extract had AChE inhibitory effects with IC_{50} values of 0.33, 0.37, and 0.45 mg/ml, respectively (Fig. 1). The DCM fraction was chosen for further investigation of chemical components.

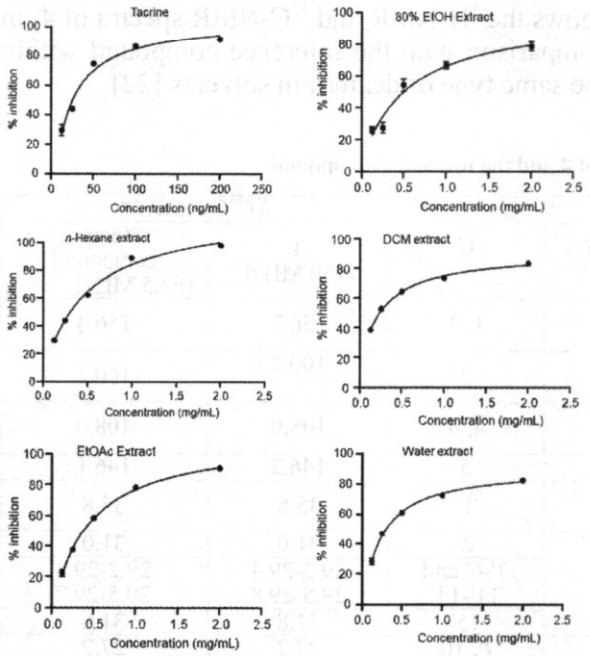

Fig. 1. AChE inhibition concentrations of the extracts and tacrine

3.2. Isolation of compounds

Kaempferol (**1**), quercetin (**2**), hydroxymethylfurfural (**3**), 5-(8′-*Z*-heptadecenyl)-resorcinol (**4**), and embelin (**5**) (Fig. 2) were isolated from the DCM fraction.

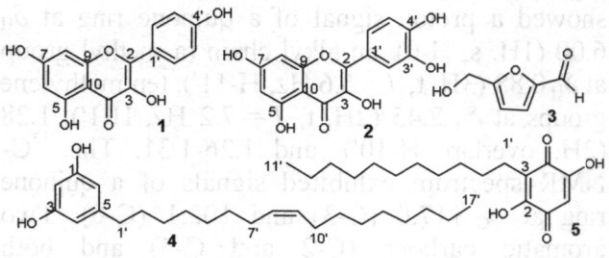

Fig. 2. Chemical structures of compounds **1-5**

The ESI-MS peak of *m/z* 287.1 $[M+H]^+$ suggested that **1** had a molecular formula of $C_{15}H_{10}O_6$. The ^{1}H-NMR showed the presence of six proton signals of two aromatic rings, of which two signals at δ_H 6.20 (1H, d, J = 2.0 Hz, H-6) and 6.41 (1H, d, J = 2.0, H-8) belonged to *meta*-protons of the A ring and four proton signals at δ_H 8.09 (2H, d, J = 8.5 Hz, H-2′, H-6′) and 6.92 (2H, d, J = 8.5 Hz, H-3′, H-5′) belonged to the B ring. The ^{13}C-NMR spectrum of **1** displayed 15 carbon signals, including one carbonyl group at δ_C 177.4 (C-4); eight non-hydrogenated carbons at δ_C 148.1 (C-2), 137.1 (C-3), 162.5 (C-5), 165.6 (C-7), 158.2 (C-9), 104.6 (C-10), 123.7 (C-1′), and 160.5 (C-4′); six methine carbons at δ_C 99.3 (C-6), 94.5 (C-8), 130.7 (C-2′, C-6′), and 116.3 (C-3′, C-5′), characteristic for a flavone. Based on these spectroscopic data and comparison to the published literature [18], compound **1** was identified as kaempferol.

