P-ISSN 1859-3585 | E-ISSN 2615-9619

INVESTIGATE THE ANTIOXIDANT AND ANTI-OSTEOPOROSIS ACTIVITIES OF COMPOUNDS ISOLATED FROM *CHRYSANTHEMUM INDICUM* FLOWERS

KHẢO SÁT HOẠT TÍNH CHỐNG OXI HOÁ VÀ CHỐNG LOÃNG XƯƠNG CỦA CÁC HỢP CHẤT PHÂN LẬP TỪ HOA CÚC HOA VÀNG

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DOI: https://doi.org/10.57001/huih5804.2023.232

ABSTRACT

In this research, five compounds were isolated from *Chrysanthemum indicum* L flowers and determined their structures to be Syringin (**1**), Dihydrosyringin (**2**), Benzyl-O-*β*-D-glucopyranoside (**3**), *β*-Phenylethoxy-O-*β*-*D*-glucopyranoside (**4**), and (*Z*)-5′-Hydroxyjasmone 5′-O-*β*-D-Glucopyranoside (**5**) by analysis 1D - NMR spectra and compared to literatures. These compounds were investigated antioxidative and anti-osteoporosis activities. Among them, compound **5** showed the best antioxidant activity at a concentration of 10μM with the value of peroxyl radical scavenging to be 11.39 ± 0.11 times higher than the protection provided by 1.0 μM Trolox and compound **2** exhibited meaningful reducing capacity, with CUPRAC values of 11.24 ± 0.01 at 10μM. Compound **5** also exhibited the best anti-osteoporosis effect at a concentration of 10.0μM with percent of inhibition on RANKL-induced osteoclast differentiation of 151.16 ± 13.13%.

Keywords: *Chrysanthemum indicum, antioxidant activity, anti-osteoporosis activity.*

TÓM TẮT

Trong nghiên cứu này, 5 hợp chất gồm Syringin (**1**), Dihydrosyringin **(2)**, Benzyl-O-*β*-D-glucopyranoside (**3**), *β*-Phenylethoxy-O-*β*-*D*-glucopyranoside (**4**) và (*Z*)-5′-Hydroxyjasmone 5′-O-*β*-D-Glucopyranoside (**5**) đã được phân lập từ hoa cúc hoa vàng (*Chrysanthemum indicum* L). Các hợp chất phân lập được được xác định cấu trúc qua các phân tích phổ cộng hưởng từ ^{1}H và ^{13}C, kết hợp so sánh với tài liệu tham khảo. Các thử nghiệm về hoạt tính chống oxi hoá và chống loãng xương của các hợp chất này đã được thực hiện. Kết quả cho thấy, hợp chất 5 thể hiện hoạt tính bắt giữ gốc tự do peroxyl tốt nhất ở nồng độ 10μM với giá trị hoạt tính cao gấp 11,39 ± 0,11 lần với khả năng chống oxi hóa của Trolox 1,0μM, hợp chất 2 thể hiện khả năng khử ion đồng (I) tốt nhất ở 10 μM với giá trị hoạt tính là 11,24 ± 0,01 tại 10μM. Hợp chất 5 cũng thể hiện hoạt tính chống loãng xương tốt nhất ở nồng độ 10,0μM với giá trị phần trăm ức chế sự giảm tế bào hủy xương do RANKL gây ra ở cùng nồng độ là 151,16 ± 13,13%.

Từ khóa: *Chrysanthemum indicum, cúc hoa vàng, hoạt tính chống oxi hoá, hoạt tính chống loãng xương.*

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Received: 15/9/2023

Revised: 30/10/2023

Accepted: 25/11/2023

1. INTRODUCTION

Chrysanthemum indicum. L (*C. indicum* L.) is a species of *Chrysanthemum*, family Compositae. In Vietnam traditional medicine, the flowers of *C. indicum* L. are widely used to treat headaches, dizziness, swelling, eye pain, bruises, sprains, snake bites, centipedes, and rhinitis., diphtheria, cholera, malaria. Clinical trials on the effects of *C. indicum* flowers in the treatment of inflammation, hypertension, and respiratory disease have been conducted [1]. To date, more than 190 chemical constituents have been isolated and identified from the flowers of this plant, including 42 flavonoids (Acacetin [2], Apigenin, Luteolin, Baicalein [3], Eupatilin, Diosmetin, Tricin, Kaempferol [4], etc.), 96 terpenoids (Clovanediol, Caryolane 1,9-β-diol, Canusesnol E, Cumambrin A [4], etc.), 21 phenylpropanoids and phenolic acids (Caffeic acid, Chlorogenic acid, Chysophanol, Vanillic acid, 4-O-β-D-glucoseoxylbenzoic acid [4], etc.) and other compounds (Chrysindins, Lupeol, α-pinene, etc.).

