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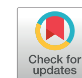
Research article

Antidiabetic and antimicrobial flavonoids from the twigs and roots of *Erythrina subumbrans* (Hassk.) Merr.



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ABSTRACT

The phytochemical investigation of the twig and root extracts of *Erythrina subumbrans* (Hassk.) Merr. (Fabaceae) resulted in the isolation and identification of a new pterocarpan, erythrinocarpan (**1**), along with 27 known compounds (**2**–**28**). All isolated compounds were evaluated for their antidiabetic, antimicrobial, and anti-inflammatory properties. Compounds **3**, **8**, **9**, and **22** had α-glucosidase inhibitory activity with IC_{50} values of 13.4 ± 0.05, 24.5 ± 0.13, 29.0 ± 0.05, and 12.8 ± 0.14 μM, respectively, while compound **2** inhibited α-amylase activity with an IC_{50} value of 67.6 ± 1.12 μM. Compounds **22** and **24** inhibited glycation activity with the IC_{50} values of 36.9 ± 0.62 and 40.5 ± 0.37 μM, respectively. From cell-based assays, compound **27** showed the highest ability to induce glucose consumption (IC_{50} 29.1 ± 0.86 μM) and glucose uptake (2.8-fold), and to inhibit nitric oxide (NO) production (IC_{50} 52.5 ± 0.56 μM) without cell toxicity. Furthermore, compound **9** showed antimicrobial activities against Gram-positive bacteria and fungi with MIC values ranging from 2–4 μg/mL.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic syndrome associated with insulin production disorder in the pancreas leading to hyperglycemia (ADA, 2009). This disease has emerged as a major health problem worldwide, with the number of DM patients having increased by 50% over the past decade. Type 2 DM (non-insulin-dependent) is found among 90–95 % of patients or approximately 451 million people from a 2017 report (Cho et al., 2018). Consistently high blood glucose levels can bring about serious health complications. DM, when not well controlled, can result in nerve damage (neuropathy), kidney failure, and blindness (retinopathy), and raises the risk of foot ulcers and inflammation which can result in a need for lower limb amputation (WHO, 2016). However, despite several DM medication reports, there is a wide range of side effects, including weight gain, hypoglycemia, and coronary heart disease (Pandey et al., 2011). Accordingly, identifying chemical entities that have the potential to become antidiabetic drugs is of importance.

One important class of natural compounds that have potential as new antidiabetic agents is flavonoids (Chen et al., 2015), which are phenolic compounds found in various species of plants (Panche et al., 2016). This group of natural products possess a diverse range of biological activities, and many of them are used as therapeutic agents to treat disease, and play an important role in the pharmaceutical industry (Brodowska, 2017). For antidiabetic activity, flavonoids show several mechanistic pathways such as the regulation of the metabolism of carbohydrates, insulin secretion and glucose uptake, the enhancement of the proliferation of β-cells, the mitigation of apoptosis, and the reduction of hyperglycemia by manipulating the liver's metabolism of glucose. Moreover, some of these compounds can reduce the risk of complications from DM (AL-Ishaq et al., 2019).

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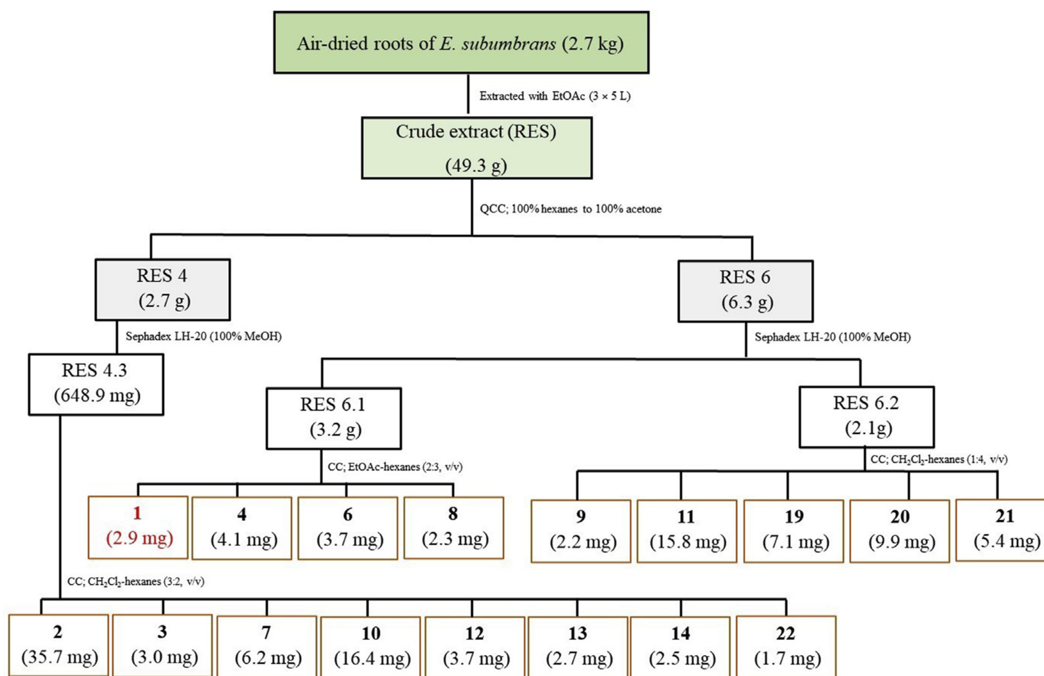

Figure 1. Flowchart of the isolation procedure from the root extract of *E. subumbrans*.

