SHORT COMMUNICATION

α-Glucosidase inhibitory and α-amylase inhibitory activities of compounds isolated from Uvaria rufa Blume

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ABSTRACT
A new C-benzylated flavone, uvariaflavone (1), along with 13 known compounds (2–14) were isolated from the twig and leaf extracts of Uvaria rufa Blume. Their structures were established by extensive spectroscopic methods. Flavones (5–8) and cyclohexene (10) were isolated from U. rufa for the first time. Most of the isolated compounds were evaluated for their α-glucosidase and α-amylase inhibitory activities. Of these, uvariaflavone (1) showed the highest α-glucosidase inhibitory activity with an IC50 value of 44.3 μM, while ferrudiol (12) displayed the highest α-amylase inhibitory activity with an IC50 value of 73.5 μM.

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1. Introduction
Uvaria rufa Blume (Annonaceae) is a woody climber tree, which is widely distributed over the tropical zones of Asia, Africa, and Australia (Tip-Pyang et al. 2011). The roots of this plant have been used in traditional medicine purposes as a stimulant for childbirth and to treat fever and kidney failure (Buncharon et al. 2016). Previous phytochemical investigations of U. rufa resulted in the isolation and identification of several types of secondary metabolites, including flavonoids (Deepralard et al. 2009; Tip-Pyang...
et al. 2011), polyoxygenated cyclohexenes (Macabeo et al. 2012), alkaloids (Tip-Pyang et al. 2011), lignans (Nguyen et al. 2015), and terpenoids (Thang et al. 2014). Some of these compounds exhibited interesting biological activities, including antimicrobial (Macabeo et al. 2012), cytotoxicity (Nguyen et al. 2015), advanced glycation end-products (AGEs) (Deepralard et al. 2009), and α-glucosidase inhibitory (Hamid et al. 2015).

As part of an ongoing investigation for anti-diabetes compounds from Thai medicinal plants (Meesakul et al. 2019; Suthiphasilp et al. 2019; Raksat et al. 2020; Phukhatmuen et al. 2021), the twig and leaf extracts of *U. rufa* were examined and these showed good α-glucosidase and α-amylase inhibitory activities with IC\textsubscript{50} values in the range of 62.6–87.1 μg/mL. These findings prompted us to further investigate their phytochemicals and anti-diabetes activities, including α-glucosidase and α-amylase inhibitory activities. This report describes the isolation and structure elucidation of a new C-benzylated flavone, uvariaruflavone (1), along with 13 known compounds (2–14) (Figure 1). The α-glucosidase and α-amylase inhibitory activities of some of the isolated compounds are also reported.

### 2. Results and discussion

The EtOAc extracts of the twigs and leaves of *U. rufa* were individually subjected to column chromatography (CC) over silica gel and Sephadex LH-20 to afford a new C-

![Figure 1. Compounds isolated from the twig and leaf extracts of *U. rufa*.](image)
benzylation of flavone (1) and 13 known compounds (2–14). The structures of the known compounds were identified as tectochrysin (2), moslossoflavone (3), moslofflavone (4) (Lojanapiwatna et al. 1981), isowogonin (5) (Zhang et al. 2020), 6-methoxy-7,8-dihydroxyflavone (6) (Ma et al. 2020), cirsimaritin (7) (Alwahsh et al. 2015), negletein (8) (Riaz et al. 2012), (−)-zeyleno (9), (−)-6-acetylzeylan (10) (Stevenson et al. 2007), (−)-elliopiopis B (11) (Kijjoa et al. 2002), ferrudiol (12) (Wirasathien et al. 2006), (−)-pinostrobin (13) (Kurkina et al. 2013), and benzyl benzoate (14) (Mou et al. 2017) by extensive NMR spectroscopic data analysis and comparisons made with spectroscopic data reported in the literature.