As an herb with a long history of use in traditional medicine, *C. indicum* flower is currently receiving much attention from the aspect of pharmacological effects in modern medicine. The extracts of *C. indicum* can scavenge DPPH free radicals or inhibit the activity of IL-1, TNF-α, and leukocyte accumulation [5]. The isolated compounds from the methanol extract of *C. indicum* such as Quercetin, Caffeic acid, and Chlorogenic acid exhibited free radical scavenging effects [6-8]; Caryolane 1,9-β-diol, Chrysanthemulide A, B, C, D, E, F, G showed obvious inhibition of NO production [9];

Quercetine, Luteolin and 5,7,3′,5′-tetrahydroxy flavanone 7-*O*-*β*-D-glucopyranoside exhibited the potent tartrate-resistant acid phosphatase (TRAP) activity in receptor activator of nuclear factor-κB ligand-induced osteoclastic RAW 264.7 cells with values of 105.95 ± 1.18, 110.32 ± 3.95, and 112.58 ± 6.42%, respectively [10]. Another bioactivities of *C. indicum* were also investigated and shown good effects such as hypotensive effect, acetylcholinesterase inhibitory activity, anti-bacterial and anti-viral effects, anti-inflammatory and immunomodulatory activities [11-13].

Even though there were a lot of international reports about *C. indicum*, there was limit research in Vietnam about this medicinal herb. Almost the research in Vietnam were choosing the extracted process of flavonoid from *C. indicum* or investigated the effect of some factors to quality of dried *C. indicum* flowers. In the aim to clarify chemical components and some bioactivities of this herb, this study describes the isolation and the structural determination of 5 compounds from the water-soluble fraction of dried *C. indicum* flower and investigated the antioxidant and anti-osteoporosis activities of isolated compounds.

2. MATERIALS AND METHODS

2.1. General experimental procedures

The NMR spectra were measured by a JEOL ECA 400 spectrometer (JEOL, Tokyo, Japan) using the internal standard TMS. CD_3OD (δ_H 4.87/δ_C 49.0) was used as solvents for NMR measurements. Silica gel (70-230, 230 - 400 mesh, Merck, Whitehouse Station, NJ), RP-18 resins (75μm, YMC Fuji Silysia Chemical Ltd., Kasugai, Japan) were used as absorbents in the column chromatography. Thin layer chromatography (TLC) plates (silica gel 60 F_{254} and RP-18 F_{254S}, 0.25μm, Merck) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation (254 and 365nm) and by spraying the plates with 10% H_2SO_4 followed by heating with a heat gun. Chemical reagents and standard compounds were purchased from Sigma-Aldrich.

2.2. Plant material

The flowers of *C. indicum* were collected in Nghia Trai, Hung Yen, Viet Nam in October 2022 and taxonomically identified by Dr. Nguyen The Cuong in the Institute of Ecology and Biological Resources, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam. A voucher specimen (CNHN-11102) was deposited at the Faculty of Chemical Technology, Hanoi University of Industry.

2.3. Extraction and isolation

The dried flowers of *C. indicum* (2.0kg) were extracted with 95% ethanol (5L × 3 times) using room temperature ultrasonic conditions. Evaporation of the solvent under reduced pressure gave ethanol extracts (140g). This extracts was suspended in H_2O (2.0L) and successively separated with dichloromethane (2.0L), ethyl acetate (2.0L), and n-butanol (2L)to yield DCM fraction, EA fraction, and n-butanol fraction, respectively. The solvents were removed using rota-vaporation under reduced pressure to obtain corresponding extracts. The n-butanol extract was then subjected on silica gel column, eluted with gradient solvent systems of dichloromethane/methanol (1/0 - 0/1, v/v) to take four fractions, CB1 - CB4. Fraction CB1 was separated on silica gel column using solvent system of ethyl acetate/acetone (3/1, v/v) to obtain compound **1** (10mg). Fraction CB2 was isolated by silica gel column eluting with dichloromethane/methanol (10/1 - 1/1) to give four fractions, CB2.1-CB2.4. Fraction CB2.2 was further purified by Sephadex column using methanol/water (1/1, v/v) to get compound **2** (10mg), **3** (8mg) and **4** (35mg). Fraction CB2.4 was purified by an RP-18 column eluting by methanol/water (1/3, v/v) to obtain compound **5** (10mg).