The ESI-MS peak at *m/z* 302.9 $[M+H]^+$ suggested the molecular formula of compound **2** as $C_{15}H_{10}O_7$. The ^{1}H-NMR showed characteristic proton signals for a flavone structure that were similar to those of **1** except for a non-protonated carbon at C-3′. The ^{13}C-NMR and DEPT spectra of **2** displayed 15 carbon signals, including one carbonyl group at δ_C 175.8 (C-4); nine non-protonated carbons at δ_C 146.8 (C-2), 135.7 (C-3), 160.7 (C-5), 163.8 (C-7), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 145.0 (C-3'), and 147.7 (C-4'); five methine carbons at δ_C 98.1 (C-6), 93.3 (C-8), 115.0 (C-2'), 115.7 (C-5'), and 119.9 (C-6'). Based on these spectroscopic data and comparison to the published literature [18, 19], compound **2** was identified as quercetin. Compounds **1** and **2** were previously identified without isolation in *E. ribes* by using ultrahigh-performance liquid chromatography analysis [20].

The ESI-MS with *m/z* 127.2 $[M+H]^+$ suggested the molecular formula of compound **3** as $C_6H_6O_3$. The ^{1}H-NMR spectrum of **3** showed the presence of 3 methylene protons, one formyl proton at δ_H 9.61 (1H, s, H-6), two mutually coupled aromatic protons at δ_H 7.22 (1H, d, J = 3.0 Hz, H-3) and 6.52 (1H, d, J = 3.0 Hz, H-4), and one hydroxymethylene group at δ_H 4.73 (2H, s, -CH_2OH). The ^{13}C-NMR spectrum of **3** showed six carbon signals, including three methine carbons at δ_C 177.6 (C-6), 7124.8 (C-3), and 110.0 (C-4), one methylene carbon at δ_C 57.7 (C-7), and two non-protonated carbons at δ_C 152.5 (C-2) and 160.4 (C-5). On the basis of the above evidence and comparison to the published literature [21], compound **3** was identified as 5-hydromethylfurfural.

Compound **4** had a molecular formula of $C_{23}H_{38}O_2$ based on the ESI-MS spectrum with a peak at *m/z* 345.2 $[M-H]^-$. In the ^{1}H-NMR spectrum, the *meta*-coupled aromatic protons at δ_H

6.24 (2H, d, J = 1.8 Hz, H-4, H-6) and 6.17 (1H, br s, H-2) suggested a 1,3,5-trisubstituted benzene ring. A long alkenyl chain was characterized by six groups of signals, including one double bond at δ_H 5.32-5.37 (2H, m, H-8′, H-9′), one methyl group at δ_H 0.88 (3H, t, J = 6.0 Hz, H-17′), one methylene at δ_H 2.48 (2H, t, J = 7.8 Hz, H-1′), two methylenes at δ_H 2.00-2.06 (4H, m, H-7′, H-10′), one methylene at δ_H 1.27 (2H, overlap, H-16′), and 10 methylenes at δ_H 1.26-1.36 (20H, m, H-(2′-6′) and H-(11′-15′)). The ^{13}C-NMR spectrum exhibited signals for a trisubstituted benzene at δ_C 156.7 (C-1, C-3), 146.2 (C-5), 108.0 (C-4, C-6), and 100.2 (C-2), and a long alkenyl chain with two olefinic carbons at δ_C 129.9 (C-9′) and 128.3 (C-8′), one methyl carbon at δ_C 14.1 (C-17′), one methylene carbon at δ_C 35.8 (C-1′), and 13 methylene carbon signals (as calculated from MS spectrum) in the range of δ_C 29.0-32.0 (C-(2′-7′) and C-(11′-16′). According to Suzuki *et al.* [22], the carbon signals of the heptadecenyl group at δ_C 27.3 and 27.2 (C-7′, C-10′) and 22.7 (C-16′) determined the *Z*-stereochemistry of the double bond. Table 1 shows the ^{1}H-NMR and ^{13}C-NMR spectra of **4**, in comparison with the reference compound within the same type of deuterium solvents [22].

