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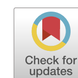
Anti-inflammatory activity of the lipophilic metabolites from Scolymus hispanicus L



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ABSTRACT

The golden thistle, Scolymus hispanicus L. (Asteraceae), is a prickly perennial herb that is widely grown in Mediterranean and in Southeastern Europe. The aim of current study was to isolate and identify the constituents of *S. hispanicus* L. chloroform fraction, and assess their anti-inflammatory activity *in-vitro*. Chemical investigation led to isolation of one new guaianolide sesquiterpene; namely iso-japonicolactone (**4**), three tritepenes namely lupeol acetate (**1**), lupeol (**2**), and oleanolic acid (**5**), and the sterol stigmasterol (**3**). The isolated compounds demonstrated free radical scavenging activity against DPPH with compound **4** being able to scavenge more than 90% of DPPH radical at 100 μM. Treatment of the peripheral blood mononuclear cells (PBMC) stimulated with phytohaemagglutinin (PHA) with the isolated compounds resulted in a decrease in nuclear factor kappa (NFκB) p65 content in a dose-dependent manner. Compound **4** was the most efficient in decreasing NFκB p65 at the lowest concentration (1 μM), followed by compound **5**. In addition, cell treatment with isolated compounds resulted in dose-related reduction of interleukins (IL-6 and IL-1β), and tumor necrosis factor (TNF-α). To the best of our knowledge this is the first report for the isolation of iso-japonicolactone and the detailed study of its anti-inflammatory activity. The potent anti-inflammatory compounds in this study could be considered as a nucleus for developing anti-inflammatory drugs through further biological studies on the molecular level.

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1. Introduction

Inflammation plays a significant role in the progress of many diseases and therefore the compounds holding antiinflammatory effects are used for the treatment of various inflammation-related diseases (Virgilio, 2004). During inflammation, stimulated inflammatory cells generate higher level of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, in addition mast cells and leukocytes are recruited to the site of damage, leading to an increased release and accumulation of reactive oxygen species (ROS) at the site of damage (Hussain et al., 2003). Therefore, it is important to maintain a balanced inflammatory status.

The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that might serve as leads for the development of novel antioxidants and antiinflammatory agents (García-Lafuente et al., 2009).

The golden thistle, Scolymus hispanicus L. (Asteraceae), is a prickly perennial herb that was found in Mediterranean and in Southeastern Europe. It is one of the most used wild vegetables in Central Spain and other Spanish regions (Polo et al., 2009). It is also consumed in other Mediterranean countries, such as Portugal, Morocco, France, Italy, Greece, Cyprus and Turkey (Polo et al., 2009). In addition, its flowers are used as a coloring alternative to saffron (Polo et al., 2009) and the whole plant is used traditionally as cholerectic (Sanz et al., 1993). Chemical investigation revealed the presence of kaempferol, quercetin, kaempferol-3-*O*-β-D-glucuronopyranoside, kaempferol-3-*O*-β-D-glucuronopyranoside-6``-methyl ester, quercetin 3-*O*-$\beta$-D-glucopyranoside, quercetin-5-*O*-$\beta$-D-glucopyranoside, quercetin-3-*O*-(2``-*O*-caffeoyl)- β -D-glucuronopyranoside in addition to phenolic acids; p-coumaric, protocaechuic, chlorogenic and isochlorogenic (Sanz et al., 1993). The presence of taraxasteryl acetate was reported as a major constituent in root bark of the plant and it showed a possible lithuretic activity (Kirimer et al., 1997).

Therefore, the aim of current study was to isolate and identify the major constituents of S. hispanicus L, in addition to assessing their anti-inflammatory activities *in-vitro* through measuring of NFκBp65, IL-6, IL-1β, and TNF-α levels in human peripheral blood mononuclear cells (PBMC) stimulated with phytohaemagglutinin (PHA).

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2. Materials and methods

2.1. General

NMR spectra (chemical shifts in ppm and coupling constants in Hz) were recorded on Bruker DRX-600 MHz Ultrashield spectrometer (Bruker BioSpin, Billerica, MA, USA) using $CDCl_3$ as solvent, with TMS as the internal reference. Column chromatographic separations were performed on Silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany). TLC analysis was performed on pre-coated TLC plates with Silica gel 60 F_{254} (Merck, Darmstadt, Germany).

2.2. Plant material

Flowering aerial parts of Scolymus hispanicus L. were collected in April 2012 from Alexandria-Matrouh road, Cairo, Egypt. The identity of the plant was authenticated by Dr. Mohamed El- Gibali, Senior Botanist, Faculty of Sciences, Cairo University, Cairo, Egypt. A voucher specimen (No. 5-12-2016) is kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

2.3. Extraction, isolation and identification

The flowering aerial parts were dried in shade under an air current for 10 days. The air-dried powdered flowering aerial parts of S. hispanicus (1 kg) were extracted with methanol (4 × 5 L) at room temperature, until exhaustion each time the plant was macerated in methanol for 24 h. The combined extracts were filtered and concentrated to give a brown residue of 60 g. The residue was suspended in distilled water (500 ml) and partitioned successively with chloroform (4 × 500 ml), and *n*-butanol (4 × 500 ml) to yield 20, and 40 g, respectively (Ezzat et al., 2016). Chloroform fraction with higher anti-inflammatory effect in rat paw edema test (supplementary data) and was chromatographed on Silica gel 60 (70–230) column (6 × 100 cm, 250 g), and eluted with hexane followed by a gradient of hexane/EtOAct mixtures until pure EtOAct. On the basis of TLC with the use of anisaldehyde-H_2SO_4 spray reagents for detection, similar fractions were pooled together to yield four collective fractions (A–C).