Erythrina subumbrans (Hassk.) Merr. (Fabaceae) or known as 'Thong Lang Pa' in Thailand has been reported to be a source of a large number of flavonoids, including flavanones, isoflavone, and pterocarpans (Rukachaisirikul et al., 2007a, 2008, 2014). This plant is a deciduous tree with spreading branches and stout prickles which is distributed in tropical and subtropical region such as, China, India, Sri Lanka, Myanmar, Thailand, Laos, Vietnam, Malaysia, Indonesia and the Philippines (Pillay et al., 2001). In Thailand, the leaves of this plant have been used as an ingredient in Thai cuisines. In contrast, the bark has been used in traditional medicinal remedies to treat microbial infections and nausea (Pillay et al., 2001) and a number of pterocarpans and flavonoids have been isolated that have antibacterial properties (Rukachaisirikul et al., 2007b). Although the isolated flavonoids from this plant show many biological activities (Rashid et al., 2019), the curative effect of the isolated compounds from *E. subumbrans* on diabetes and their complications has not been reported. In the current study, the twig and root extracts of *E. subumbrans* were used to investigate their chemical components and to evaluate their antidiabetic, anti-inflammatory, and antimicrobial activities. The project aimed to discover primary bioactive compounds for developing antidiabetic therapeutic drugs in the future.

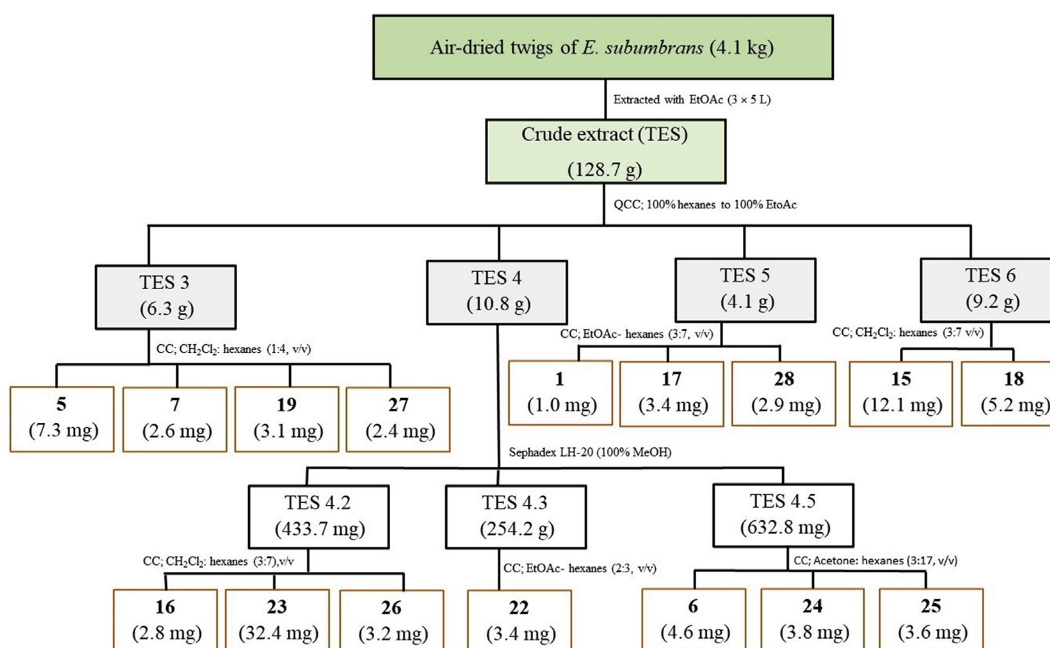

Figure 2. Flowchart of the isolation procedure from the twig extract of *E. subumbrans*.

Table 1. NMR Spectroscopic data of compound **1** (500 MHz in $CDCl_3$).

position	δ_C	δ_H (J in Hz)	COSY (^1H–^1H)	HMBC ($^1H \rightarrow ^{13}C$)
1	153.2	-	-	-
2	114.3	-	-	-
3	156.3	-	-	-
4	101.2	6.30 (s)	-	C-1, C-2, C-3, C-4a, C-11b
4a	154.0	-	-	-
6	65.0	5.44 (s)	-	C-4a, C-6a, C-6b, C-11a
6a	107.3	-	-	-
6b	118.7	-	-	-
7	116.1	6.80 (d, 8.2)	H-8	C-6b, C-8, C-9
8	112.8	7.09 (d, 8.2)	H-7	C-6a, C-6b, C-9, C-10, C-10a
9	152.1	-	-	-
10	110.0	-	-	-
10a	154.7	-	-	-
11a	145.9	-	-	-
11b	104.7	-	-	-
1′	22.5	3.42 (d, 7.2)	H-2′, H-4′, H-5′	C-1, C-2, C-3, C-1′, C-2′, C-3′
2′	122.2	5.25 (t, 7.2)	H-1′, H-4′, H-5′	-
3′	135.2	-	-	-
4′	18.0	1.84 (s)	H-1′, H-2′	C-2′, C-3′, C-5′
5′	25.9	1.76 (s)	H-1′, H-2′	C-2′, C-3′
1″	23.4	3.73 (d, 7.1)	H-2″, H-4″, H-5″	C-9, C-10, C-10a, C-2″, C-3″
2″	121.2	5.41 (t, 7.1)	H-1″, H-4″, H-5″	-
3″	135.7	-	-	-
4″	18.0	1.87 (s)	H-1″, H-2″	C-2″, C-3″, C-5″
5″	25.9	1.77 (s)	H-1″, H-2″	C-2″, C-4″
OMe-1	62.5	3.89 (s)	-	C-1
OH-3		5.46 (s)		C-2, C-3, C-4
OH-9		5.32 (s)		C-8, C-9, C-10

2. Materials and methods

2.1. General procedure

The general information on the instrumentation used and chemicals were the same as in previous reports (Phukhatmuen et al., 2020; Raksat et al., 2020; Tantapakul et al., 2016).