Table 1. Comparison of NMR spectra of **4** and the reference compound

H (at C)	δ (^{1}H-NMR) *		C	δ (^{13}C-NMR) *	
	4 (600 MHz)	reference compound (270 MHz)		**4** (150 MHz)	reference compound (67.5 MHz)
1, 3	4.80-5.00 (2H, br s)	5.24 (2H, br s)	1, 3	156.7	156.4
2	6.17 (1H, br s)	6.17 (1H, t, J = 2.3 Hz)	2	100.2	100.1
4, 6	6.23 (2H, d, J = 1.8 Hz),	6.25 (2H, d, J = 2.3 Hz)	4, 6	108.0	108.0
5	-	-	5	146.2	146.1
1′	2.48 (2H, t, J = 7.8 Hz)	2.47 (2H, t, J = 7.2 Hz)	1′	35.8	35.8
2′-6′ and 11′-15′	1.26-1.36 (20H, overlap)	1.26-1.36 (20H, br s)	2′	31.0	31.0
			3′-6′ and 11′-14′	29.2-29.4 29.5-29.8	29.2-29.4 29.5-29.7
			15′	31.8	31.8
7′, 10′	2.00-2.06 (4H, m)	2.00 (4H, m)	7′, 10′	27.2	27.2
8′, 9′	5.32-5.37 (2H, m)	5.35 (2H, m)	8′, 9′	128.3 129.9	129.8 129.9
16′	1.28 (2H, overlap)	1.55 (2H, m)	16′	22.7	22.6
17′	0.88 (3H, t, J = 6.0 Hz)	0.89 (3H, t, J = 6.7 Hz)	17′	14.1	14.0
(*) solvents: $CDCl_3$; δ: ppm; reference compound in [22]					

The HMBC correlation of H-1′ (δ_H 2.48) with C-5 (δ_C 146.2) confirmed the linkage of the alkenyl chain at C-5 of the benzene ring. The position of the double bond was confirmed to be at C-8′ and C-9′ based on the HMBC correlations of H-7′ (δ_H 2.00-2.06) with C-8′ (δ_C 128.3) and H-10′ (δ_H 2.00-2.06) with C-9′ (δ_C 129.9). The benzene ring was 1,3,5-trisubstituted based on the following HMBC correlations: H-4, H-6 (δ_H 6.24) with C-1, C-3 (δ_C 156.7), C-2 (δ_C 100.2), C-4, C-6 (δ_C 108.0), and C-1′ (δ_C 35.8). Based on these spectroscopic data and their comparison to the published literature [22], compound **4** was identified as 5-(8′-*Z*-heptadecaenyl)-1,3-benzenediol. Compound **4** was reported in the aerial part of *E. ribes* [16].

The ESI-MS peak of **5** with *m/z* 293.6 $[M-H]^-$ suggested the formula of $C_{17}H_{26}O_4$. The IR spectrum of **5** showed absorptions at 3309 cm^{-1} (OH), 2921 and 2849 cm^{-1} (aliphatic CH stretching), 1615 cm^{-1} (α,β-unsaturated C=O), and at 982 cm^{-1} (C-O). The ^{1}H-NMR spectrum of **5** showed a proton signal of a quinone ring at δ_H 6.00 (1H, s, H-6), an alkyl chain (a methyl group at δ_H 0.88 (3H, t, J = 6.6 Hz, H-11′), ten methylene groups at δ_H 2.45 (2H, t, J = 7.2 Hz, H-1′), 1.28 (2H, overlap, H-10′), and 1.26-1.31. The ^{13}C-NMR spectrum exhibited signals of a quinone ring at δ_C 117.0 (C-3) and 102.1 (C-6). Two aromatic carbons (C-2 and C-5) and both carbonyl groups (C-1 and C-4) did not appear in the ^{13}C-NMR spectrum due to the loss of signals. The signal at δ_C 13.9 was identified as the terminal methyl group C-11′; two signals at δ_C 22.5 and 22.4 were attributed to two methylene groups at C-1′ and C-10′, respectively. Moreover, the signal at δ_C 31.8 was assigned to C-9′. The

overlapping signals in the region of δ_C 29.2-29.5 were contributed to the remaining 7 CH_2 groups (C-2′ - C8′). On the basis of spectral analysis and comparison to the literature [23], compound **5** was identified as embelin.