Fraction A (10% EtOAct) was chromatographed on a Silica gel 60 (70–230) column using Hexane: EtOAct, 97:3 as an eluent to afford compounds **1** (20 mg). Fraction B (20% EtOAct) was subjected to Silica gel 60 (70–230) column using Hexane: EtOAct, 95:5 as an eluent to afford compounds, **2** (15 mg) and **3** (12 mg). Finally, Fraction C (30% EtOAct) was subjected to silica gel column (25 cm × 2 cm, 50 g) and eluted with a Hexane: EtOAct, (9:1, v/v) to give compounds **4** (14 mg) and **5** (22 mg).

2.4. Biological study

2.4.1. Material for biological study

The inflammatory interleukins IL-6, IL-1β and TNF-α were assessed using enzyme-linked immunosorbent assay (ELISA) kits obtained from Orgenium Laboratories' (Vantaa, Finland). The NFκB p65 was assessed

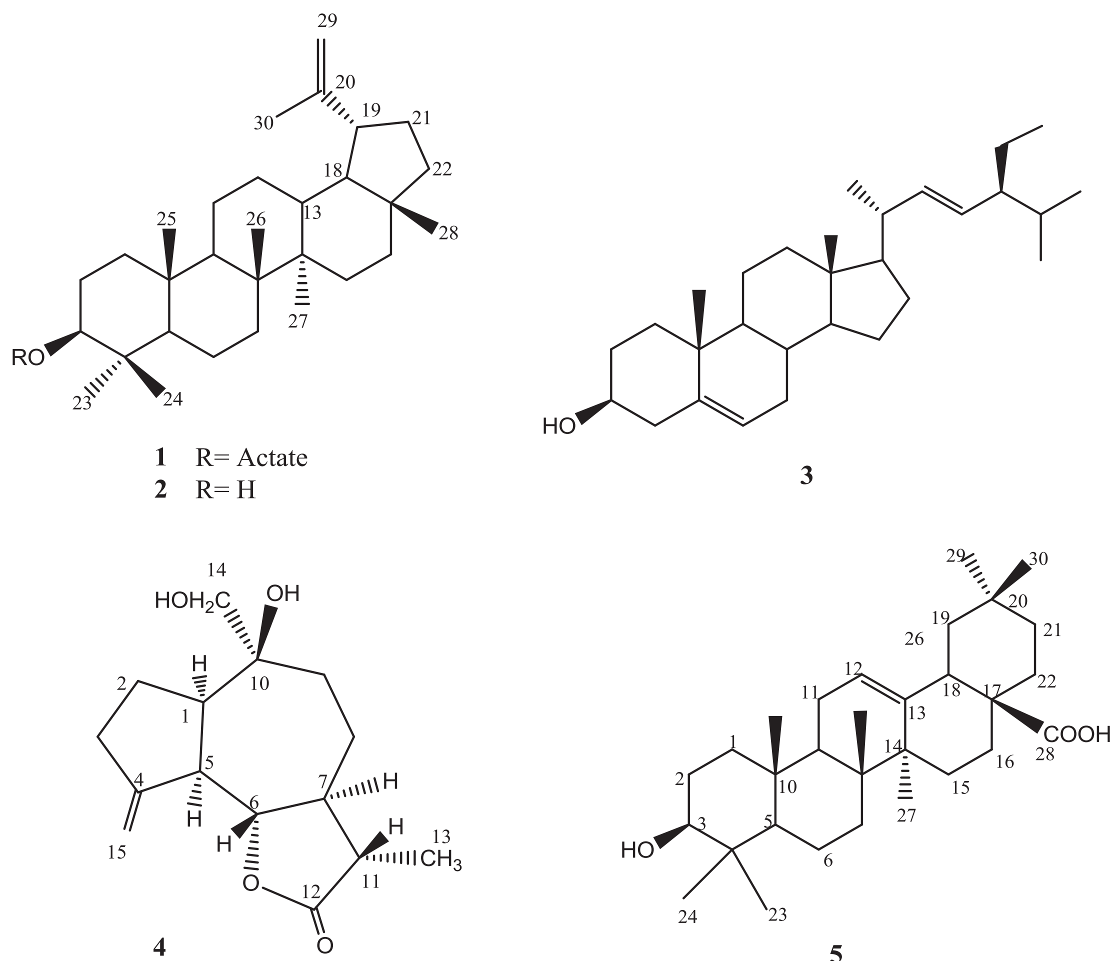

Fig. 1. Structure of the isolated compounds.

using ELISA kit obtained from abcam (#: ab 176648; MA, USA) according to the manufacturer instructions. Cell culture reagents including: fetal bovine serum (FBS), DMEM, penicillin/streptomycin, and phosphate-buffered saline (PBS) were obtained from Lonza (Basel, Switzerland). Ficoll-Paque was obtained from GE Healthcare (Biosciences AB, Uppsala, Sweden) and phytohemagglutinin (PHA) from Gibco (Germany). All other chemicals were of the highest available commercial grade.

