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Xanthine Oxidase Inhibitory Activity of *Tetracera Indica*

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Abstract: Preliminary screening study revealed that the methanolic extract of the stem of *T. indica* showed xanthine oxidase inhibitory activity in a concentration-dependent manner. EA fraction was selected to be further study due to its potential to inhibit xanthine oxidase enzyme with IC_{50} value of 21.14 µg/ml which in lower than IC_{50} value of MeOH extract, 42.02 µg/ml. Further separation and purification of EA fraction led to the isolation of two known compounds. Those compounds were identified by analysis of their spectroscopic data and comparisons with literature data to be betulinic acid and 5,7-dihydroxyl-8-methoxyflavone.

Keywords: Tetracera indica, xanthine oxidase.

1. INTRODUCTION

Xanthine oxidase (XO) is a key enzyme that catalyzes the last step in the conversion of purines to uric acid, and plays a vital role in producing hyperuricemia and gout [1]. Allopurinol, the medication prescribed for gout prevention, is a xanthine oxidase inhibitor [2]. However, due to unwanted side effects of allopurinol, new alternatives with fewer side effects are desired. In folk remedies, leaves of *Tetracera indica* Merr. (Dilleniaceae) are effectively used in the treatment of diabetes and anti-inflammatory related diseases. Some studies have proven scientific evidence for the traditional use of leaves of *T. indica* in the management of diabetes in Malaysia. However, there is no scientific claim about its efficacy in the treatment of anti-inflammatory related diseases [3]. Based on literature, *Tetracera* spesies are widely used for the treatment of anti-inflammatory related diseases. Therefore, the aim of this study is to investigate potential anti-inflammatory activity of *T. indica* via xanthine oxidase inhibitory assay. Our preliminary screening study revealed that the methanolic extract of the stem of *T. indica* showed xanthine oxidase inhibitory activity in a concentration-dependent manner.

2. MATERIALS AND METHODOLOGY

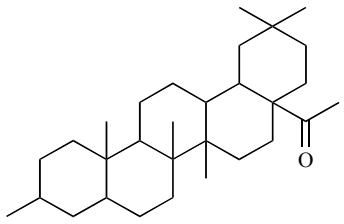
2.1. Plant Material

The stem of *T. indica* was purchased from Intan Gaharu Sdn. Bhd. in October 2011 and was identified by Miss Tan Ai Lee, Bio resources Programme, Natural Product Division, Forest Research Institute Malaysia. A voucher sample of the aerial part has been deposited at the Herbarium of Forest Research Institute Malaysia

2.2. Extraction and Isolation

The dried stem of *T. indica* was extracted with methanol (MeOH), the MeOH solution was evaporated under pressure to give a MeOH extract (73.6g; IC_{50} 42.02 µg/ml).The MeOH extract was suspended in water (H_2O) and partitioned successively with hexane, (dichloromethane) DCM, and (ethyl acetate) EA to yield hexane (1.89 g; IC_{50} > 100 µg/ml), DCM (2.78 g; IC_{50} > 100 µg/ml), EA (3.80 g; IC_{50} 21.14 µg/ml) and aqueous (59.17 g; IC_{50} 35.36 µg/ml) fractions, respectively. EA fraction was selected to be further study due to its potential to inhibit xanthine oxidase enzyme with IC_{50} value of 21.14 µg/ml which in lower than IC_{50} value of MeOH extract, 42.02 µg/ml.

The EtOAc fraction (3.89 g) was subjected to Medium Pressure Liquid Chromatography (MPLC) using Injection Column of silica gel 70µm, 14g, size M (20 x 75mm) and Hi-Flash Column of silica gel 40µm, 120g, size 3L (46 x 130mm) eluted with $CHCl_3$- MeOH (0-100%) to give 11 fractions. Fraction 4 was white crystal of betulinic acid (**1**, 0.10mg) [4]. Fraction 6 was light yellow powder of 5,7-dihydroxyl-8-methoxy-flavone (**2**, 0.03mg) [5].