Syringin (**1**): white amorphous powder, $\mathbf{C_{17}H_{24}O_9}$, ^{1}H NMR (600MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data are given in Table 1.

Dihydrosyringin (**2**): white amorphous powder, $\mathbf{C_{17}H_{26}O_9}$, ^{1}H NMR (600MHz, CD_3OD) and ^{13}C NMR (150MHz, CD_3OD) spectral data are given in Table 1.

Benzyl-O-*β*-D-glucopyranoside (**3**): white amorphous powder, $\mathbf{C_{13}H_{18}O_6}$, ^{1}H NMR (400MHz, CD_3OD) and ^{13}C NMR (100MHz, CD_3OD) spectral data are given in Table 1.

***β*-Phenylethoxy-O-*β*-*D*-glucopyranoside** (**4**): white amorphous powder, $\mathbf{C_{14}H_{20}O_6}$, ^{1}H NMR (400MHz, CD_3OD) and ^{13}C NMR (100MHz, CD_3OD) spectral data are given in Table 1.

(*Z*)-5′-Hydroxyjasmone 5′-O-*β*-D-Glucopyranoside (**5**): white amorphous powder, $\mathbf{C_{17}H_{26}O_7}$, ^{1}H NMR (400MHz, CD_3OD) and ^{13}C NMR (100MHz, CD_3OD) spectral data are given in Table 1.

2.4. Bioactive investigation method

Oxygen Radical Absorbance Capacity (ORAC) Assay

To investigate antioxidant activity, the ORAC assay was used. In this assay, 2,2-azobis dihydrochloride (AAPH) and fluorescein were used as a peroxyl radical generator and a target of free radical, respectively. The positive control was trolox (1.0μM) and this one was prepared fresh daily. The assay was carried out on a Tecan GENios multi-functional plate reader (Tecan, Salzburg, Austria) with fluorescent filters to get excitation and emission wavelengths of 485nm and 535nm, respectively. In the final assay mixture, 40nM fluorescein attack with 20mM of AAPH in the presence of different concentrations of tested compound or positive control [14]. The analyzer was instructed to record the fluorescence of fluorescein every 2 min during 200 min incubation at 37°C after addition of AAPH. All fluorescence measurements were expressed relative to the initial reading. Results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. $ORAC_{ROO\cdot}$ was expressed as micromoles of Trolox equivalents (TE). Therefore, one ORAC unit is equivalent to the net protection area provided by 1.0μM of trolox.

Determination of Reduction Capacity

To determine the reducing abilities of samples, the cupric ion reducing antioxidant capacity (CUPRAC) method was carried out [15]. Firstly, 40µL of different concentrations (1 and 10.0µM) of the compounds were mixed with 160µL of the mixture containing 0.5mM of $CuCl_2$ and 0.75mM of neocuproine in 10.0mM of phosphate buffer, pH 7.4. The resulting mixtures were incubated at room temperature for 1 hour before measuring the absorbance with a micro-plate reader at wavelength of 454nm. The reduction led to the change in solution color, therefore increasing absorbance of the mixture indicated the increasing reducing power because of the conversation of Cu (II) ions into Cu (I) ion. The CUPRAC values were stated as µM of Cu (I) ion reduced from Cu (II) by antioxidant.

Tartrate-resistant acid phosphatase (TRAP) activity measurement

RAW 264.7 (macrophages (pre-osteoclasts) from BALB/c mice) cells were cultured in 96-well plates (1×10^4 cells/mL) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) FBS for 2 days. Medium was then replaced with test samples in differentiation medium containing 50ng/mL RANKL (receptor activator of nuclear factor κB ligand). The differentiation medium was replaced every 2 days. After differentiating the RAW 264.7 cells into osteoclasts for 5 days, the medium was removed, and the cell monolayer was gently washed twice using ice-cold PBS. The cells were fixed in 3.5% formaldehyde for 10 min and ethanol-acetone (1:1) for 1 min. Subsequently, the dried cells were incubated in 50.0mM citrate buffer (pH 4.5) containing 10mM sodium tartrate and 6mM p-nitrophenyl phosphate (PNPP). After incubation for 1 hour, the reaction mixtures were transferred to new well plates containing an equal volume of 0.1N NaOH. Absorbance was then measured at 408nm using an ELISA reader. TRAP activity was expressed as a percentage of the control.