2.4.2. Assay for DPPH free-radical scavenging potential

The nitrogen centered stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ 517 nm (Amarowicz et al., 2004). One hundred microliter from each sample in HPLC methanol was added to 3.9 mL, 0.004% (w/v) DPPH in methanol so that 3 different concentrations (1, 10 and 100 μM) of each compound were tested. The tubes were kept at an ambient temperature for 30 min and the absorbance was measured at λ 517 nm. The scavenging activity (SCA) was expressed as a percentage of scavenging activity on DPPH:

SCA% = [(A control − A test)/A control)] X 100%, where A control is the absorbance of the control (DPPH solution without test sample) and A test is the absorbance of the test sample (DPPH solution plus scavenger).

2.4.3. Experimental design

Based on a previous pilot study, the plant extract was primarily screened among different other plants for their anti-inflammatory activity using the animal model “carrageenin-induced paw edema in rats”. The total extract exhibited good anti-inflammatory activity. On continuation of this work; the experiment was repeated on the total extract, chloroform and aqueous fractions on the same animal model for their anti-inflammatory efficacy. The chloroform fraction had anti-inflammatory effect much more than the negligible effect of the aqueous fraction. The results of pilot *in-vivo* experiment were added as a *supplementary file*. Then, the compounds under investigation were isolated from the chloroform fraction (yielding few milligrams for each compound) and tested for their cytotoxic anti-inflammatory activities *in-vitro* (assay of cytokines released from isolated human PBMC).

2.4.4. Isolation of human peripheral blood mononuclear cells (PBMCs)

PBMCs were separated from whole blood obtained from healthy volunteers of whom informed consent was obtained that their donated blood might be used specifically for isolation of white blood cells to investigate the anti-inflammatory effect of some natural compounds. The separation method was approved by the Research Ethical Committee, Faculty of Pharmacy; King Abdulaziz University before blood samples collection. Separation of blood cells was performed using density centrifugation (Lan et al., 2007). Briefly, the diluted blood sample was carefully layered on Ficoll-Paque Plus. The mixture was centrifuged under at 400 x *g* for 15 min at 18–20 °C. The undisturbed lymphocyte layer was carefully transferred out. The cells were washed and pelted down with three volumes of PBS-BSA-EDTA twice. Then, they were maintained in DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 0.1% of penicillin/streptomycin, in a humidified atmosphere containing 5% CO_2 for 48 h. PBMCs were freshly prepared.

4.4.5. Cytotoxicity assay

Trypan Blue (TB) exclusion assay was carried out (Avelar-Freitas et al., 2014). Freshly isolated PBMCs cells were incubated with all compounds in DMEM at final concentrations of (100, 10 & 1 μM) for 96 h, to mimic the same experimental conditions in the study. Viable cells actively excluded TB and appeared bright colorless under light microscope, while dead cells could not and so appear blue colored.

Table 1
^{1}H and ^{13}C NMR data for compounds 4 ($CDCl_3$, ^{1}H NMR 600MHZ, ^{13}C NMR 150 MHZ, δppm, *J* in Hz).

	4	
	δ_H (*J* in Hz)	δ_C
1	2.34 m	49.2 (CH)
2	1.68 m	25.5 (CH_2)
	1.75 m	
3	2.39 m	29.9 (CH_2)
	2.50 m	
4		149.9 (C)
5	2.73 m	51.7 (CH)
6	4.00 (1H, t, *J* = 9.8 Hz)	81.4 (CH)
7	2.22 (1H, dd, *J* = 9.8, 10.4 Hz)	43.8 (CH)
8	1.39 m	25.1 (CH_2)
	2.10 m	
9	1.66 m	28.9 (CH_2)
	1.83 m	
10		75.8 (C)
11	2.31 m	47.4 (CH)
12		178.5 (C)
13	1.23 d (7.0)	13.3 (CH_3)
14	3.37 d (10.8)	68.7 (CH_2)
	3.45 d (10.8)	
15	4.99 br. s	110.1 (CH_2)
	5.18 br. s	

br., broad; s, singlet; d, doublet; dd, doublet doublet; m, multiplet; t: triplet.

Table 2
Effect of compounds isolated from Scolymus hispanicus on DPPH Free-Radical 1 Scavenging.

Compound	DPPH SCA%		
	1 μM	10 μM	100 μM
Compound 1	25.34 ± 0.18	31.289 ± 0.31	36.91 ± 0.24
Compound 2	27.62 ± 0.23	33.25 ± 0.18	36.01 ± 0.13
Compound 3	27.27 ± 0.35	32.31 ± 0.18	36.57 ± 0.23
Compound 4	86.68 ± 0.24	87.95 ± 0.11	93.30 ± 0.29
compound 5	25.14 ± 0.31	31.24 ± 0.18	49.82 ± 0.11

Data are presented as Mean ±S.D. *n* = 3.