Uvariaruflavone (1) was obtained as a yellow powder, mp 194–195°C. Its molecular formula, C_{23}H_{18}O_{5}, was determined on the basis of NMR data and HRESITOFMS, which showed a [M+H]^+ ion peak at m/z 375.1219 (calcd. for C_{23}H_{19}O_{5}^+, 375.1227). UV absorption bands at λ_{max} 249, 274, and 313 nm agreed with a flavone skeleton (Lojanapiwatna et al. 1981; Meesakul et al. 2019; Ma et al. 2020). The IR spectrum of 1 displayed absorption bands at 3389 and 1654 cm\(^{-1}\) consistent with hydroxy and conjugated ketone functionalities, respectively. The ^13C and DEPT NMR spectroscopic data indicated that compound 1 contained 23 carbons, including one methyl (δ_C 56.3), one methylene (δ_C 23.0), 11 methines (δ_C 132.0, 131.7, 129.1 (× 2), 128.0 (× 2), 126.3, 120.0, 116.7, 105.9, and 90.7), and 10 quaternary carbons (δ_C 182.5, 164.3, 163.2, 157.1, 156.5, 154.7, 131.2, 125.6, 112.1, and 105.6). The ^1H NMR spectroscopic data (Table S1) displayed an olefinic proton [δ_H 6.71 (1H, s, H-3)], an aromatic proton [δ_H 6.57 (1H, s, H-8)], a monosubstituted aromatic ring [δ_H 7.89 (2H, dd, J = 8.1, 1.4 Hz, H-2’, H-6’), 7.51 (2H, m, H-3’, H-5’), and 7.54 (1H, m, H-4’), a hydrogen-bonded hydroxy proton [δ_H 13.83 (1H, s, OH-5’)], a hydroxy proton [δ_H 7.57 (1H, s, OH-3’)], a methyl group [δ_H 4.01 (3H, s, OMe-7), and a 3”-hydroxybenzyl unit [δ_H 3.96 (2H, s, H-1’”), 6.88 (1H, m, H-4’”), 7.10 (1H, td, J = 8.1, 1.6 Hz, H-5’’), 6.82 (1H, td, J = 8.1, 1.6 Hz, H-6’’), and 7.44 (1H, dd, J = 8.1, 1.6 Hz, H-6’’)]. These NMR data suggested that compound 1 had a flavone skeleton (Meesakul et al. 2019). The HMBC correlations between the hydrogen-bonded hydroxy proton (δ_H 13.83) and C-4a (δ_C 105.6), C-5 (δ_C 157.1) and C-6 (δ_C 112.1) confirmed that the hydrogen-bonded hydroxy proton was located at C-5. The location of methoxy group at C-7 was confirmed by the HMBC correlations between OMe-7 (δ_H 4.01) and H-8 (δ_C 6.57) with C-7 (δ_C 163.2). The linkage of the hydroxybenzyl group at C-6 of flavone moiety was deduced from the 3J HMBC of H-1” (δ_H 3.96) to C-5 (δ_C 157.1), C-6 (δ_C 112.1), C-7 (δ_C 163.2), C-2” (δ_C 125.6), C-3” (δ_C 154.7), and C-7” (δ_C 131.7) (Table S1). Furthermore, HMQC cross-peaks of H-8 (δ_H 6.57) to C-6 (δ_C 112.1) supported the position of the hydroxybenzyl moiety at C-6. Therefore, compound 1 had been identified as a new C-benzylation of flavone and named uvariaruflavone.

Compounds 1, 3–8, and 10–14 were evaluated for their α-glucosidase inhibitory activities. All tested compounds displayed α-glucosidase inhibitory activity with IC_{50} values in the range of 44.3-646.7 μM. Of these, uvariaruflavone (1) showed the highest α-glucosidase inhibitory activity with IC_{50} value of 44.3 μM, which is comparable to that of the positive control (acarbose, IC_{50} = 77.2 μM). The α-glucosidase inhibition of compounds 1, 5, 6, and 10–12 are reported for the first time in this study.

In the case of α-amylase inhibitory activity, compounds 1–4, 9, and 11–14 showed α-amylase inhibitory activity with an IC_{50} values ranging from 73.5-265.5 μM and
compounds 1 (uvariaruflavone) and 12 (ferrudiol) displayed the activity better than that of positive control (acarbose, IC$_{50} = 103.4 \mu M$) with the IC$_{50}$ values of 92.5 and 73.5 $\mu M$, respectively (Table S2). The $\alpha$-amylase inhibition of compounds 1–4, 9, 11 and 12 are reported for the first time in this study.

3. Experimental

For the details of all experimental parts see the Supplementary material.

4. Conclusion

The phytochemical investigation of the twig and leaf of $U. rufa$ afforded one new C-benzylated flavone, uvariaruflavone (1), together with 13 known compounds (2–14). Flavones (5–8) and a cyclohexene (10) were isolated for the first time from this plant. The biological activities of tested compounds were evaluated using $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities. C-benzylated flavone (1) exhibited the significant $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities. An analysis of the structure-activity relationship suggested that C-hydroxybenzyl moiety at C-6 may enhances the $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities of this family of flavonoids.

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Disclosure statement

The authors declare no conflicts of interest.

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