Statistical analysis

All data represent the mean ± S.D. of at least three independent experiments performed in triplicates. Statistical significance is determined by one-way ANOVA followed by Dunnett's multiple comparison test, $P < 0.05$, using GraphPad Prism 6 program (GraphPad Software Inc., San Diego, CA, USA).

3. RESULTS AND DISCUSSION

Compounds **1-5** were identified by comparing the ^1H, ^{13}C-NMR data with the literatures values and determined to be Syringin (1) [16], Dihyrosyringin (2) [16], Benzyl-O-*β*-D-glucopyranoside (**3**) [17], *β*-Phenylethoxy-O-*β*-*D*-glucopyranoside (**4**) [18] and (*Z*)-5'-Hydroxyjasmone 5'-O-*β*-D-Glucopyranoside (**5**) [19] (see Fig. 1). The isolated compound's NMR data in comparison to literature were listed in Table 1.

The potential antioxidant activities of isolated compounds were first investigated using ORAC assays. In this method, the antioxidant inhibition of a molecule is considered based on the ability to reflect classical radical breaking antioxidant activity by hydrogen atom transfer. [20]. The result is shown in Table 2. As the result, compound **2** - **5** exhibited the weak antioxidant activity at the concentration of 1.0µM while compound **1** was almost inactive at the same concentration. However, when the concentration was increased to 10µM, compounds **1** and **5** were significant activity with the ORAC values of 5.02 ± 0.23 and 11.39 ± 0.11 times higher than control, respectively. Other compounds also showed the increasing in ORAC values - meaned that improving in antioxidant activity against peroxyl radicals - but this increasing was not much, corresponded to double or triple in compared to these values of previous concentration. Then, to investigate their single electron transfer activity, the reducing capacity of these compounds was examined by copper reduction assay. These assays measured the concentration of Cu (I) ions reduced from Cu (II) ions by the combined action of reducing agents (antioxidants) in a sample [21]. The reducing capacity at concentration of 10µM were remarkable with the CUPRAC values of 6.04 ± 0.06 and 11.24 ± 0.01µM corresponded to compound **1** and **2**, respectively. Compound **5** exhibited well peroxyl radical scavenging activity, yet low capacity for reducing Cu (II) ions to Cu (I) ions. That means compound **5** can donate hydrogen strongly but its single electron transfer activity was weak while compound **2** showed the reversed result with the weakness in peroxyl radical scavenging capacity but potent transferring single electron activity. This research also found the ability of compound **1** to transfer hydrogen atoms or electrons to peroxyl radicals and change them into stable compounds, proved by the relatively balanced values of ORAC and CUPRAC at the tested concentrations.

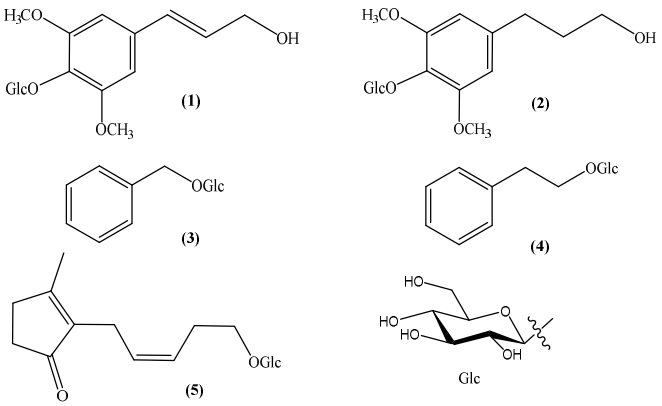

Figure1. Chemical structures of compound 1 - 5 isolated from soluble-n-butanol fraction of *Chrysanthemum indicum* flowers