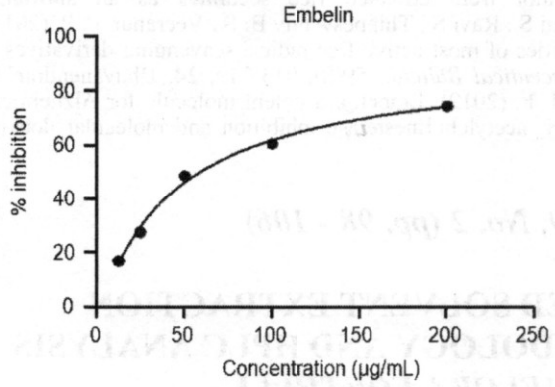

Fig. 3. AChE inhibition concentration of embelin

In the *in vitro* test, embelin (**5**) showed inhibition of AChE with an IC_{50} of 0.204 µM (60.0 µg/ml) (Fig. 3.). This result was in a similar range to the published value (49.6 µg/ml) [24]. We isolated embelin in good yield (1.11%) (20 g embelin was isolated from 1.8 kg of the dried fruits of *E. ribes*). In our method, the raw material was extracted two times (one day at a time) with DCM at room temperature. The extracts were then filtered. After the elimination of the solvent, the crude product was continuously washed (seven times) with *n*-hexane or petroleum ether, then recrystallized in the mixture of DCM/MeOH (9/1) to obtain embelin in lamellar crystallized form.

4. Conclusion

The DCM fraction from *E. ribes* showed the strongest inhibitory effect on AChE. From the DCM fraction, five compounds were isolated, including kaempferol (**1**), hydroxymethylfurfural (**2**), quercetin (**3**), 5-(8′-*Z*-heptadecenyl) resorcinol (**4**), and embelin (**5**). The structures of these compounds were established by spectral data analysis and literature references. To the best of our knowledge, compounds **1-3** were isolated for the first time from the *E. ribes* fruits collected in southern Vietnam. In our test, embelin demonstrated AChE inhibition with an IC_{50} of 0.204 µM (60 µg/ml). Embelin (**5**) was then isolated and purified by recrystallization in a large quantity for further use.

