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Bioactive compounds from organic extracts of *Helianthus tuberosus* L. flowers

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ABSTRACT

The chromatographic separation of *Helianthus tuberosus* L. (Jerusalem artichoke) flowers led to the isolation of twenty three compounds (**1**-**23**). They were characterized into four classes, terpenoids, flavonoids, coumarins and chromones, based primarily on the analysis of spectral data including IR, 1D and 2D NMR (^{1}H, ^{13}C, COSY, NOSEY, HMQC, and HMBC). The antiproliferative activity against colon cancer cell lines HT29 and HCT116, cytotoxicity against Vero cells and antimicrobial activity against *E. Faecium* ATCC 51559, *P. aeruginosa* PAO1, *A. Baumannii* ATCC 19606, *K. Neumoniae* ATCC 700603 and *M. tuberculosis* H_{37}Ra (TB) were evaluated. Feradiol (**9**) exhibited a significant growth inhibitory effect against both cell lines (IC_{50} values of 3.93 ± 0.52 and 6.02 ± 0.33 µg/mL at 72 h respectively). The *ent*-kaur-16-en-19-oic acid (**1**) and *β*-sitostenone (**14**) exhibited weak antimicrobial activity against *E. faecium* with the same MIC values in the range of 6.25–12.50 µg/mL. In addition, **14** showed weak anti-TB with a MIC of 25.00 µg/mL. To the best of our knowledge, this is the first report on the chemical constituents and their biological activities from *H. tuberosus* L. flowers.

1. Introduction

The *Helianthus tuberosus* L. (Jerusalem artichoke) is a member of the Asteraceae family, a sunflower species that originated from North America. Nowadays it is cultivated all over the world, thanks to its ability to adapt to various growth conditions such as temperatures, soils and diseases (Pan et al., 2009; Yang et al., 2015). The comprehensive study on the natural products of *H. tuberosus* established four applications including production of biofuels; ethanol and butanol (Sarchami and Rehmann, 2014; Williams and Ziobro, 1982), chemicals; lactic acid, butyric acid and citric acid (Chi et al., 2011), functional foods; inulin and fructose and bioactive compounds; antimicrobial, anticancer and antioxidant compounds (Yang et al., 2015). A number of reports concerned the bioactive compounds from the leaves, aerial parts and the whole plant *H. tuberosus*. Terpenoids and flavonoids were among the more commonly reported compounds found, many of which were biologically active. For example there were germacrane-type sesquiterpene lactones such as 4,15-isoatriplicolide angelate, 4,15-isoatriplicolide methylacrylate, both of which exhibited significant cytotoxicity against cancer cell lines MCF-7, A549 and HeLa (Pan et al., 2009; Yuan et al., 2013), 4,15-isoatriplicolide tiglate, 4,15-isoatriplicolide isobutyrate, budlein A methylacrylate, and budlein A tiglate. Two flavones 5,8-dihydroxy-6,7,4′-trimethoxy pedunculin and 5,8-dihydroxy-6,7dimethoxy-2-(3,4-diMeOPh)-4-benzopyrone exhibited cytotoxic against HeLa cell lines (Yuan et al., 2013). The phenolic contents such as 3-*O*-caffeoylquinic acid, 1,5-dicaffeoylquinic acid caffeic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid methyl ether exhibited radical scavenging abilities and some showed antifungal activity (Chen et al., 2013; Yuan et al., 2012). The bright yellow flowers of *H. tuberosus*, however, have never been studied separately and there have been no reports on their chemical constituents or biological screening, which led to our interest in the bioactive compounds from *H. tuberosus* flowers.

In this work, we studied the chemical constituents from organic extracts of *H. tuberosus* flowers and their potential antiproliferative activity against colon cancer cell lines HT29 and HCT116 and cytotoxicity against Vero cells (African green monkey kidney cell ATCC CCL-81). The antimicrobial activity against *Enterococcus faecium* ATCC 51559, *Pseudomonas aeruginosa* PAO1, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumonia* ATCC 700603, and *Mycobacterium tuberculosis* H_{37}Ra were also evaluated. We believe that this research provides information on promising anti-colon cancer candidates,

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antibiotics for drug resistant bacteria and anti-TB agents from *H. tuberosus* flowers, and the possible use of these flowers as functional food applications such as remedies, supplementary capsules and herbal teas.