Osteoroposis is a "silent" disease related to the decreasing in mineral density and mass of bone, leading to an increase in the risk of bone fragility and fracture. The bone formation and bone resorption were responsible by specialized cells named osteoblasts and osteoclasts, respectively [22]. Osteoclasts are multinucleated cells which are derived from monocyte/macrophage lineage precursors

Table 1. NMR spectral data for compounds 1-5 in CD_3OD

No	1			2			3			4			5		
	$^{\#}\delta_C$	$^{a}\delta_C$	$^{b}\delta_H$ (mult., *J* in Hz)	$^{\#}\delta_C$	$^{a}\delta_C$	$^{b}\delta_H$ (mult., *J* in Hz)	$^{\#\#}\delta_C$	$^{c}\delta_C$	$^{d}\delta_H$ (mult., *J* in Hz)	$^{\#\#\#}\delta_C$	$^{c}\delta_C$	$^{d}\delta_H$ (mult., *J* in Hz)	$^{\#\#\#\#}\delta_C$	$^{a}\delta_C$	$^{b}\delta_H$ (mult., *J* in Hz)
1	136.0	135.9	-	139.1	140.5		138.0	139.2		139.3	140.1		174.6	174.7	
2	105.5	105.4	6. 75 (s)	106.0	107.4	6.56 (s)	127.5	129.3	7.40 (d, 7.2)	128.6	129.4	7.23-7.25 (overlapped)	139.8	139.8	
3	154.5	154.3 154.0	-	152.7	154.1	-	128.0	129.4	7.31 (t, 7.6)	129.3	130.1	7.23-7.25 (overlapped)	212.0	212.1	
4	135.3	135.3	-	133.1	134.5	-	127.2	128.8	7.26 (d, 7.2)	126.4	127.3	7.15-7.19 (overlapped)	35.2	35.2	2.36 (m)
5	154.5	154.3 154.0	-	152.7	154.1	-	128.0	129.4	7.31 (t, 7.6)	129.3	130.1	7.23-7.25 (overlapped)	32.6	32.6	2.56 (m)
6	105.5	105.4	6.75 (s)	106.0	107.4	6.56 (s)	127.5	129.3	7.40 (d, 7.2)	128.6	129.7	7.23-7.25 (overlapped)			
7	131.3	131.3	6.55 (d, 5.4)	32.0	33.4	2.64 (m)	70.2	71.7	4.90 (d, 12.0) 4.63 (d, 11.6)	36.6	37.2	2.92 (m)			
8	130.2	130.0	6.33 (dt, 15.6, 5.7)	34.1	35.4	1.83 (m)				71.6	71.7	4.09 (m); 3.75 (m)			
9	63.7	63.6	4.22 (dd, 1.8; 5.4)	60.8	62.1	3.58 (t, 6.0)									
3,5-OCH_3	57.1	57.0	3.85 (s)	55.6	57.0	3.84 (s)									
1-CH_3													17.5	17.5	2.12 (s)
1′													22.1	22.1	2.96 (d, 7.2)
2′													128.7	128.7	5.31-5.36 (brq)
3′													127.5	127.5	5.42-5.47 (brq)
4′													29.0	29.0	2.51 (brq)
5′													70.2	70.2	3.58 (m) 3.91 (m)
OGlc															
1'	105.6	105.4	4.87 (m)	104.3	105.7	4.80 (d, 7.8)	102.1	103.3	4.32(d, 7.6)	104.6	104.4	4.30 (d, 8.0)	104.3	104.4	4.28 (d, 7.8)
2'	75.9	75.7	3.47 (m)	74.4	775.7	3.48(m)	73.5	75.2	3.21-3.35 (overlapped)	75.0	75.1	3.17-3.38 (overlapped)	75.1	75.1	3.27-3.37 (overlapped)
3'	78.5	78.3	3.20 (m)	77.0	78.3	3.21 (m)	76.9 76.8	78.1	3.21-3.35 (overlapped)	78.3	77.9 78.1	3.17-3.38 (overlapped)	78.1	78.1	3.27-3.37 (overlapped)
4'	71.49	71.3	3.41 (m)	70.0	71.3	3.42 (dd, 2.7; 7.2)	69.4	71.7	3.21-3.35 (overlapped)	70.5	71.6	3.17-3.38 (overlapped)	71.7	71.7	3.27-3.37 (overlapped)
5'	78.0	77.8	3.41 (m)	76.4	77.8	3.42 (dd, 2.7; 7.2)	76.8 76.9	78.1	3.21-3.35 (overlapped)	78.3	77.9 78.1	3.17-3.38 (overlapped)	77.9	78.0	3.27-3.37 (overlapped)
6'	62.8	62.6	3.67 (dd, 5.1; 11.4) 3.7 (dd, 2.4; 12.0)	61.2	62.5	3.68 (dd, 4.8; 12.0) 3.79 (dd, 2.4;12.0)	61.2	62.8	3.66 (dd, 5.6; 12.0) 3.88 (dd, 2.4; 12.0)	62.8	62.7	3.65 (dd, 5.4, 11.8) 3.87 (dd, 1.8; 12.0)	62.8	62.8	3.67 (dd, 5.4; 12.0) 3.87 (dd, 1.8; 12.0)