(1)

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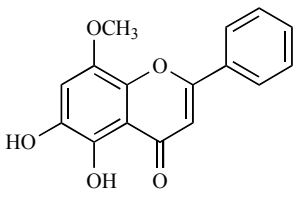

(2)

Betulinic acid (**1**) White crystal, ^{1}H-NMR ($CDCl_3$–CD_3OD, 300 MHz) δ: 0.73 (3H, s, H-25), 0.83 (3H, s, H-23), 0.95 (3H, s, H-24), 0.98 (3H, s, H-27), 1.38 (3H, s, H-26), 1.68 (3H, s, H-30), 3.03 (2H, t, H19), 4.68 (1H, dd, H-3) 4.78 (3H, s, H-29), ^{13}C-NMR ($CDCl_3$ – CD_3OD, 300 MHz) δ: 15.1 (C-27), 15.8 (C-24), 16.3 (C-25), 16.5 (C-26), 18.9 (C-6), 21.5 (C-11), 26.2 (C-12), 27.4 (C-2), 28.3 (C-23), 30.3 (C-21), 31.2 (C-15), 32.9 (C-16), 35. (C-22), 37.7 (C-7), 37.8 (C-10), 39.0 (C-13), 39.4 (C-4), 39.5 (C-1), 43.1 (C-8), 43.3 (C-14), 47.7 (C-18), 49.8 (C-19), 51.3 (C-9), 56.2 (C-5), 56.9 (C-17), 79.2 (C-3), 113.5 (C-29), 154.9 (C-20), 183.4 (C-28).

5,7-dihydroxyl-8-methoxyflavone (wogonin) (2) light yellow powder

NMR spectrum (CDCl3– CD_3OD, 300 MHz) δ: 3.18 (3H, s, OCH3), 6.16 (1H, s, H-3), 6.60 (1H, s, H-6), 7.47 (3H, m, H-3,4,5), 8.04 (2H, d, J = 8.0, H-2,6), 12.99 (1H, s, 5-OH). ^{13}C-NMR ($CDCl_3$ – CD_3OD, 300 MHz) δ: 62.9 (C-8), 100.5 (C-6), 105.1 (C-3), 105.7 (C-10), 127.2 (C-2', C-5'), 129.3 (C-8), 130.3 (C-3', C-5'), 132.3 (C-1'), 133.0 (C-4'), 151.2 (C-7), 159.3 (C-5), 165.2 (C-2), 183.8 (C-4)

2.3. XO Inhibitory Assay

Xanthine oxidase inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Nuki 2006 [6]. In assay protocol, 130 µl of 0.05 M potassium phosphate buffer (pH 7.5), 10 µl of test-compound solution and 10 µl of xanthine oxidase enzyme solution were mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 100 µl xanthine (substrate) solution. The enzymatic conversion of xanthine to form uric acid and hydrogen peroxides measured at absorbance of 295 nm. Test compounds and reference standards were dissolved in DMSO. All reactions were performed in triplicates in a 96-well UV microplate

3. RESULT AND DISCUSSION

The dried stem of *T. scandens* was extracted with MeOH, the MeOH solution was evaporated under pressure to give a MeOH extract. The MeOH extract was suspended in H_2O and partitioned successively with hexane, CH_2Cl_2, and EtOAc to yield hexane, CH_2Cl_2, EtOAc, and H_2O fractions. Among them, the EtOAc fractions showed strong XO inhibitory activity with IC_{50} values of IC_{50} value of 21.14 µg/ml. EA fraction was selected to be further study due to its potential to inhibit xanthine oxidase enzyme with IC_{50} value of 21.14 µg/ml which in lower than IC_{50} value of MeOH extract, 42.02 µg/ml. Further separation and purification of EA fraction led to the isolation of two known compounds. Those compounds were identified by analysis of their spectroscopic data and comparisons with literature data to be betulinic acid (**1**) [4] and 5,7-dihydroxyl-8-methoxyflavone (**2**) [5].

4. CONCLUSIONS

In this paper, we have reported xanthine oxidase inhibitory activity in a concentration-dependent manner of MeOH crude extract, hexane, DCM, EA and H2O fractions of *T. indica*. MeOH extract and EA fraction showed xanthine oxidase inhibitory activity in a concentration-dependent manner. Interstingly, betulinic acid (**3**) – a naturally occurring pentacyclic triterpenoid which has been shown to exhibit avariety of biological activities including inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic and antioxidant properties [7], was found in high yield from this plant. These results suggested that the traditional use of *T. indica* for the treatment of rheumatism and inflammatory diseases may be attributable to the XO inhibitory activity of lupane triterpene and flavonoid constituents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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