2. Material and methods

2.1. Plant material

The *H. tuberosus* tubers (genotype HEL65) were cut into small pieces with 2–3 active buds and incubated in moist charred rice husk. After 3 days, seedlings, 2–3 cm in length, were transferred into plug trays containing soil and charred rice husk at a ratio of 1:1 (v:v) for 7 days (4–6 leaves). Uniform seedlings were transplanted into a loamy-sand plot of 1600 m^2 (0.504% of organic matter and total nitrogen, potassium and phosphorus at 249, 134, and 193 mg/kg, respectively), at the Khon Kaen University agronomy farm from September-January. Fertilizer (15-15-15: NPK) was applied 25 days after transplanting at the rate of 25 kg per 1600 m^2. The flowers were hand-picked 70–80 days after planting, air dried for 5 days and kept at 4 °C before the extraction procedure. The plant material was identified by Prof. Dr. Sanun Jogloy and the sample specimen was deposited at the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand.

2.2. General procedures

Column chromatographic methods were performed on silica gel 60 (Merck, Germany), 0.063–0.200 mm meshes for Column Chromatography (CC), and 0.040–0.063 mm meshes, for Flash Column Chromatography (FCC). Preparative Layer Chromatography (PLC) was prepared using silica gel PF254 (Merck, Germany). Sephadex LH-20 (25–100 μ, SIGMA) was used for the size separation. Analytical TLC was performed on silica gel 60 F254 pre-coated aluminum sheets (Merck, Germany), visualized under UV light (254 and 366 nm), further sprayed with anisaldehyde reagent and heated until spots of compound appeared. All organic solvents were commercial grade and were distilled before being used. The IR technique was used for analyzing functional groups and recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. The NMR data, including ^{1}H and ^{13}C NMR and 2D NMR, were recorded on a Varian Mercury Plus 400 spectrometer, with TMS as the internal standard. The samples were prepared by dissolution in a single or mixture of deuterated solvents ($CDCl_3$, CD_3OD and DMSO-d_6). The chemical shift (δ) was reported in ppm and the coupling constant (J) is in Hz.

2.3. Extraction and isolation

The 1.5 kg of air dried flowers were cut into small pieces, put in a muslin sack and macerated three times with hexane (5L) in a covered stainless steel container (30L) at room temperature for 72 h each time. After being filtered and concentrated *in vacuo*, a yellow viscous oil of crude hexane extract (70.5 g, 4.70%) was obtained. The same procedure was carried out on the residue with EtOAc (5L) and then MeOH (5L) to yield the brown viscous oil and dark viscous liquid of crude EtOAc (42.6 g, 3.11%) and MeOH (150 g, 10%) extracts, respectively. All three crude extracts were individually subjected to CC eluted with a gradient system (400 mL each) from hexane-EtOAc (100:0 to 0:100 v/v), followed by EtOAc-MeOH (100:0 to 0:100 v/v). The eluent was continuously collected at 75 mL at a time. The fractions were combined based on TLC profiles and after being concentrated *in vacuo*, fractions H1-H8, E1-E9 and M1-M12 from the crude hexane, EtOAc and MeOH extracts were obtained, respectively.

Fractions H2 and H3 were fractionated individually on FCC, eluted with hexane-CH_2Cl_2 (100:0 to 0:100 v/v) to obtain fractions H2.1-H2.8 and H3.1-H3.8, respectively. Fractions H2.8 and H3.3 were further purified on FCC, eluted with hexane-CH_2Cl_2 (1:4 v/v) and hexane-CH_2Cl_2-EtOAc (7.5:2:0.5 v/v) to yield **10** (273.6 mg in total, 0.0182%). Fraction H4 was fractionated on FCC, eluted with hexane-CH_2Cl_2 (100:0 to 0:100 v/v) to obtain fractions H4.1-H4.7. Fractions H4.4 and H4.5 were separately subjected on FCC eluted with hexane-CH_2Cl_2-EtOAc (6:3.5:1.5 v/v) to yield subfractions H4.4.1-H4.4.6 and H4.5.1-H4.5.6, respectively. Compound **11** (72.3 mg, 0.0048%) was obtained from H4.4.3 when separated on FCC eluted with hexane-EtOAc (100:0 to 0:100 v/v). Compound **1** (22.3 mg, 0.0015%) was obtained from H4.4.5 and H4.5.3 when purified on Sephadex LH-20 CC (MeOH). Fraction H5 was separated on FCC, eluted with hexane-EtOAc (100:0 to 0:100 v/v) to obtain fraction H5.1-H5.6. Fraction H5.4 was further purified on Sephadex LH-20 CC (MeOH) to yield **12** (10.4 mg, 0.0007%). Fraction H6 was subjected to FCC, eluted with hexane-CH_2Cl_2-EtOAc (5:3:2 v/v) to obtain fractions H6.1-H6.9. Compound **9** (169.8 mg, 0.0113%) was obtained from H6.6 when fractionated on Sephadex LH-20 CC (MeOH) and further purified on FCC eluted with hexane-EtOAc (7:3 v/v). Compounds **2** and **3** were obtained as a mixture from H6.7 when it was purified on Sephadex LH-20 CC (MeOH) and recrystallized (EtOAc).