*Measured at [a]150MHz, [b]600MHz, [c]100MHz, [d]400MHz; [#)]Prevously reported δ_C data for (**1**) and (**2**)* [16]*; [##)]Prevously reported δ_C data for* (**3**) [17]*; [###)]Prevously reported δ_C data for* (**4**) [18]*; [####)]Prevously reported δ_C data for* (**5**) [19]

in the presence of the polypeptide growth factor CSF-1 (colony-stimulating factor-1) and receptor activator of nuclear factor κB ligand (RANKL). Two factors, CSF-1 and RANKL are required to induce the expression of genes

involved in osteoclast maturation. Effect to the development of osteoclasts, there are some components including TRAP, cathepsin K (CATK), calcitonin receptor, and β3-integrin [23]. TRAP is an abundant enzyme in osteoclasts. This enzyme produces reactive oxygen species (ROS) which can destroy type I collagen - the major protein in the matrix of bone [24]. That means the more inhibited osteoporosis, the less in generating of ROS, or the less increasing in activity of TRAP - measured by the percent of inhibition. All investigated compounds were confirmed no toxicity to RAW 264.6 macrophage cells at the examined concentration (10μM) during the tested time (five days - data not shown). Osteoclastic difference from murine macrophage RAW 264.7 cells was induced by RANKL. The RANKL treatment significantly induced the formation of osteoclast from pre-osteoclast RAW 264.7 cells and led to the enhancement of TRAP activity up to 183.58 ± 11.54 relative to the control. The inhibitory effects of the isolated compounds on the activity of TRAP were presented in Table 3. Compounds **1** and **2** were inactive with the value inhibition the development of TRAP up to 187.79 ± 15.27 and 220.63 ± 11.76%, respectively, while the other compounds showed significant TRAP inhibitory activity with the values of 156.84 ± 5.93%, 164.42 ± 5.34% and 151.16 ± 13.13% corresponded to compounds **3**, **4**, **5**, respectively. Therefore, compound **5** was the most potential candidate for anti-osteoporosis inhibitor. There was a difference in chemical structure of inactive and active anti-osteoporosis compounds. Firstly, β-D-glucopyranoside moiety in compounds **3** - **5** were not linked directly to benzene part, while in case of compounds **1** and **2** were linked with benzene ring in directed way. Secondly, there were some substituted groups that appeared in phenyl part of compounds **1** and **2** while the other did not contain these ones. To determine the reason for the difference in anti-osteoporosis ability of compounds, there were many factors that need to be concerned, and chemical structure was one of these reasons. So, this result will be useful for further research and design of effective compounds with desired bioactivities.

Table 2. Antioxidant activities of the isolated compounds from C. *indicum* flowers

Compounds	Peroxyl radical scavenging (TE, μM)		Reduction power (Copper(I) ion, μM)	
	1μM	10μM	1μM	10μM
1	0,01 ± 0.1	5.02 ± 0.23	0.17 ± 0.11	6.04 ± 0.06
2	0.62 ± 0.03	1.58 ± 0.04	0.75 ± 0.06	11.24 ± 0.01
3	0.47 ± 0.06	1.29 ± 0.03	0.21 ± 0.11	1.29 ± 0.10
4	0.39 ± 0.08	1.41 ± 0.04	0.29 ± 0.11	1.27 ± 0.03
5	0.48 ± 0.21	11.39 ± 0.11	0.08 ± 0.06	0.75 ± 0.13