2.4.6. Stimulation of PBMCs

Isolated PBMCs were seeded in 6-well plates at a density of 1.5×10^6 cells/ml in supplemented DMEM according to the published methods (Abdallah and Esmat, 2017). Then, cells were pretreated with the test compounds at 3 different concentrations (100, 10 and 1 μM), at which most isolated compounds have shown their *in-vitro* biological

Table 3
Effect of compounds isolated from *Scolymus hispanicus* on Trypan Blue exclusion from PBMCs, expressed as cell viability%.

Compound	PBMC viability%		
	1 μM	10 μM	100 μM
Control	95.56 ± 0.87		
1	93.90 ± 0.61	92.63 ± 1.85	93.40 ± 1.01
2	91.53 ± 3.52	90.80 ± 2.09	90.72 ± 2.88
3	92.62 ± 1.81	90.95 ± 2.42	91.20 ± 2.45
4	93.40 ± 1.91	92.90 ± 3.11	90.83 ± 2.54
5	92.64 ± 1.37	90.76 ± 1.26	91.10 ± 2.70

Data are presented as Mean ± S.D. *n* = 3.
Freshly isolated cells were incubated with all compounds in DMEM at final concentrations of (100, 10 & 1 μM) for 96 h. In brief, 10 μL of cell suspension was mixed with 10 μL TB (0.4% in PBS) on a hemocytometer, then examined under the light microscope. Viable cells actively excluded TB, appeared colorless & bright, while dead cells could not, and so appear blue colored.
Viability% was calculated as follows:
Viability% = (Number of viable cells/Number of total cells) × 100.

activities in literature (Abdallah and Esmat, 2017; Ashalatha et al., 2010). After 24 h, they were stimulated with 10 μg/ml of mitogen. Cells treated with PHA but not the extracts were used as a positive control group. Negative control group was treated with DMSO in a final concentration equal to test wells. DMSO concentration never exceeds 0.1%. Indomethacin was used as a reference standard at 10 μM concentration. After 3 days of incubation, supernatant media were used for measuring the levels of IL-6, IL-1β and TNF-α. Cells were collected and their lysates were used for measuring NFκB p65 concentration.

2.4.7. Assessment of IL-1β, IL-6 and TNF-α levels

The quantitative sandwich immunoassay technique was used for measuring IL-1β, IL-6 and TNF-α levels. Briefly, each kit had a specific microplate pre-coated with monoclonal antibody against its analyte. After adding the standards and samples, the analyte was sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for it. The later was recognized by a streptavidin–peroxidase conjugate. All unbound material was then washed away. Thereafter, a peroxidase enzyme substrate was added (Virella and Litwin, 2001) followed by the stop solution. Finally, the color intensity was measured at 450 nm using a microplate reader (ChroMate-4300, FL, USA). The intensity of the color is directly proportional to the sample concentration.

2.4.8. Assessment of NFκB p65 concentration

This assay employed an affinity tag-labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is - in turn - immobilized via immunoaffinity of an anti-tag antibody coating the well. Briefly, samples or standards were added to the wells, followed by the antibody mix. After incubation, the wells were washed to remove unbound material. TMB substrate was added and incubation was catalyzed by HRP, generating blue coloration. This reaction was then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal was generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm using a microplate reader (ChroMate-4300, FL, USA).

2.5. Statistical analysis

Data are presented as mean ± S.D. Comparisons were carried out using one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. Statistical significance was acceptable to a level of $p < 0.05$. All statistical analyses were performed using GraphPad InStat software, version 3.05 while graphs were plotted using GraphPad Prism software, version 5.00 (GraphPad Software, Inc. La Jolla, CA, USA).