Fraction E4 was fractionated on FCC, eluted with hexane-EtOAc (100:0 to 0:100), followed by EtOAc-MeOH (100:0 to 0:100 v/v) to obtain fractions E4.1-E4.8. Compounds **4** (5.4 mg, 0.0004%) and **14** (35.7 mg, 0.0024%) together with an additional **1** (3.2 mg, 0.0002%) were obtained from E4.2 when separated on FCC, eluted with hexane-EtOAc (1:1 v/v) and individually purified on Sephadex LH-20 CC (MeOH). Additional **2** and **3** (4.8 mg, 0.0003% in total) were obtained from E4.5 when separated on FCC eluted with hexane-EtOAc (1:1 v/v) and further purified on Sephadex LH-20 CC (MeOH). Fractions E6 and E7 were fractionated separately on FCC, eluted with the same system of hexane-EtOAc (100:0 to 0:100 v/v), followed by EtOAc-MeOH (100:0 to 0:100 v/v) to obtain E6.1-E6.11 and E7.1-E7.8, respectively. Compound **5** (64.2 mg, 0.0041%) was recrystallized (EtOAc) from E6.2 and E7.2. Compounds **7** (5.9 mg, 0.0004%) and **13** (2.8 mg, 0.0002%) were obtained from E6.3 when fractionated on FCC, eluted with hexane-EtOAc (3:2 v/v) and then purified on PLC developed with 100% CH_2Cl_2. Compounds **17** (9.0 mg, 0.0006%) and **19** (6.8 mg, 0.0005%) were obtained from E6.5 when eluted with hexane-EtOAc-CH_2Cl_2-MeOH (6:2:1.5:0.5 v/v) and further purified on PLC developed with hexane-EtOAc (1:1 v/v). Fraction E7.4 was separated on FCC eluted with hexane-EtOAc (100:0 to 0:100 v/v) to yield E7.4.1-E7.4.5. Compounds **8** (18.0 mg, 0.0012%) and **20** (14.8 mg, 0.0010%) were obtained from E7.4.1 when purified on FCC, eluted with hexane-CH_2Cl_2-EtOAc (5:3:2 v/v). Fraction E7.4.2 appeared as a mixture of yellow liquid and a white solid, which was washed and recrystallized (EtOAc) to obtain **6** (5.8 mg, 0.0004%). Compound **16** (59.5 mg, 0.0040) was recrystallized (EtOAc) from fraction E7.6.

Fractions M7, M8 and M9 were individually fractionated on Sephadex LH-20 CC (MeOH), to obtain fractions M7.1-M7.7, M8.1-M8.8 and M9.1-M9.7 respectively. Additional **20** (27.1 mg, 0.0018%) was obtained from M7.5 by recrystallization (EtOAc) and from M8.5 when purified on PLC developed with hexane-CH_2Cl_2-EtOAc (2:4:4 v/v). Compound **18** (5.1 mg, 0.0003%) together with an additional **17** (27.5 mg, 0.0018%) were obtained from M7.6 when purified on PLC developed with hexane-CH_2Cl_2-EtOAc (4:3:3 v/v). Fraction M8.6 appeared as a single spot of **22** on the TLC (19.0 mg, 0.0013%). Compound **15** (11.0 mg, 0.0007%) was recrystallized (EtOAc) from M9.2. Compound **21** (3.4 mg, 0.0002%) was obtained from M9.5 when separated on Sephadex LH-20 CC (MeOH) and recrystallized (EtOAc). Compound **23** (40.4 mg, 0.0027%) was obtained from M10 when fractionated on Sephadex LH-20 CC (MeOH) and recrystallized (EtOAc).

2.4. Antiproliferative activity

The antiproliferative activity was evaluated against two colon cancer cell lines HT29 and HCT116 by MTT colorimetric assay in