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References

1. Yang L. J., He M. Z., Huang W. P., Xie Y. Y., Wang Q., Li Y., Li Z., F. Feng Y., L., Yang S., L. (2005), Advances in the research of the plants of *Embelia*, *Journal of Chinese Medicine and Materials,* 38, 1761-1767. **2.** Institute of Medicinal Materials (2006), *Medicinal Plants and Animals Used as Medicine in Vietnam* (Vol. 1), Science and Technology Publishing House, Hanoi. **3.** Do T. L. (2001), *Vietnamese Medicinal Plant*, Medicine Publisher: Hanoi. **4.** Zappavigna S., Cossu A. M., Grimaldi A., Bocchetti M., Ferraro G. A., Nicoletti G. F., Filosa R., Caraglia M. (2020), Anti-Inflammatory Drugs as Anticancer Agents (a review), *International Journal of Molecular Science*, 21, 2605-2634. **5.** Durg S., Veerapurb V. P., Neelimac S., Dhadde S. B. (2017), Antidiabetic activity of *Embelia ribes*, embelin and its derivatives: A systematic review and meta-analysis, *Biomedicine & Pharmacotherapy*, 86, 195-204. **6.** Kundap U. P., Bhuvanendran S., Kumari Y., Othman I., Shaikh M. F. (2017), Plant Derived Phytocompound, Embelin in CNS Disorders: A Systematic Review, *Frontiers in Pharmacology,* 8(76), 1-13, doi: 10.3389/fphar.2017.00076. **7.** Mori T., Doi R., Kida A., Nagai K., Kami K., Ito D., Eiji T., Yoshiya K., Shinji U. (2007), Effect of the XIAP inhibitor Embelin on TRAIL□induced apoptosis of pancreatic cancer cells, *Journal of Surgical Research*, 142, 281□286. **8.** Chen J., Nikolovska□Coleska Z., Wang G., Qiu S., Wang S., Design, synthesis, and characterization of new embelin derivatives as potent inhibitors of X□linked inhibitor of apoptosis protein (2006), *Bioorganic & Medicinal Chemistry Letters*, 16, 5805-5808. **9.** Ansari M. N., Bhandari U. (2008), Protective effect of *Embelia ribes* Burm on methionine-induced hyperhomocysteinemia and oxidative stress in rat brain, *Indian Journal of Experimental Biology*, 46(7), 521-527. **10.** Thippeswamy B. S., Nagakannan P., Shivasharan B. D., Mahendran S., Veerapur V. P., Badami S. (2011), Protective effect of embelin from *Embelia ribes* Burm. against transient global ischemia-induced brain damage in rats, *Neurotoxicity Research*, 20, 379-386. **11.** Nuthakki V. K., Sharma A., Kumar A., Bharate S. B. (2019), Identification of embelin, a 3-undecyl-1,4-benzoquinone from *Embelia ribes* as a multitargeted anti-Alzheimer agent, *Drug Development Research*, 80, 655-665. **12.** Ayaz M., Ullah F., Sadiq A., Ok Kim M. and Ali T. (2019), Editorial: Natural Products-Based Drugs: Potential Therapeutics Against Alzheimer's Disease and Other Neurological Disorders, *Frontiers in Pharmacology*, 10, 1417. **13.** Lin P., Li S., Wang S., Yang Y., Shi J. (2006), A nitrogen-containing 3-alkyl-1,4-benzoquinone and a gomphilactone derivative from *Embelia ribes*, *Journal of Natural Products*, 69(11), 1629-1632. **14.** Haq K., Ali M., Siddiqui A. W. (2005), New compounds from the seeds of *Embelia ribes* Burm., *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 60(1), 69-71. **15.** Dang P. H., Nguyen N. T., Nguyen H. X., Nguyen L. B., Le T. H., Do T. N. V., Can M. V., Nguyen M. T. T., (2015), α-Glucosidase inhibitors from the leaves of *Embelia ribes. Fitoterapia*, 100, 201-207, doi: 10.1016/j.fitote.2014.12.004. **16.** Dang P. H., Nguyen H. X., Nguyen N. T., Nguyen L. B., Le H. N. T., M. T. T. Nguyen (2014), α-Glucosidase inhibitors from the stems of *Embelia ribes. Phytotherapy Research*, 28(11), 1632-1636. **17.** Ellman G. L., Courtney K. D., Andres V. (J.R.) and Featherstone R. M. (1961), A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochemical Pharmacology*, 7, 88-95. **18.** Tram N. C. T., Cuong N. M. (2016), Kaempferol and kaempferol glycosides from *Phyllanthus acidus* leaves, *Vietnam Journal of Chemistry*, 54(6), 790-790. **19.** Shen C. C., Chang Y. S., Ho L. K. (1993), Nuclear-magnetic-resonance studies of 5,7-dihydroxyflavonoids, *Phytochemistry*, 34(3), 843-