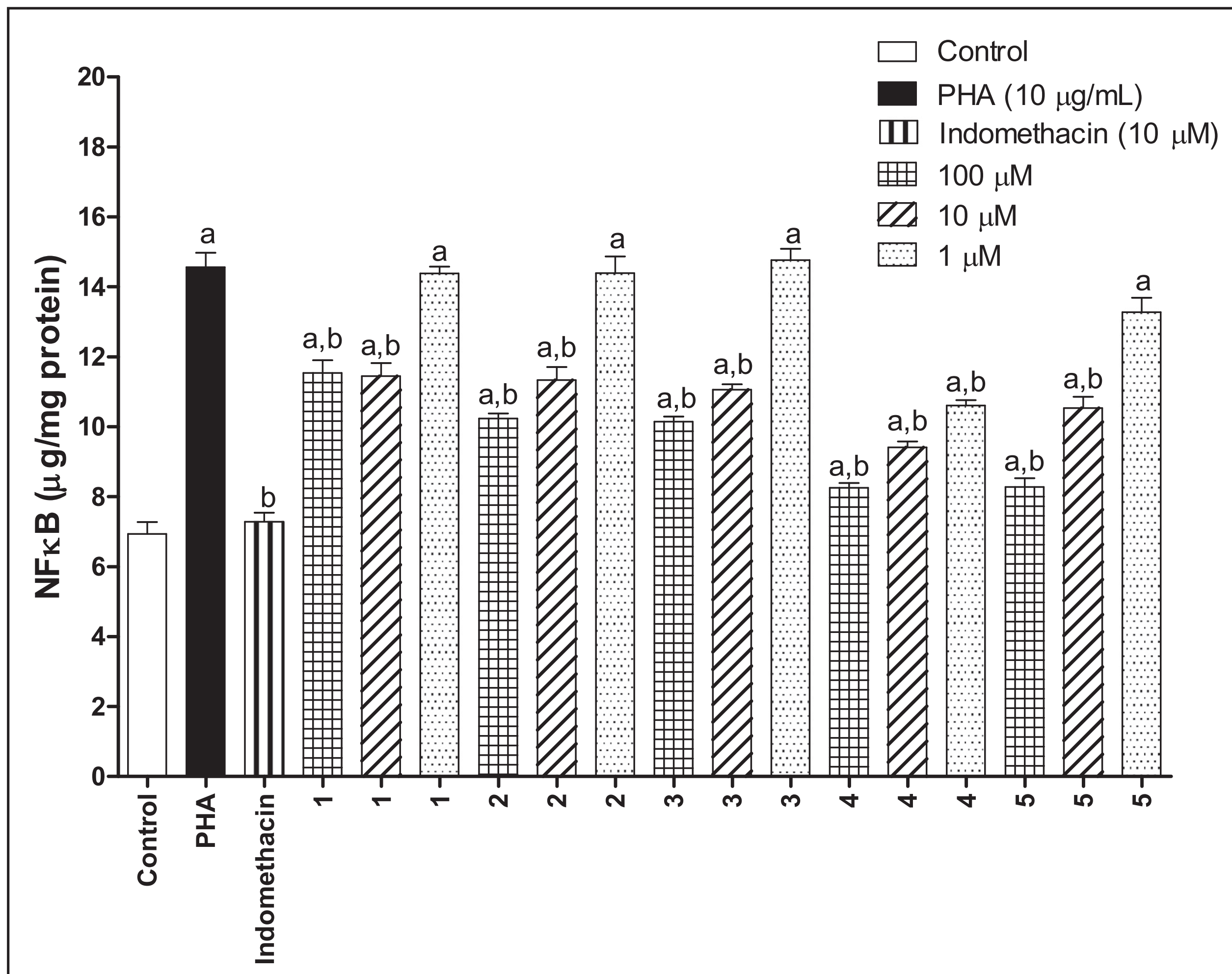

Fig. 2. Effect of compounds isolated from *S. hispanicus* on NFκB p65 content in PHA-stimulated PBMCs at concentrations: 100, 10 and 1 μM.
Data are presented as Mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test.
$n = 3$ (different donors).
[a] statistically different from the corresponding control group at $p < 0.001$.
[b] statistically different from the corresponding PHA-treated group at $p < 0.001$.

3. Results and discussion

Phytochemical investigation of the anti-inflammatory lipophilic fraction of the flowering aerial parts of S. hispanicus led to the isolation of a new guaianolide sesquiterpene; iso- japonicolactone (**4**) in addition to four major known metabolites (Fig. 1).

Compound **4** was obtained as yellow oil. Its molecular formula was determined as $C_{15}H_{22}O_4$ by HRESIMS (*m/z* 267.3481, $[M + H]^+$) (supporting file). The ^1H NMR spectrum of compound **4** (Table 1) exhibited one doublet methyl group at δ 1.23 (3H, d, J = 7 Hz, H-13), pair of exomethylene proton signal at δ 4.99 and δ 5.18 (each 1H, broad singlet) and 6,7-lactone group at δ 4.00 (1H, t, J = 9.8 Hz, H-6), 2.22 (1H, dd, J = 9.8, 10.4 Hz, H-7). Moreover, the spectrum showed two doublet protons at δ 3.37 and δ 3.45 each integrated as 1 H, with J = 10.8 Hz indicated two proton on oxy carbon (an oxy methylene group) this was assigned to the two protons of C-14 appeared at δ_c 68.7 as deduced from HSQC (supporting file); therefore compound 4 was confirmed to be guaianolide sesquiterpene (Costa et al., 2018). The ^{13}C NMR spectrum (Table 1) showed 15 carbon signals, consisting of two olefinic carbon signals at δ 110.1, 149.9, a carbonyl carbon signal at δ 178.5, and three oxygenated carbon signals at δ 75.8, δ 68.7, and δ 81.4 (for carbons 10, 14 and 6 respectively), one methyl signal at δ 13.3. By analyzing the $^1H-^1H$ COZY, HSQC and HMBC spectra (supporting file), compound **4** was closely comparable to those of japonicolactone (Jia et al., 1991). The relative configuration of **4** was determined by the NOE correlations observed in the NOESY spectrum (supporting file). From previous publication, if H-11 is α, it should appear at δ 2.6 and CH_3-11 at δ 1.18 (Jia et al., 1991), our results revealed the appearance of H-11 at 2.23, indicating its β orientation. Moreover, the NOE correlations of H-1/H-5, H-1/H-14, H-6/H-11, H-7/CH_3-11 revealed α-orientation of H-1, H-5, H-7 and methyl at C-11, while H-11 and H-6 revealed β-orientation. Based on the previous discussion compound 4 could be identified as iso- japonicolactone. Japonicolactone which is the isomer of our compound has been isolated and identified from other related plants species of the same family (Asteraceae) such as *Lactuca sativa* L var. *anagustata* (Han et al., 2010) and *Saussurea involucrata* (Kar. et Kir. ex Maxim) (Xiao et al., 2011).

