RJC

RASĀYAN *J. Chem.*
Vol. 17 | No. 3 |832-837| July - September | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ISOLATION AND INHIBITION STUDY OF ERYVARIN D AND E FROM THE ROOT BARKS OF *Erythrina variegata* AGAINST RECEPTOR TYROSINE KINASES

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ABSTRACT

Erythrina sp. contains alkaloids and flavonoids, which are the primary bioactive compounds found in these species. These organisms are a rich source of secondary metabolites. Two known pterocapans in this study, eryvarin D (**1**) and eryvarin E (**2**), were successfully isolated from the root bark extract of *Erythrina variegata*. The isolated compounds were elucidated using 1D- and 2D-NMR spectroscopy. Among the two compounds, only compound **2** was evaluated for its inhibitory activity against eight receptor tyrosine kinases (EGFR, HER2, HER4, IGFR, InsR, KDR, PDGFRα, and PDGFRβ). Compound **2** exhibited weak insulin receptor inhibitory activity. Thus, compound 2 could be a lead compound as an anticancer drug targeting insulin receptors for future development.

Keywords: *Erythrina variegata*, Inhibitor, Isoflavonoid, Pterocarpan, Receptor Tyrosine Kinase.

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INTRODUCTION

The Fabaceae family, which has over 115 species, includes *Erythrina* species. These species are found across the world's tropical and warm-temperate regions. The genus name, which is derived from the Greek term "erythros," meaning "red color" and alludes to the hue of the flowers, serves as the source of its name.[1] Some *Erythrina* species are utilized for decorative purposes, as shade, and to enhance soil in yards and pavements.[2, 3] These plants are collectively referred to as "coral trees".[4] *Erythrina* species have been traditionally employed in the field of medicine to treat a wide range of ailments. For instance, the Tenek tribe of San Luis Potosi, Mexico, uses *Erythrina herbacea* as a sedative because of its alkaloids.[5] In Indonesia, a bark or leaf decoction was used to treat malaria.[6]

Alkaloids and flavonoids are the main bioactive compounds found in *Erythrina* sp., which are a rich source of secondary metabolites. Furthermore, *Erythrina* alkaloids have anticonvulsants, smooth muscle relaxants, neuromuscular blockers, and antidepressant properties and *Erythrina* flavonoids have anticancer, anti-inflammatory, antidiabetic, antimicrobial, anti-HIV, and anti-plasmodial properties.[7-9] Receptor tyrosine kinases (RTKs) are a subcategory of tyrosine kinase enzymes that play crucial roles in facilitating cell-to-cell communication and regulating various biological processes. The irregular RTK expression can cause a discrepancy in cell growth.[10]

Thus, RTKs have become an important target in the clinical treatment of cancer.[11-14] Several flavonoid derivatives were reported to have tyrosine kinase inhibitory activities.[10,15] Moreover, *Erythrina* flavonoids showed potent inhibitory activities against several cancer cell lines,[7,16] but there was still a lack of information about the inhibition study against receptor tyrosine kinases (RTKs).

Thus, flavonoid derivatives in this study were isolated from the root bark of *Erythrina variegata* and evaluated their inhibitory activities against eight receptor tyrosine kinases, such as epidermal growth factor (EGFR, HER2, HER4), insulin (IGF1R, InsR), kinase insert domain (KDR), and platelet-derived growth factor (PDGFRα, PDGFRβ) receptors.

http://doi.org/10.31788/RJC.2024.1738790

EXPERIMENTAL

Material and Methods

All compounds were analyzed using NMR spectroscopy on an Agilent DD2 system operating at 500 MHz in $CDCl_3$. Additionally, vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were conducted on Merck silica gel 60 GF254 (codes 7731 and 7749, respectively). Thin layer chromatography (TLC) analysis was performed using precoated silica gel plates (Merck Kielselgel 60 GF254, 0.25 mm thickness), which were detected by UV irradiation and spraying with 10% vanillin in H_2SO_4, followed by heating. The solvents used for extraction, fractionation, and purification were acetone, EtOAc, n-hexane (technical grade, distilled before use), and $CHCl_3$ (pro analysis grade). The Kinase Selectivity Profiling System (KSPS) Receptor Tyrosine Kinases TK1 (EGFR, HER2, HER4, IGF1R, InsR, KDR, PDGFRα, and PDGFRβ) were purchased from Promega and stored at -80 °C as one-time use aliquots. The kinase enzyme assay was carried out using a Pipetmax automatic liquid handler (Gilson), while luminescence measurements were taken using a GloMax Explorer.