2.2. Plant material

The roots and twigs of *E. subumbrans* (Fabaceae) were collected in 2019 from plants growing in Doi Tung, Chiang Rai Province, Thailand (20°20′ 27.00″ N and 99° 50′ 2.39″ E). The plant was authenticated by Mr. Martin Van de Bult, a botanist at Doi Tung Development Project, Chiang Rai, Thailand. A herbarium specimen number MFU-NPR0188 has been deposited at the Natural Products Research Laboratory, Mae Fah Luang University.

2.3. Extraction and isolation

Air-dried roots (2.7 kg) and twigs (4.1 kg) of *E. subumbrans* were extracted with ethyl acetate (EtOAc) (3 × 5 L) over a period of 3 days at room temperature. Removal of the solvent under reduced pressure yielded the EtOAc extract of the roots (49.3 g) and the twigs (128.7 g), respectively. The root extract (49.3 g) was fractionated by quick column chromatography (QCC) on silica gel eluting with a gradient of hexanes-acetone to give seven fractions (RES1-RES7). Compounds **2** (35.7 mg), **3** (3.0 mg), **7** (6.2 mg), **10** (16.4 mg), **12** (3.7 mg), **13** (2.7 mg), **14** (2.5 mg) and **22** (1.7 mg) were isolated from RES4 (2.73 g). Compounds **1** (2.9 mg), **4** (4.1 mg), **6** (3.7 mg), **8** (2.3 mg), **9** (2.2 mg), **11** (15.8 mg), **19** (7.1 mg), **20** (9.9 mg), and **21** (5.4 mg) were obtained from RES6 (6.3 g). For a flowchart of the isolation procedure, see Figure 1.

The twig extract (128.7 g) was subjected to QCC on silica gel eluting with a gradient of hexanes-EtOAc to obtain eight fractions (TES1-TES8). Compounds **5** (7.3 mg), **7** (2.6 mg), **19** (3.1 mg) and **27** (2.4 mg) were isolated from TES3 (6.3 g). Fraction TES4 (10.8 g) was subjected to CC on Sephadex LH-20 using 100% MeOH as eluent to afford six fractions (TES4.1-TSE4.6). Compounds **16** (2.8 mg), **23** (32.4 mg), and **26** (3.2 mg) were isolated from TES4.2 (433.7 mg) while compound **22** (3.4 mg) was obtained from TES4.3 (254.2 mg). Fraction TES4.5 (632.8 mg) was further purified by CC on silica gel yielding compounds **6** (4.6 mg), **24** (3.8 mg), and **25** (3.6 mg). Compounds **1** (1.0 mg), **17** (3.4 mg), and **28** (2.9 mg) were obtained from fraction TSE5 (4.1 g) whereas compounds **15** (12.1 mg) and **18** (5.2 mg) were obtained from TSE6 (9.2 g). For a flowchart of the isolation procedure, see Figure 2.

Erythrinocarpan (1). Light yellow viscous oil. UV λ_{max} (log ε): 246 (2.10), 286 (3.19) nm; IR (KBr) υ_{max}: 3467, 1613, 1491, 1169 cm^{-1}; ^1H and ^{13}C-NMR spectral data, see Table 1; HRESITOFMS m/z 443.1829, $[M + Na]^+$ (calcd for $C_{26}H_{28}NaO_5$, 443.1835).

2.4. α-Glucosidase inhibition activity

The α-glucosidase inhibitory assay was performed using the same method as described in our previous reports (Phukhatmuen et al., 2020; Raksat et al., 2020). The positive controls were acarbose, voglibose, and quercetin.

2.5. α-Amylase inhibition activity

The α-amylase inhibitory assay was modified from a previous report (Kusano et al., 2011). Acarbose, voglibose, and quercetin were used as positive controls.