The other four compounds were identified as lupeol acetate (**1**) (Gupta et al., 2005), lupeol (**2**) (El Sayed et al., 2016), stigmasterol (**3**) (Forgo and Kövér, 2004), and oleanolic acid (**5**) (Seebacher et al., 2003). The structures of the isolated compounds (Fig. 1) were determined by comprehensive analyses of their NMR and comparison with previously known analogs (supporting file).

The antioxidant activity of the isolated compounds from Scolymus hispanicus was tested *in-vitro* using DPPH assay for free radical scavenging activity. As shown in Table 2, compound **4** has exhibited the highest antioxidant activity, being able to scavenge more than 90% of DPPH radical at 100 μM, followed by compound **5** with 49% DPPH scavenging activity at 100 μM. Conversely, other compounds have shown about 36% scavenging activity even at the highest concentration (100 μM). These findings provide some clues about the relative antioxidant activities of those compounds isolated from *S. hispanicus*. The compounds demonstrated antioxidant effect with compound **4** showing exceptional activity. These results were in accordance with the reported data for the antioxidant activity of lupeol and its ester (Sudhahar et al., 2006), oleanolic acid (Somova et al., 2003) and sesquiterpenes (Gonzalez-Burgos and Gomez-Serranillos, 2012).

To initially assess the safety of all compounds on isolated PBMCs, their cytotoxic effects were carried out by TB exclusion test, as shown in (Table 3). In fact, all tested compounds did not significantly decrease cell viability from the control cells. This confirms that the potential anti-inflammatory effects of these compounds were not

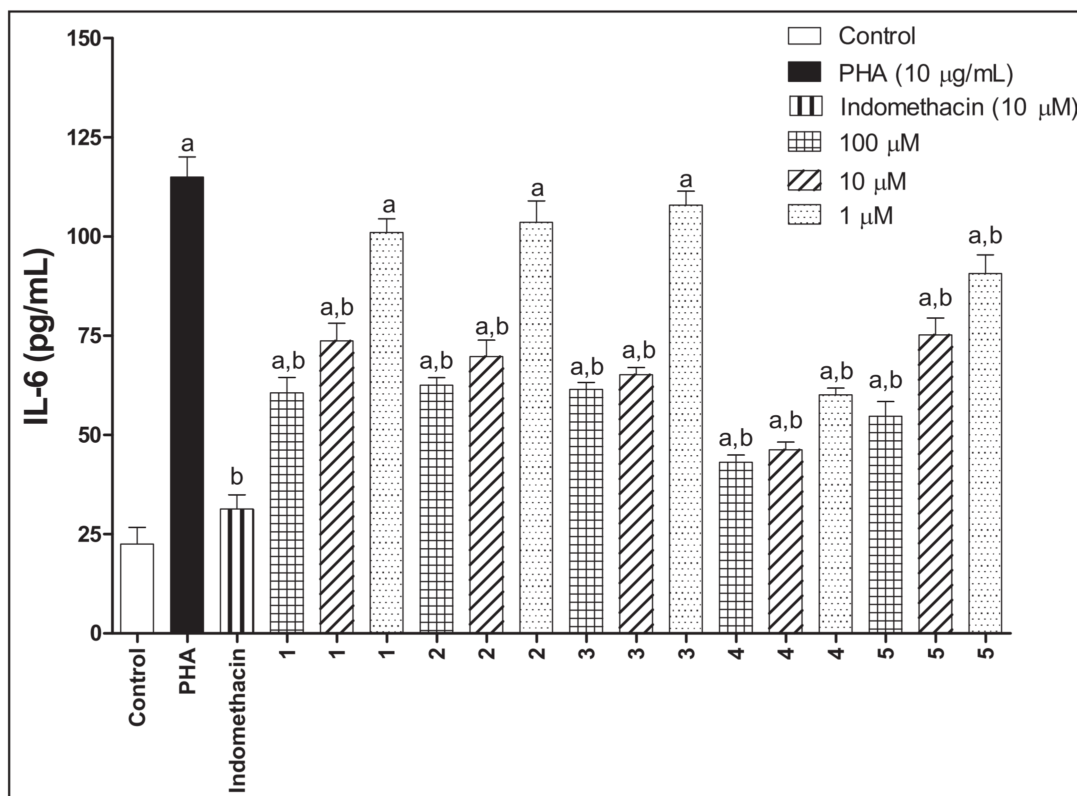

Fig. 3. Effect of compounds isolated from *S. hispanicus* on IL-6 release from PHA-stimulated PBMCs at concentrations: 100, 10 and 1 μM.
Data are presented as Mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test.
n = 3 (different donors).
[a] statistically different from the corresponding control group at $p < 0.001$.
[b] statistically different from the corresponding PHA-treated group at $p < 0.001$.

associated with cytotoxicity. It is worth mentioning that NFκB represents an important linkage between oxidative stress and inflammation (Kakrani et al., 2011). Therefore, it was assessed in the lysates of PHA-stimulated PBMCs after treatment with different concentrations (1, 10, 100 μM) of the tested compounds, using indomethacin as a standard anti-inflammatory drug. As shown in Fig. 2, PHA-stimulated cells showed significant 2-fold increase of NFκB p65 subunit content, compared to the control. The standard anti-inflammatory drug indomethacin was able to normalize NFκB p65 content, with insignificant change from the control. Cell treatment with the tested compounds resulted in a decrease in NFκB p65 content in a dose-dependent manner. Particularly, compounds **4** was the most efficient in decreasing NFκB p65 at the lowest concentration (1 μM) followed by compound **5**. On contrast, the other 3 compounds did not significantly decrease NFκB p65 compared to PHA-challenged group.