Sample Collection

In January 2021, root bark samples of *Erythrina variegata* L were gathered from Bandung, located in the West Java Province of Indonesia, and transported to the BSCA Building within the Faculty of Mathematics and Natural Sciences at the Institut Teknologi Bandung.

Isolation of Bioactive Compounds

The root bark of *Erythrina variegata L* was dried and powdered, and 1 kg of it was extracted with 3 × 10 L of acetone overnight at room temperature to produce a dried extract, which was then subjected to VLC using gradient elution with n-hexane-EtOAc (10:0 to 1:1, 10% stepwise EtOAc addition, followed by EtOAc and MeOH, each 2 × 200 mL) to yield 14 fractions (A1-A14). Fractions A11-A13 were combined and further refractionated using the same method (gradient elution: n-hexane-EtOAc = 9:1, 8.5:1.5, 7.5:2.5, 7:3, followed by EtOAc, each 2 × 50 mL), resulting in 15 subfractions (B1-B15). Subfractions B7-B9 were combined and purified using CPC eluted with $CHCl_3$-EtOAc (7:3) to yield compounds 1 (50 mg) and 2 (25 mg), which were determined using 1D and 2D NMR methods and compared with literature data.

Receptor Tyrosine Kinase Assay

The assay was done according to the previous study.[17,18] The compound was prepared by mixing it with DMSO and 4x kinase buffer, with 62.5 µL and 175 µL of nuclease-free water, respectively, to achieve a final concentration of 10 µM. Each kinase stock was diluted with 2.5 × reaction buffer, comprising 95 µL, while the substrate/cofactor stock was diluted with 20 µL of 80 µM ATP solution. The reaction was carried out by adding 2 µL of the kinase, 2 µL of the ATP/substrate, and 1 µL of the tested compound to each well of a 384-well plate and allowing them to react for 1 hour at 22-25°C. After this, 5 µL of ADP-Glo reagent was added and incubated for 40 minutes at 22-25°C, followed by adding 10 µL of kinase detection reagent, which was then incubated for another 30 minutes. The reaction was then measured for luminescence using a Glomax Explorer, which was used to determine the kinase activity. The negative control was the well without the tested compound solution, and the background luminescence was the well without the enzyme solution. The percentage of kinase activity was calculated by subtracting the background luminescence and dividing it by the negative control. Erlotinib (1 µM) was used as the positive control.

RESULTS AND DISCUSSION

In our study, two known compounds with pterocarpan structures (eryvarin D (1) and eryvarin E (2)) were successfully isolated from the root bark of acetone extract of *Erythrina variegata* as shown in Fig.-1. Compounds 1 and 2 had been reported previously[19,20] as shown in Tables-1 & 2. Thus, compound 2 was selected to address the structural elucidation. The ^{13}C-NMR spectrum of compound 2 showed 25 signals representing 26 carbon atoms and the ^{1}H-NMR spectrum appeared with the presence of 28 protons as shown in Table-2. Moreover, methylene proton at δ_H 5.5 ppm showed a singlet signal bound with carbon at δ_C 65.6 ppm which was typical of the pterocarpene skeleton. As presented in Table-2, the HMBC spectrum provided a typical pterocarpene signal of two proton signals at δ_H 5.5 ppm showing a correlation of 2-3 bonds to carbon at δ_C 106.0, 147.5, and 153.7 ppm. The position of the two prenyls was also confirmed by

the correlation of the proton at δ_H 3.34 ppm with δ_C 119.9 ppm. Thus, the COSY experiment confirmed the overlapping proton signals for H-2' and H-2" with multiplet signal at δ_H 5.3 ppm representing two C-H signals (Fig.-S7, supplementary data). The obtained result from the COSY experiment was a correlation between H-1'/H-2' and H-1"/H-2" at δ_H 3.34 and 3.63 ppm, respectively. The structure and correlation are presented in Fig.-2.