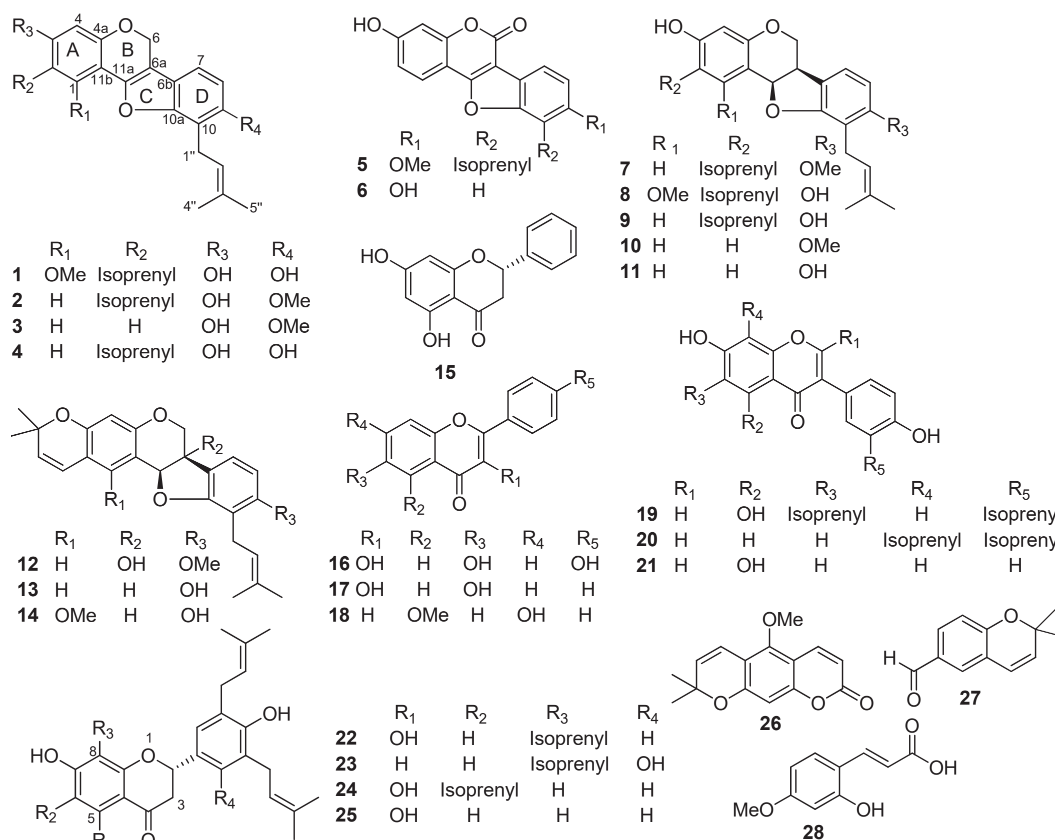

Figure 3. Compounds isolated from twigs and roots of *E. subumbrans*.

2.6. Glycation inhibition activity

The procedure for the glycation inhibition assay was performed using the same procedure as the previous report (Justino et al., 2016). The standard control was quercetin.

2.7. Glucose uptake activity

The glucose uptake assay was conducted using the same procedure as the previous report with slight alteration (Sharma et al., 2019), and metformin was used as the standard control.

2.8. Glucose consumption activity

The glucose consumption assay was performed using the same procedure as our previous report (Phukhatmuen et al., 2020), and metformin was used as standard control. Cell viability was carried out by 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, as previously described (Ahmed et al., 1994).

2.9. Nitric oxide production inhibition activity

The nitric oxide production (NO) inhibition assay was performed using the same procedure as previously reported (Dong et al., 2017), and aminoguanidine was used as a positive control and 0.1% DMSO as a negative control. Cell viability studies were evaluated by the MTT assay (Ahmed et al., 1994).

2.10. Antimicrobial activity

Bacterial strains including Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* DMST 15503, methicillin resistant *Staphylococcus aureus* NPRC 001R, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC, 19615), Gram-negative bacteria (*Shigella flexneri* DMST 4423, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* DMST 22842, *Salmonella typhimurium* DMST 562, *Escherichia coli* TISTR 780) and fungi (*Candida albicans* ATCC 10231) used in these experiments were obtained from the Microbiological Resources Center, Thailand. A 2-fold serial dilution method (Nutrient broth) was used for the antibacterial assays and the determination of minimum inhibitory concentrations (MICs) (Wikler et al., 2006; Tantapakul et al., 2016). The antimicrobial assays were tested in triplicate, and the standard compounds were vancomycin and gentamycin.

3. Results and discussion

3.1. Isolation and structure elucidation

The EtOAc extracts of the twigs and roots of *E. subumbrans* were purified by repeated chromatographic techniques to obtain 28 compounds (Figure 3) including 14 pterocarpans (**1**–**14**), five flavanones (**15** and **22**–**25**), three flavones (**16**–**18**), three isoflavones (**19**–**21**) and three phenolic derivatives (**26**–**28**). The known compounds were identified as eryvarin E (**2**), eryvarin D (**3**) (Tanaka et al., 2001a), erycristagallin (**4**) (Hegde et al., 1997), erythribyssin N (**5**) (Nguyen et al., 2010), coumestrol (**6**) (Yuk et al., 2011), erycristin (**7**) (Mitscher et al., 1988), 1-methoxyerythrabyssin II (**8**) (Rukachaisirikul et al., 2008), erythrabyssin II (**9**) (Tanaka et al., 1998), sandwicensin (**10**) (Mckee et al., 1997), phaseollidin (**11**) (Dagne et al., 1993), erypoejin J (**12**) (Tanaka et al., 2003), orientanol C (**13**) (Tanaka et al., 1998), gangetin (**14**) (Purushothaman et al., 1986), pinocembrin (**15**) (Ramirez et al., 2013), 3,6,4′-trihydroxyflavone (**16**), 3,6-dihydroxyflavone (**17**) (Park et al., 2006), 5-methoxy-7-hydroxyflavone (**18**) (Dao et al., 2003), lupalbigenin (**19**) (Pistelli et al., 1996), erysubin F (**20**) (Tanaka et al., 2001b),

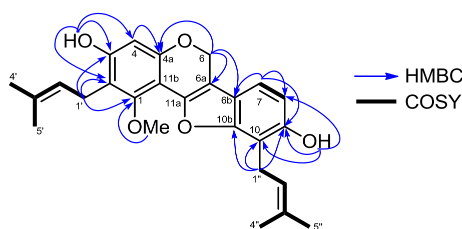

Figure 4. ^1H–^1H COSY and HMBC key correlations of erythrinocarpan (**1**).

genistein (**21**) (Ma et al., 2013), 5-hydroxysophoranone (**22**) (Matsuura et al., 1994), 7,2′,4′-trihydroxy-8,3′,5′-(3″-methyl- but-2″-enyl)flavanone (**23**) (Xia et al., 2019), 4′-hydroxy-6,3′,5′-triprenylisoflavanone (**24**), abyssinone V (**25**) (Hegde et al., 1997), xanthoxyletin (**26**) (Wu and Furukawa, 1983), 2,2-dimethyl-2H-chromene-6-carboxaldehyde (**27**) (Smith et al., 2003) and 2-hydroxy-4-methoxy-cinnamic acid (**28**) (Hofer et al., 1986) by comparisons with the literature reported spectroscopic data.