Table 3. Inhibitory effects of the isolated compounds on RANKL-induced osteoclast differentiation at a concentration of 10.0μM

Compound	Inhibition (%)
1	187.79 ± 15.27
2	220.63 ± 11.76
3	156.84 ± 5.93
4	164.42 ± 5.34
5	151.16 ± 13.13
RANKL	183.58 ± 11.54
Control	100.00 ± 0.00

4. CONCLUSION

Five compounds including Syringin (**1**), Dihydrosyringin (**2**), Benzyl-O-β-D-glucopyranoside (**3**), β-Phenylethoxy-O-β-D-glucopyranoside (**4**) and (Z)-5'-Hydroxyjasmone 5'-O-β-D-glucopyranoside (**5**) were isolated from the water extract of dried *C. indicum* flowers. Their chemical structures were determined via 1D and 2D-NMR spectral analysis and comparison with literature. Their antioxidant activities were investigated using ORAC and CUPRAC procedures. Compound **5** showed the reasonable antioxidant effect with peroxyl radical scavenging value of 11.39 ± 0.11μM at the examined concentration of 10μM, while compound **2** exhibited the good in single electron transfer activity with the value of 11.24 ± 0.01μM at the tested concentration. Anti-osteoporosis was the other bioactivity was studied and compound **5** showed the best result with the development of TRAP was reduced to 151.16 ± 13.13% compared to 183.58 ± 11.54% of RANKL. These research hence will be the evident for the usefulness of *C. indicum* in the natural antioxidant and anti-osteoporosis functional food and pharmaceutical industry.

SUPPORTING INFORMATION

Supplementary data associated with this article including NMR spectral data of isolated compounds can be found in the Supporting information.

ACKNOWLEDMENT

This work was supported by the Hanoi University of Industry under grant number of 49-2022-RD/HĐ-ĐHCN.

REFERENCES

[1]. M. Dong, D. Yu, V. Duraipandiyan, N. Abdullah Al-Dhabi, 2017. *The protective effect of Chrysanthemum indicum extract against ankylosing spondylitis in mouse models*. Biomed. Res. Int. 2017, 8206281.

[2]. P. Li, Z. Huang, Y. She, S. Qin, W. Gao, Y. Cao, X. Liu, 2020. *An assessment of the interaction for three Chrysanthemum indicum flavonoids and α-amylase by surface plasmon resonance*. Food Sci. Nutr., 8 (1), 620-628.

[3]. L. Bai, X. Li, L. He, Y. Zheng, H. Lu, J. Li, L. Zhong, R. Tong, Z. Jiang, J. Shi, J. Li, 2019. *Antidiabetic Potential of Flavonoids from Traditional Chinese Medicine: A Review*. Am. J. Chin. Med. 47 (5), 933-957.

[4]. A. Amel, B. Ouahiba, B. Nassima, B. Kenza, D. Radja, B. Nawal, Z. Djamila, B. Fadila, B. Samir, 2016. *Impact of Chrysanthemum fontanesii extract on sodium valproate mediated oxidative damage in mice kidney*. J. Appl. Pharm. Sci., 6 (4), 067-071.

[5]. D. Y. Lee, G. Choi, T. Yoon, M. S. Cheon, B. K. Choo, H. K. Kim, 2009. *Anti-inflammatory activity of Chrysanthemum indicum extract in acute and chronic cutaneous inflammation*. J. Ethnopharmacol, 123 (1), 149-154.

[6]. I. B. Afanas'ev, A. I. Dcrozhko, A. V. Brodskii, V. A. Kostyuk, A. I. Potapovitch, 1989. *Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation*. Biochem. Pharmacol, 38 (11), 1763-1769.

[7]. L. Y. Zhang, G. Cosma, H. Gardner, V. Vallyathan, V. Castranova, 2003. *Effect of chlorogenic acid on hydroxyl radical*. Mol. Cell. Biochem., 247 (1), 205-210.

[8]. J. H. Chen, C. T. Ho, 1997. *Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds.* J. Agric. Food Chem., 45 (7), 2374-2378.

[9]. G. M. Xue, X. Q. Li, C. Chen, K. Chen, X. B. Wang, Y. C. Gu, J. G. Luo, L. Y. Kong, 2018. *Highly oxidized guaianolide sesquiterpenoids with potential anti-inflammatory activity from Chrysanthemum indicum*. J. Nat. Prod., 81 (2), 378-386.