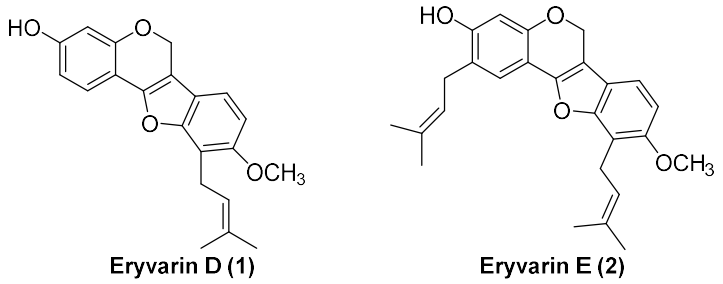

Fig.-1: Structures of the Compounds Isolated from Root Barks of *E. variegata*

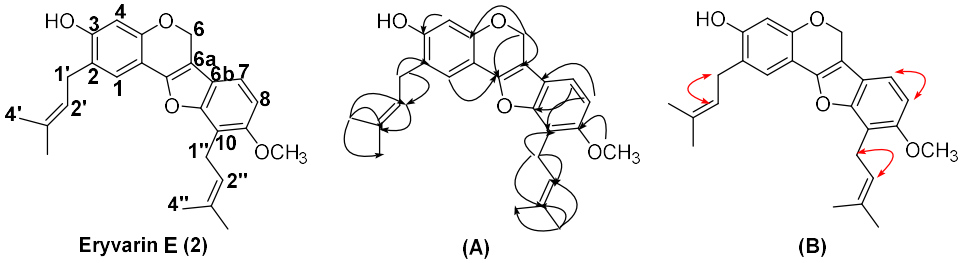

Fig.-2: Correlation of HMBC (A) and COSY (B) on Compound 2

Table-1: NMR Data of Compound 1 in $CDCl_3$

Position	δ_H, [mult., *J* (Hz)][a]	δ_C[b]	HMBC (H→C)	δ_H, [mult., *J* (Hz)][c]	δ_C[c]
1	7.37 (*d*, 8.2)	121.4	11a, 4a, 3	7.37 (*d,* 8.1)	121.3
2	6.46 (*dd*, 8.1; 2.2)	108.5	11b	6.45 (*dd*, 8.1; 2.2)	108.4
3	-	156.9	-	-	156.9
4	6.43 (*d*, 2.2)	103.9	3	6.43 (*d*, 2.2)	103.9
4a	-	155.3	-		155.2
6	5.54 (*s*)	65.8	6a, 11a, 4a	5.54 (*s*)	65.7
6a	-	105.9	-	-	105.8
6b	-	119.7	-	-	119.6
7	7.10 (*d*, 8.45)	115.4	10a	7.09 (*d*, 8.4)	115.3
8	6.84 (*d*, 8.85)	108.0	10, 6b	6.85 (*d*, 8.4)	107.9
9	-	154.9	-	-	154.9
10	-	114.5	-	-	114.4
10a	-	154.7	-	-	154.7
11a	-	147.3	-	-	147.3
11b	-	110.2	-	-	110.1
1'	3.63 (*d*, 7.4)	23.01	10, 2', 3', 10a, 9	3.62 (*d*, 7.3)	22.9
2'	5.35 (*t*, 7.3)	122.0	4'	5.35 (*t*, 7.3),	121.9
3'	-	132.1	-	-	132.0
4'	1.88 (*s*)	17.9	5', 2', 3'	1.88 (*s*)	17.8
5'	1.69 (*s*)	25.9	4', 2', 3'	1.69 (*s*)	25.8
OCH_3	3.89 (*s*)	56.8	9	3.88 (*s*)	56.7

[a]Data were recorded at 400 MHz
[b]Data were recorded at 500 MHz
[c]Tanaka *et al*. (2001)