In addition, the anti-inflammatory activities of compounds isolated from the lipophilic fraction of S. hispanicus were assessed by measuring the levels of IL-6, IL-1*β* and TNF-*α* in supernatant media of PHA-stimulated PBMCs. As indicated in Fig. 3, PHA-challenged cells showed significant elevation of IL-6 level compared with the control. Treatment with isolated compounds resulted in dose-related decline in IL-6 level. At 1 μM concentrations, only compounds **4** and **5** significantly decrease IL-6 level from PHA treatment. However, at 100 μM, all compounds significantly decreased IL-6 compared to PHA treatment. Interestingly, compounds **4** and **5** decreased IL-6 level to the extent that was not statistically different from the control untreated group. Assessing IL-1*β* has demonstrated a similar pattern of activity, as shown in Fig. 4. The 4 compounds (**1**–**3** and **5**) did not significantly decrease IL-1*β* level at (1 μM) compared to PHA-challenged group. At 100 μM, all compounds significantly decreased IL-1*β*. Likewise, cell treatment with isolated compounds resulted in dose-related reduction of TNF-*α* level. As shown in Fig. 5, all isolated compounds (at 100 μM) significantly decreased TNF-*α* level compared to PHA-treated group. This effect was reduced as the concentration decreases till (1 μM) where only compounds **4** and **5** still showing significant difference from PHA-challenged group.

The obtained results are in agreement with many published data. Although sesquiterpenoids are known for their anti-inflammatory effects (Hall et al., 1980), this is the first report on anti-inflammatory activity of iso-japonicolactone. Current available literature recorded the anti-inflammatory activity of lupeol (**2**) and its acetate (**1**) (Ashalatha et al., 2010; Fernández et al., 2001). Lupeol showed anti-inflammatory effect through inhibition of the production of some pro-inflammatory mediators (PGE_2, TNF-*α* and IL-1*β*) (Fernández et al., 2001). Lupeol acetate (LA) inhibit pro-inflammatory cytokines and the NO system and down regulate TNF-*α* and IL-2 (Ashalatha et al., 2010; Lucetti et al., 2010). Moreover, stigmasterol (**3**) was reported for its antioxidant and local anti-inflammatory effects through inhibition of inhibit 12-O-tetradecanoylphorbol acetate-induced edema (Conforti et al., 2008). Oleanolic acid was reported to inhibit PGE2 production, and suppress protein expression of COX-2, and NF-*κ*B p65 (Tsao and Yin, 2015).