[10]. B. T. T. Luyen, B. H. Tai, N. P. Thao, Y. M. Lee, S. H. Lee, H. D. Jang, Y. H. Kim, 2015. *The anti-steoporosis and antioxidant activities of chemical constituents from Chrysanthemum indicum flowers*. Phytother. Res., 29 (4), 540-548.

[11]. W. Cheng, J. Li, T. You, C. Hu, 2005. *Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of Chrysanthemum indicum Linne*. J. Ethnopharmacol, 101 (1-3), 334-337.

[12]. M. S. Cheon, T. Yoon, D. Y. Lee, G. Choi, B. C. Moon, A. Y. Lee, B. K. Choo, H. K. Kim, 2009. *Chrysanthemum indicum Linne extract inhibits the inflammatory response by suppressing NF-kappaB and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophages*. J. Ethnopharmacol, 122 (3), 473-477.

[13]. S. Zhu, Y. Yang, H. Yu, Y. Yue, G. Zou, 2005. *Chemical composition and antimicrobial activity of the essential oils of Chrysanthemum indicum.* J. Ethnopharmacol, 96 (1-2), 151-158.

[14]. H. Kurihara, H. Fukami, S. Asami, Y. Toyoda, M. Nakai, H. Shibata, X.S. Yao, 2004. *Effects of Oolong tea on plasma antioxidative capacity in mice loaded with restraint stress assessed using the oxygen radical absorbance capacity (ORAC) assay*. Biol. Pharm. Bull., 27 (7), 1093-1098.

[15]. O. I. Aruoma, M. Deiana, A. Jenner, B. Halliwell, H. Kaur, S. Banni, F. P. Corongiu, M. A. Dessi, R. Aeschbach, 1998. *Effect of Hydroxytyrosol Found in Extra Virgin Olive Oil on Oxidative DNA Damage and on Low-Density Lipoprotein Oxidation*. J. Agric. Food Chem., 46 (12), 5181-5187.

[16]. P. V. Kiem, C. V. Minh, N. T. Dat, X. F. Cai, J. J. Lee, Y. H. Kim, 2003. *Two new phenylpropanoid glycosides from the stem bark of Acanthopanax trifoliatus.* Arch. Pharm. Res., 26 (12), 1014-1017.

[17]. A. Min Tong, W. Ya Lu, J. He Xu, G. Qiang Lin, 2004. *Use of apple seed meal as a new source of β-glucosidase for enzymatic glucosylation of 4-substituted benzyl alcohols and tyrosol in monophasic aqueous-dioxane medium.* Bioorg. Med. Chem. Lett., 14 (9), 2095-2097.

[18]. K. Umehara, I. Hattori, T. Miyase, A. Ueno, S. Hara, C. Kageyama, 1988. *Studies on the constituents of leaves of Citrus unshiu MARCOV.* Chem. Pharm. Bull., 36 (12), 5004-5008.

[19]. D. Yan, L. Chun, C. Eun Mi, R. Jeong Chan, K. Young Ho, 2009. *Chemical constituents from artemisia iwayomogi Increase the function of osteoblastic MC3T3-E1 cells*. Nat. Prod. Sci., 15 (4), 192-197.

[20]. R. L. Prior, X. Wu, K. Schaich, 2005. *Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements*. J. Agric. Food Chem., 53 (10), 4290-4302.

[21]. R. Apak, K. Guclu, M. Ozyurek, S. E. Karademir, 2004. *Novel total antioxidant capacity index for dietary polyphenols and Vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method.* J. Agric. Food Chem., 52 (26), 7970-7981.

[22]. X. Zeng, J. Tian, L. Cui, Y. Wang, Y. Su, X. Zhou, X. He, 2014. *The phenolics from the roots of Livistona chinensis show antioxidative and obsteoblast differentiation promoting activity*. Molecules, 19 (1), 263-278.

[23]. W. J. Boyle, W. S. Simonet, D. L. Lacey, 2003. *Osteoclast differentiation and activation*. Nature, 423 (6937), 337-342.

[24]. J. M. Halleen, S. R. Raisanen, S. L. Alatalo, H. K. Vaananen, 2003. *Potential function for the ROS-generating activity of TRACP*. J. Bone Miner. Res., 18 (10), 1908-1911.

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