Compound (**1**) was obtained as a light yellow viscous oil. The molecular formula of $C_{26}H_{28}O_5$ was determined from its HRESITOFMS, which showed an apparent molecular ion at *m/z* 443.1829 $[M + Na]^+$ (calcd for $C_{26}H_{28}NaO_5$, 443.1835). The IR spectrum displayed the hydroxy group at 3467 cm^{-1}, while the UV spectrum showed maximum absorptions at λ_{max} 246 and 286 nm. The ^{13}C NMR and DEPT spectroscopic data (Table 1) displayed resonances for 26 carbons including five methyls (δ_C 18.0, 18.0, 25.9, 25.9, and 62.5), three methylenes (δ_C 65.0, 23.4, and 22.5), five methines (δ_C 122.2, 121.2, 116.1, 112.8, and 101.2), and 13 quaternary carbons (δ_C 156.3, 154.7, 154.0, 153.2, 152.1, 145.9, 135.7, 135.2, 118.7, 114.3, 110.0, 107.3, and 104.7). The ^1H NMR spectroscopic data (Table 1) revealed resonances for three aromatic protons [δ_H 7.09 (1H, d, $J = 8.2$ Hz, H-7), 6.80 (1H, d, $J = 8.2$ Hz, H-8), and 6.30 (1H, s, H-4)], one methylene [δ_H 5.44 (2H, s, H-6)]. The NMR spectroscopic data suggested that the structure of **1** had a pterocarpan core structure, which was closely related in structure to that of erycristagallin (**4**) (Hegde et al., 1997). The difference between compounds **1** and **4** was that compound **1** displayed one extra a methoxy group at C-1 [δ_H 3.89 (3H, s, 1-OMe)]. In addition, two sets of isoprenyl units [δ_H 5.25 (1H, t, $J = 7.2$ Hz, H-2′), 3.42 (2H, d, $J = 7.2$ Hz, H-1′), 1.84 (3H, s, H-4′), and 1.76 (3H, s, H-5′) and 5.41 (1H, t, $J = 7.1$ Hz, H-2″), 3.73 (2H, d, $J = 7.1$ Hz, H-1″), 1.87 (3H, s, H-4″), and 1.77 (3H, s, H-5″)] were identified. The following HMBC correlations (Figure 4) established the attachments of a methoxy, a hydroxy, and an isoprenyl unit on the A ring at C-1, C-3, and C-2, respectively: MeO-1 (δ_H 3.89) with C-1 (δ_C 153.2); HO-3 (δ_H 5.46) with C-2 (δ_C 114.3), C-3 (156.3), and C-4 (δ_C 101.2); H_2-1′ (δ_H 3.42) with C-1 (153.2), C-2 (δ_C 114.3), and C-3 (δ_C 156.3); H-4 (δ_H 6.30) with C-2, C-3, C-4a (δ_C 154.0), and C-11b (δ_C 104.7). In addition, the HMBC correlations between H_2-1″ (δ_H 3.73) with C-9 (δ_C 152.1), C-10 (δ_C 110.0), and C-10a (δ_C 154.7) and HO-9 (δ_H 5.32) with C-8 (δ_C 112.8), C-9 (δ_C 152.1), and C-10 (δ_C 110.0) confirmed the other isoprenyl unit and hydroxy group where on the D ring at C-10 and C-9, respectively. Therefore, the structure of **1** was characterized as erythrinocarpan.

Table 2. α-Glucosidase, α-amylase and glycation inhibition activities of compounds **1**–**28** isolated from *E. subumbrans* roots and twigs.

Compound	α-Glucosidase inhibition (IC_{50}, μM)	α-Amylase inhibition (IC_{50}, μM)	Glycation inhibition (IC_{50}, μM)
1	60.8 ± 0.25	Inactive	Inactive
2	Inactive	67.6 ± 1.12	Inactive
3	13.4 ± 0.05	Inactive	Inactive
4	90.8 ± 2.86	Inactive	Inactive
5	99.9 ± 0.38	Inactive	Inactive
6	Inactive	Inactive	Inactive
7	Inactive	Inactive	Inactive
8	24.5 ± 0.13	Inactive	53.1 ± 1.65
9	29.0 ± 0.05	Inactive	Inactive
10	Inactive	Inactive	Inactive
11	Inactive	Inactive	Inactive
12	Inactive	Inactive	Inactive
13	Inactive	Inactive	Inactive
14	Inactive	Inactive	95.6 ± 1.02
15	Inactive	Inactive	83.1 ± 0.94
16	Inactive	Inactive	Inactive
17	Inactive	Inactive	Inactive
18	Inactive	Inactive	Inactive
19	Inactive	Inactive	Inactive
20	Inactive	Inactive	Inactive
21	Inactive	Inactive	Inactive
22	12.8 ± 0.14	Inactive	36.9 ± 0.62
23	Inactive	Inactive	Inactive
24	Inactive	Inactive	40.5 ± 0.37
25	Inactive	Inactive	Inactive
26	Inactive	Inactive	Inactive
27	Inactive	Inactive	Inactive
28	Inactive	Inactive	Inactive
Acarbose	76.7 ± 1.42	103.4 ± 0.89	NT
Voglibose	134.2 ± 0.10	201.2 ± 1.94	NT
Quercetin	28.0 ± 0.18	174.3 ± 0.68	59.1 ± 0.78

Inactive at >100 μM.