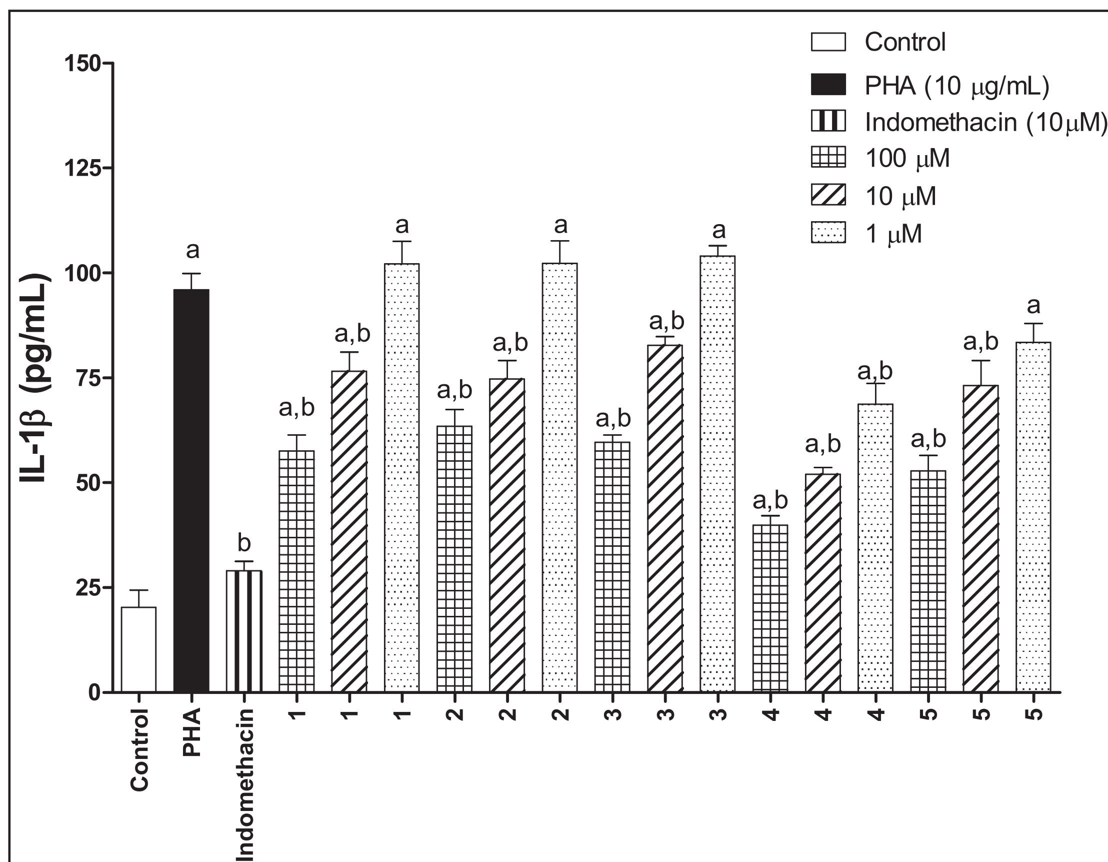

Fig. 4. Effect of compounds isolated from *S. hispanicus* on IL-1*β* release from PHA-stimulated PBMCs at concentrations: 100, 10 and 1 μM.
Data are presented as Mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test.
n = 3 (different donors).
[a] statistically different from the corresponding control group at $p < 0.001$.
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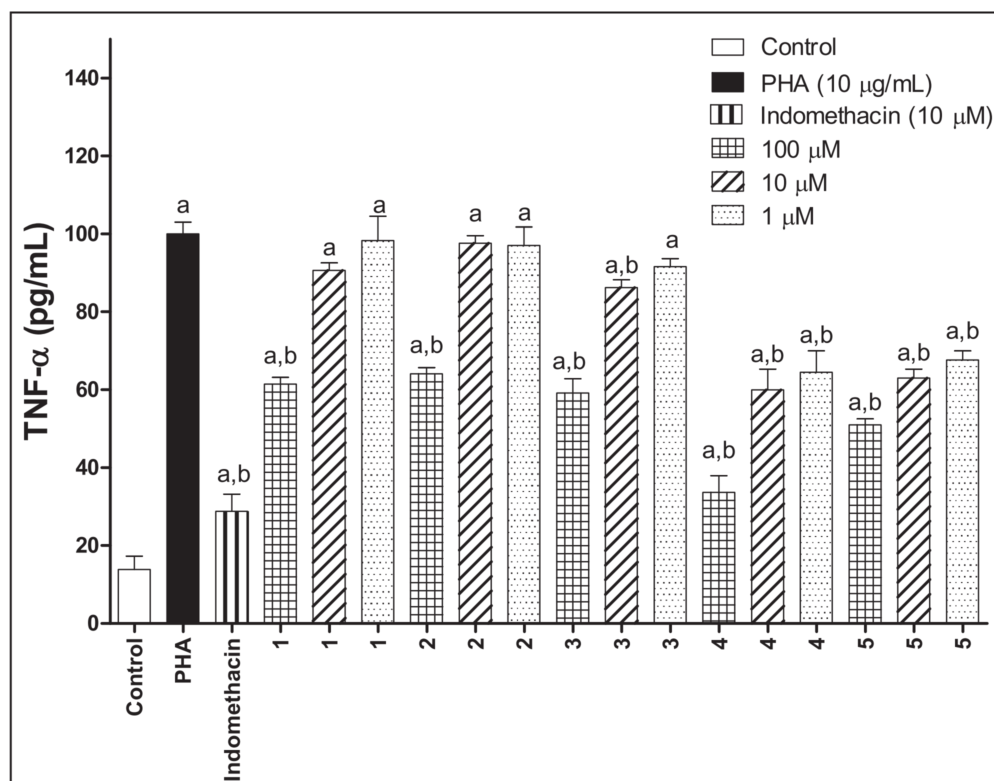

Fig. 5. Effect of compounds isolated from *S. hispanicus* on TNF-α release from PHA-stimulated PBMCs at concentrations: 100, 10 and 1 μM.
Data are presented as Mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test.
$n = 3$ (different donors).
[a] statistically different from the corresponding control group at $p < 0.001$.
[b] statistically different from the corresponding PHA-treated group at $p < 0.001$.

4. Conclusion

One new guaianolide sesquiterpene; iso-japonicolactone in addition to four major triterpene metabolites were isolated from the bioactive lipophilic fraction of S. hispanicus. The isolated compounds exhibited anti-inflammatory activities *in-vitro*, manifested by inhibition of NFκB p65 expression and subsequent decrease of inflammatory cytokines: IL-6, IL-1β, and TNF-α in PHA-stimulated with human PBMCs. To the best of our knowledge this is the first report for the isolation of iso-japonicolactone and the detailed study of its *in-vitro* anti-inflammatory activity. However, an extended future work concerning the isolated compounds might be necessitated not only to evaluate their acute toxicities to determine their LD_{50} in rats, but also to confirm their anti-inflammatory activities *in-vivo* using animal models - like rat paw edema or ear edema – in optimum safe doses. Moreover, the isolated compounds could be considered as a scaffold for developing anti-inflammatory drugs through further biological studies on the molecular level.

Declarations of Competing Interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2020.01.022.

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