845. **20.** Guo S., He M., Liu M., Huang W., Ouyang H., Feng Y., Zhong G., Yang S. (2020), Chemical profiling of *Embelia ribes* by ultra-high-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry and its antioxidant and anti-inflammatory activities *In vitro*, *Journal of Chromatographic Science*, 58(3), 241-250. **21.** Lin S. M., Wu J. Y., Su C., Ferng S., Lo C. Y., Chiou R. Y. Y. (2012), Identification and mode of action of 5-hydroxymethyl-2-furfural (5-HMF) and 1-methyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (MTCA) as potent xanthine oxidase inhibitors in vinegars, *Journal of Agricultural and Food Chemistry*, 60(39), 9856-9862. **22.** Suzuki Y., Esumi Y., Hyakutake H., Kono Y., Sakurai A. (1996), Isolation of 5-(8′ Z-heptadecenyl)-resorcinol from etiolated rice seedlings as an antifungal agent, *Phytochemistry*, 41(6), 1485-1489. **23.** Mahendran S., Badami S., Ravi S., Thippeswamy B. S., Veerapur V. P. (2011), Synthesis and evaluation of analgesic and anti-inflammatory activities of most active free radical scavenging derivatives of Embelin-A Structure-Activity relationship, *Chemical and Pharmaceutical Bulletin*, 59(8), 913-919. **24.** Bhuvanendran S., Hanapi N. A., Ahemad N., Othman I., Yusof S. R. and Shaikh M. F. (2019), Embelin, a potent molecule for Alzheimer's disease: A proof of concept from blood-brain barrier permeability, acetylcholinesterase inhibition and molecular docking studies, *Frontiers in Neuroscience*, 13(495), 1-10.

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OPTIMIZATION OF PRESSURIZED SOLVENT EXTRACTION USING RESPONSE SURFACE METHODOLOGY AND HPLC ANALYSIS OF VITEXIN FROM *PASSIFLORA FOETIDA* L.

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Summary

Optimization of Pressurized Solvent Extraction Using Response Surface Methodology and HPLC Analysis of Vitexin from *Passiflora Foetida* L.

The study aims to optimize the automated sample preparation step using accelerated solvent extraction (ASE) and response surface methodology (RSM) and integrate it with a simple, quick, precise, and accurate high performance liquid chromatography (HPLC) technique for vitexin measurement in *P. foetida*. The RSM results demonstrated that the optimal conditions for vitexin extraction were an extraction temperature of 150.59°C, an extraction time of 19.16 min, and a solvent volume of 140.91 mL; The actual values at the optimal conditions according to the model showed the maximum value of the objective function of vitexin yield was 11.18 ± 0.15 (mg/g). The validated HPLC method was as follows: A mixture of acetonitrile-0.1% formic acid solution with the elution gradients were: 0 - 40 min, 15% acetonitrile; 40 - 45 min, 100% acetonitrile. Other running conditions included the detection wavelength (342 nm), the flow rate (1 mL/min), the injection volume (20 µL), and the column temperature (40°C).

Keywords: *Passiflora foetida* L., *Vitexin, Accelerated solvent extraction (ASE), High performance liquid chromatography (HPLC), Response surface methodology (RSM).*

1. Introduction

With over 500 species, *Passiflora* is the most common genus in the Passifloraceae family [1]. *Passiflora foetida* L. (*P. foetida*) is a plant native to South America that has extended to many tropical locations, including Vietnam regions. The plant has been exploited as an ingredient for traditional medicine related to its bioactive action like antiproliferative, sedative, anti-anxiety, antibacterial, antispasmodic, emetic, dressing for wounds, and antiulcer [2]. In Vietnam, dry leaves are consumed in the form of tea to treat sleep disorders. The major phytochemical constituents of *P. foetida* are chrysoeriol, apigenin, luteolin, kaempferol, isoschaftoside, 2"-xylosylvitexin, isovitexin, and vitexin. Vitexin also has been chosen as the marker for quantitation of *P. foetida* while was shown to have powerful hypotensive, anti-inflammatory, and anti-spasmodic effects [3],[4],[5].

Numerous approaches, including spectroscopic and chromatographic methods, have been used to measure the levels of vitexin in various plants [4],[6],[7],[8],[9]. Among them, high performance liquid chromatography (HPLC) is overwhelmingly the most popular method for determining vitexin. The high precision of HPLC primarily results from its automated processes, except for the sample preparation phase. Even though manual sample preparation methods have been in use for years, a significant number of analytical chemists continue to depend on them, consuming a substantial portion of the overall analysis time. In fact, sample preparation alone