Table 3. Glucose consumption, glucose uptake and NO inhibition of compounds **1**–**28** isolated from *E.subumbrans* roots and twigs.

Compound	Glucose consumption (IC_{50}, μM)	Glucose uptake (fold)	NO Inhibition (IC_{50}, μM)	Cell viability of 3T3-L1 cells (%)	Cell viability raw 264.7 cells (%)
1	Inactive	Inactive	Inactive	54.1 ± 0.9	99.4 ± 0.6
2	Inactive	Inactive	Inactive	76.5 ± 1.1	56.1 ± 2.4
3	Inactive	Inactive	Inactive	84.3 ± 1.6	13.2 ± 0.7
4	Inactive	Inactive	Inactive	94.4 ± 0.5	97.3 ± 0.3
5	Inactive	Inactive	Inactive	34.2 ± 0.9	98.6 ± 1.6
6	Inactive	Inactive	Inactive	65.5 ± 1.2	3.7 ± 0.2
7	Inactive	Inactive	Inactive	85.2 ± 1.8	3.1 ± 0.9
8	Inactive	Inactive	Inactive	40.5 ± 0.9	2.9 ± 0.6
9	Inactive	Inactive	Inactive	78.3 ± 0.7	3.3 ± 0.2
10	Inactive	Inactive	Inactive	64.2 ± 0.3	3.0 ± 0.1
11	Inactive	Inactive	Inactive	43.9 ± 0.9	18.9 ± 0.3
12	Inactive	Inactive	Inactive	56.5 ± 1.3	95.1 ± 0.7
13	Inactive	Inactive	Inactive	36.9 ± 0.8	3.4 ± 0.1
14	Inactive	Inactive	Inactive	43.1 ± 0.4	2.8 ± 0.1
15	Inactive	Inactive	Inactive	77.3 ± 1.4	92.9 ± 0.8
16	Inactive	Inactive	96.2 ± 1.61	39.7 ± 1.9	98.4 ± 0.5
17	Inactive	Inactive	Inactive	43.6 ± 1.1	97.7 ± 0.6
18	Inactive	Inactive	Inactive	41.6 ± 1.8	5.7 ± 1.1
19	Inactive	Inactive	Inactive	79.6 ± 1.8	3.8 ± 0.1
20	Inactive	Inactive	Inactive	84.7 ± 0.9	93.7 ± 0.7
21	Inactive	Inactive	Inactive	74.5 ± 1.4	81.3 ± 0.5
22	Inactive	Inactive	Inactive	80.8 ± 1.2	3.67 ± 0.9
23	Inactive	Inactive	Inactive	26.9 ± 0.9	12.1 ± 1.1
24	Inactive	Inactive	Inactive	39.7 ± 0.7	3.7 ± 0.1
25	Inactive	Inactive	Inactive	62.4 ± 1.4	3.4 ± 0.2
26	33.4 ± 0.34	1.3 ± 0.21	Inactive	97.5 ± 0.3	21.6 ± 1.5
27	29.1 ± 0.86	2.8 ± 0.47	52.5 ± 0.56	98.2 ± 1.7	97.9 ± 1.2
28	Inactive	Inactive	Inactive	40.6 ± 1.1	97.1 ± 0.2
Metformin	47.2 ± 1.17	3.7 ± 0.96	NT	96.1 ± 0.9	99.4 ± 0.6
Aminoguanidine	NT	NT	22.4 ± 0.78	NT	97.9 ± 0.8

Inactive at >100 μM.

3.2. Antidiabetic activities (enzymatic based assays)

Various natural products directly or indirectly affect diabetes pathways as enzyme inhibitors (Alam et al., 2019). Several enzymes are involved in the pathogenesis of the disease. In this study, the properties of α-glucosidase inhibition, α-amylase inhibition, and glycation inhibition of all isolated compounds (**1**–**28**) from *E. subumbrans* were evaluated (Table 2).

In the α-glucosidase inhibition assay, seven compounds including six pterocarpans (**1**, **3**–**5**, **8** and **9**), and one flavanone (**22**), showed α-glucosidase inhibition activities with IC_{50} values ranging from 12.8 ± 0.14–99.9 ± 0.38 μM. Of these compounds, compounds **22** (IC_{50} of 12.8 ± 0.14 μM), **3** (IC_{50} of 13.4 ± 0.05 μM), and **8** (IC_{50} of 24.5 ± 0.13 μM), showed inhibitory activity greater than that of the positive controls [acarbose (IC_{50} of 76.7 ± 1.42 μM), voglibose (IC_{50} of 134.2 ± 0.10 μM), and quercetin (IC_{50} of 28.0 ± 0.18 μM)] (Table 2). Compound **9** (IC_{50} of 29.0 ± 0.05 μM) also showed inhibitory activity better than that of acarbose and voglibose but was less active than quercetin. Compound **22** was the only flavanone that revealed significant α-glucosidase inhibitory activity. It is interesting to note that the structures of compounds **1** and **8** only differ by the degree of unsaturation at the B/C ring junction ($\Delta^{6a(11a)}$). The more unsaturated compound (**1**) showed significantly less α-glucosidase inhibition activity than its more saturated counterpart (**8**) (Table 2). Similarly, the presence of the prenyl unit at C-2 in compound **2** (IC_{50} > 100 μM) is crucially important to reduce the α-glucosidase inhibition activity when compared to compound **3** (IC_{50} of 13.4 μM) containing a proton.