Table-2: NMR Data of Compound 2 in $CDCl_3$

Position	δ_H, [mult., *J* (Hz)][a]	δ_C[b]	HMBC (H→C)	δ_H, [mult., *J* (Hz)][c]	δ_C[c]
1	7.23 (s)	121.5	2, 11a, 3a, 4a	7.23 (s)	121.4
2	-	119.9	-	-	119.8a
3		155.3	-	-	155.3
4	6.42 (s)	104.2	11b, 2, 11a, 4a	6.42 (s)	104.2
4a	-	153.7	-	-	153.6
6	5.51 (s)	65.6	6a, 11a, 4a	5.51 (s)	65.5
6a	-	106.0	-	-	105.9
6b	-	119.8	-	-	119.7a
7	7.09 (d, 8.45)	115.3	9	7.09 (d, 8.3)	115.2
8	6.85 (d, 8.5)	107.9	10, 6b	6.85 (d, 8.3)	107.8
9	-	154.8	-	-	154.8
10	-	114.4	-	-	114.4
10a	-	154.7	-	-	154.7
11a	-	147.5	-	-	147.4
11b	-	110.0	-	-	109.9
1'	3.34 (d, 7.45)	29.2	2, 1, 3'	3.34 (d, 7.3)	29.1
2'	5.3 (m)	122.0	4', 5'	5.34 (t, 7.3)	122.0b
3'	-	135.1	-	-	135.0
4'	1.81 (s)	17.9	5', 2', 3'	1.80 (s)	17.8c
5'	1.81 (s)	25.9	4', 2', 3'	1.80 (s)	25.8
1"	3.63 (d, 7.5)	23.0	10, 2", 3", 9	3.36 (d, 7.3)	22.9
2"	5.3 (m)	121.9	4', 5'	5.34 (d, 7.3)	121.8b
3"	-	132.0	-	-	131.9
4"	1.90 (s)	18.0	5", 2", 3"	1.80 (s)	17.9c
5"	1.70 (s)	25.9	4", 2", 3"	1.80 (s)	25.8
OCH_3	3.89 (s)	56.8	9	3.89 (s)	56.7

[a]Data were recorded at 400 MHz.
[b]Data were recorded at 500 MHz
[c]Tanaka *et al*. (2001).

Table-3: *In vitro* Tyrosine Kinase Activities of 1, 2, and Erlotinib at 10 μM

	% Activity[c]							
	EGFR	HER2	HER4	IGF1R	InsR	KDR	PDGFRα	PDGFRβ
Eryvarin D (1)	NA[b]	NA	NA	NA	NA	NA	NA	NA
Eryvarin E (2)	106	99	123	110	88	112	121	114
Erlotinib[a]	0	6	23	103	98	4	13	13

[a]Positive control at 1 μM. [b]NA= not analysis.
[c]Strong: <20%, Moderate: 20 – 60%, Weak or not active: >60%.

According to Table-1, compound 2 was evaluated for its inhibitory properties against eight RTKs including EGFR, HER2, HER4, IGF1R, InsR, KDR, and PDGFRα with erlotinib used as a positive control, except compound 1 due to unstable. Moreover, in a previous study, compound 1 showed cytotoxic activities against three cancer cell lines, such as KB, MCF-7, and NCI-H187 cell lines.[16] Compound 2 exhibited weak inhibitory activity by decreasing insulin receptor (InsR) activity by around 88% better than erlotinib by around 98% at 10 μM. This result shows that compound 2 selectively inhibited the insulin receptor (InsR) by around 12%. Therefore, pterocarpan skeletons could be potent as a lead compound for the development of insulin receptor inhibitors to treat several cancers, such as prostate, breast, osteosarcoma, and thyroid carcinoma cancers.[21,22]

CONCLUSION

In conclusion, eryvarin D (1) and eryvarin E (2) were isolated from the acetone extract of the root bark of *E. variegata*. Compound 2 showed a weak inhibitory activity against insulin receptors. These data indicate

that pterocarpan derivatives could be potential lead compounds for anticancer targeting of the insulin receptors (InsR).

ACKNOWLEDGMENTS

The author expresses his deepest gratitude to Prof. Yana Maolana Syah, MS, and integrated chemistry laboratory, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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