In the case of α-amylase inhibitory activity, only pterocarpan **2** showed inhibitory activity with an IC_{50} value of 62.6 ± 1.12 μM, which was stronger than that of the positive controls [acarbose (IC_{50} 103.4 ± 0.89 μM), voglibose (IC_{50} 201.2 ± 1.94 μM), and quercetin (IC_{50} 174.3 ± 0.68 μM)] (Table 2). The other compounds had IC_{50} values of >100 μM. It should be noted that the structure of pterocarpan **2** differed from pterocarpans **3**, **4** and **7** only at C-2 (R_2 = H), C-9 (R_4 = OH), and the B/C ring junction ($\Delta^{6a(11a)}$), respectively. These differences have a much greater effect on reducing the α-amylase inhibitory activity of pterocarpans **3**, **4** and **7**.

Glycation inhibition assay was evaluated by advanced glycation end products (AGEs) analysis by emulating the conditions of proteins under hyperglycaemic conditions. This process is significantly related to diabetes long-term complications such as diabetic nephropathy, inflammatory conditions, and fibrosis (Uribarri et al., 2010). Among the tested compounds, flavanones **22** and **24** showed the best glycation inhibitory activities with IC_{50} values of 36.9 ± 0.62 and 40.5 ± 0.37 μM, respectively, which were better than the positive control (quercetin, IC_{50} value of 59.1 ± 0.78 μM, Table 2). Interestingly, flavanones **22** and **24** in which the A-ring prenyl unit is at C-8 and C-6, respectively, have very similar glycation inhibition activities.

3.3. Antidiabetic activities (cell-based assays)

Previous studies indicated that some natural products, especially flavonoids, can enhance glucose uptake activity in L-6 cell lines (Narmatha and Maneemegalai, 2019). In this study, cell-based assays on the

Table 4. Antimicrobial activity of compounds **1**–**28** isolated from *E. subumbrans* roots and twigs.

Compound	MIC (μg/mL)										
	Gram-positive bacteria					Gram-negative bacteria					Fungi
	EF	MT	MRSA	SA	SP	SF	PA	ST	STR	EC	CA
1	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	128	128	128	Inactive
2	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	Inactive
3	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	128	128	Inactive
4	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	128	Inactive	128	Inactive
5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	128	Inactive	128	Inactive
6	128	Inactive	32	Inactive	Inactive	128	128	128	Inactive	128	128
7	128	128	128	64	128	128	128	128	Inactive	128	128
8	128	128	128	64	128	Inactive	128	128	Inactive	128	128
9	2	2	4	2	2	Inactive	128	128	Inactive	Inactive	4
10	8	8	8	64	8	128	128	Inactive	128	Inactive	16
11	128	128	64	64	64	Inactive	128	128	Inactive	Inactive	128
12	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	128	Inactive	Inactive	Inactive
13	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	128	Inactive	128	Inactive
14	128	128	64	32	64	Inactive	128	128	Inactive	128	128
15	Inactive	Inactive	Inactive	128	Inactive	128	128	Inactive	Inactive	128	Inactive
16	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	Inactive	Inactive	128	Inactive
17	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	Inactive	Inactive	Inactive	Inactive
18	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	128	Inactive	128	Inactive
19	Inactive	Inactive	128	128	64	Inactive	128	Inactive	Inactive	128	128
20	128	Inactive	Inactive	16	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	16
21	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	Inactive	Inactive	Inactive
22	Inactive	Inactive	Inactive	128	128	128	128	Inactive	Inactive	128	Inactive
23	Inactive	Inactive	Inactive	128	Inactive	Inactive	128	Inactive	Inactive	128	Inactive
24	Inactive	Inactive	128	128	128	Inactive	128	128	Inactive	128	128
25	Inactive	128	Inactive	128	128	128	128	128	128	Inactive	Inactive
26	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	Inactive	Inactive	Inactive
27	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	Inactive	Inactive	Inactive
28	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	128	Inactive	Inactive	Inactive	Inactive
Vancomycin	2	2	1	0.5	2	-	-	-	-	-	4
Gentamicin	-	-	-	-	-	4	4	1	0.5	4	-

Inactive at >128 μg/mL. EF = *Enterococcus faecalis* (ATCC 29212); MT = *Micrococcus luteus* (DMST 15503); MRSA = Methicillin Resistant *Staphylococcus aureus* (NPRC 001R); SA = *Staphylococcus aureus* (ATCC 25923); SP = *Streptococcus pyogenes* (ATCC, 19615); SF = *Shigella flexneri* (DMST 4423); PSA = *Pseudomonas aeruginosa* (ATCC 10145); STP = *Salmonella typhi* (DMST 22842); STM = *Salmonella typhimurium* (DMST 562); EC = *Escherichia coli* (TISTR 780); CA = *Candida albicans* (ATCC 10231).

antidiabetic properties of the isolated compounds via glucose consumption and glucose uptake assays were determined and the results are shown in Table 3. Only two compounds, compounds **26** and **27**, illustrated the ability to induce glucose consumption into 3T3-L1 adipocyte cells with IC_{50} values of 33.4 ± 0.34 and 29.1 ± 0.86 μM, respectively without toxicity to the cells. To confirm the activity of promoting glucose consumption, these compounds were further evaluated for glucose uptake induced by L6 myotube cells. The results point out the relationship between glucose consumption with a ratio of glucose uptake at 1.3-fold and 2.8-fold compared with metformin positive control (Table 3). The outcome means that these compounds can transport glucose into cells to supply cells with energy in the form of adenosine triphosphate (ATP) and play an important role in many other cellular functions (Busik et al., 2002).

3.4. Nitric oxide production inhibitory activity

Nitric oxide (NO) is a general signaling molecule considered a pro-inflammatory mediator that plays a part in inflammation pathogenesis (Luiking et al., 2010). This signaling induces inflammation due to over-production in abnormal situations (Sharma et al., 2007). Prolonged DM is one of the factors that is positively associated with inflammation by activating pathways for the production of pro-inflammatory cytokines (NO) (Kim et al., 2008). Only compounds **16** and **27** showed nitric oxide production inhibitory activity with IC_{50} values of 96.2 ± 1.61 and 52.5 ± 0.56 μM, respectively, which were less active than the standard control (aminoguanidine, IC_{50} value of 22.4 ± 0.78 μM, Table 3) Compared with prior studies, compounds in the same group of active compounds, including flavones, showed the greatest potential to inhibit nitric oxide production with IC_{50} values of 3.8–25.1 μM (Menini et al., 2020). The compounds in groups of phenylpropanoid derivatives similar to compound **27** have also been reported to inhibit nitric oxide production with IC_{50} values in the range of 26.3–31.6 μM (Cai et al., 2020). Our data can infer that compounds **16** and **27** are only moderately active inhibitors of LPS-stimulated NO production in RAW 264.7 cells.

3.5. Antimicrobial activity

The most severe complications of diabetes are foot ulcer infections, which can ultimately lead to lower limb amputations (Ramirez-Acuña et al., 2019). Therefore, compounds **1**–**28** were also evaluated for their antimicrobial activities against Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* DMST 15503, methicillin resistant *Staphylococcus aureus* NPRC 001R, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC, 19615), Gram-negative bacteria (*Shigella flexneri* DMST 4423, *Pseudomonas aeruginosa* ATCC 10145,

Salmonella typhi DMST 22842, *Salmonella typhimurium* DMST 562, *Escherichia coli* TISTR 780) and fungi (*Candida albicans* ATCC 10231) using the broth microdilution assay. Compound **9** showed the best activities against Gram-positive bacterial and fungi with MIC values ranging from 2-4 μg/mL, whereas compound **10** was less active with MIC values ranging from 8-64 μg/mL (Table 4). Antibacterial activities against *S. aureus* and MRSA of pterocarpans **9** and **10** have been reported with MIC values in the range of 0.78–1.56 (Rukachaisirikul et al., 2007b) and 8–64 μg/mL (Innok et al., 2010), respectively. In this study, the MIC values against *S. aureus* and MRSA of pterocarpans **9** and **10** were 2–4 and 8–64 μg/mL, respectively, similar to that found in the previous study (Rukachaisirikul et al., 2007b; Innok et al., 2010). All remaining compounds were found to have weak activities or were inactive against all strains of bacteria and fungi. It should be noted that compounds **9** and **10** shared the same core structure but have a different number of hydroxy and isoprenyl groups. Compound **9** contains two hydroxy groups (C-3 and C-9) and two isoprenyl groups (C-2 and C-10) while compound **10** comprises a hydroxy (C-3), a methoxy (C-10), and an isoprenyl (C-10) group. From this information we might conclude that compound **9** has better antimicrobial activity than **10**, due to the extra hydroxy and isoprenyl groups in the former compound.

4. Conclusion

In conclusion, a new pterocarpan (**1**), was isolated together with 27 known compounds (**2**–**28**) from the root and twig extracts of *E. subumbrans*. The compounds were identified as 14 pterocarpans (**1**–**14**), five flavanones (**15** and **22**–**25**), three flavones (**16**–**18**), three isoflavones (**19**–**21**) and three phenolic derivatives (**26**–**28**). Five compounds (**1**, **6**, **7**, **19**, and **22**) were obtained from both extracts, whereas 12 compounds (**2**–**4**, **8**–**14**, **20**, and **22**) and 11 compounds (**5**, **15**–**18**, and **23**–**28**) were obtained only from the root and twig extracts, respectively. Compounds **5**, **6**, **7**, **12**, **13**, **15**, **16**, **17**, **18**, **21**, **23**, **24**, **25**, **26**, **27**, and **28** were isolated for the first time from this plant. All isolated compounds were evaluated for their potential antidiabetic activities (enzyme and cells-based assays) along with anti-inflammatory effects through nitric oxide production inhibitory activities and their antimicrobial activities. Compounds **22**, **3**, **8**, and **9** displayed promising α-glucosidase inhibition activity, while compound **2** showed the best α-amylase inhibition activity. Furthermore, compounds **22** and **24** were effective to prevent AGEs. Compound **27** showed the best ability to induce glucose consumption and to enhance glucose uptake. It also had NO production inhibitory activity without causing toxicity to cells. These bioactive compounds might be considered as a group of antidiabetic agents with potential for further development as new drugs to treat diabetes mellitus and complications. Compounds **9** and **10** were identified as the most promising compounds showing potent activity against all Gram-positive strains and fungi.

Declarations

Author contribution statement

Piyaporn Phukhatmuen, Pornphimol Meesakul: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Virayu Suthiphasilp: Analyzed and interpreted the data; Wrote the paper.

Rawiwan Charoensup, Tharakorn Maneera, Sarot Cheenpracha: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Thunwadee Limtharakul, Stephen G. Pyne: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Surat Laphookhieo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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