Full Length Research Paper

**In vivo TNF-α and IL-1β inhibitory activity of phenolics isolated from Trachelospermum jasminoides (Lindl.) Lem**

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A bio-guided fractionation of the defatted ethanolic extract (DEE) of the aerial parts of *Trachelospermum jasminoides* and its fractions: ethyl acetate fraction (EAF), chloroform fraction (CF) and n-butanol fraction (BF) were carried out, using carragenan induced rat paw edema method, to evaluate the *in vivo* acute anti-inflammatory potential at a given dose of 100 mg/kg body weight compared to indomethacin (20 mg/kg body weight). The EAF revealed the highest anti-inflammatory activity (76.92%) relative to the DEE and the CF (63.82 and 48.75%, respectively) at the same tested dose, while the BF was significantly inactive. The EAF and its major isolated compounds were investigated to determine the level of pro-inflammatory cytokines using enzyme-linked immunosorbant assay (ELISA). Seven major compounds were isolated from the EAF, identified as: trachelogenin, nor-trachelogenin and tracheloside, in addition to apigenin, luteolin, quercetin and luteolin-7-O-β-D-glucopyranoside. Identification of the isolated compounds was achieved by their physico-chemical properties and spectral analysis (1D and 2D NMR). The EAF and the isolated compounds inhibited the excessive production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β. Furthermore, the liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS) for the bioactive EAF was carried out to complete the phytochemical picture. The results of this study verified that the EAF and the major compounds exert their action through inhibition of TNF-α and IL-1β.

**Key words:** *Trachelospermum jasminoides*, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS).

**INTRODUCTION**

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents (Nadkarni, 2000). The classic signs of inflammation are local redness, swelling, pain, heat and loss of function (Pervical, 1999). There are mainly two types of inflammation; acute inflammation which is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes while,
chronic inflammation is usually associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. Cytokines represent a group of multifunctional substances that are involved in the inflammatory response (Sacca et al., 1997). Pro-inflammatory cytokines as interleukin (IL-1β), tumour necrosis factor (TNF-α), IL-6 and IL-18 are involved in the initiation and amplification of inflammation (Dinarello, 2008). Overproduction of pro-inflammatory cytokines in macrophages is responsible for many inflammatory diseases, including rheumatoid arthritis, atherosclerosis, and hepatitis (Opal and DePalo, 2000; Srinivasan et al., 2011; Verma and Singh, 2008). Therefore, inhibiting their production may serve to prevent or suppress a variety of inflammatory diseases. Inflammation is usually treated by Nonsteroidal Anti-inflammatory Drugs (NSAIDs). Unfortunately, these drugs cause increased risk of blood clots resulting in heart attacks and strokes (Nadkarni, 2000). Only protein-based drugs are available for the clinical inhibition of TNF-α activity. TNF-α inhibitors from natural origins are being advanced for the treatment of inflammatory disorders. Natural products have been, and continue to be, a major source of pharmacologically active substances from which drugs can be developed (Paul et al., 2006). The screening and development of drugs for their anti-inflammatory activity is the need of hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants (Bingtao et al., 1995). Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants (Verma and Singh, 2008).

Family Apocynaceae (Li et al., 1995) (Dogbane) is one of the largest plant families comprising over 424 genera and up to 1500 species. Plants belonging to this family are distributed in tropical and temperate areas. Trachelospermum jasminoides (Lindl.) Lem. is a member of family Apocynaceae. Many of which is a woody climber plant found in the tropics and subtropics. It is an evergreen woody liana growing to 50 cm in height. It is also known as Luoshi or Chinese jasmine (Endress and Bruyns, 2000; Nishibe et al., 2002).

Bioactive metabolites flavonoids, lignans, sterols and triterpenes, alkaloids were reported in the plant (Zhang et al., 2013; Zhu et al., 2013; Tan et al., 2010; Xing-qf et al., 2006; Tan et al., 2005; Jing et al., 2005; Atta-ur-Rahman et al., 1988; Fatima et al., 1987).

The plant is reputed for its anti-inflammatory, analgesic activities and its anti cancer activities (Li et al., 2003; Nishibe and Han, 2002; Nishibe et al., 1987). The ethanol extract of T. jasminoides showed potent inhibitory activities against both COX-1 and PLA₂ (Lai et al., 2003). Moreover, its lignans content significantly inhibited lipid peroxidation (Fujimoto et al., 1992) evidencing its anti-oxidant activity. Also, the lignans exhibited relaxation effects on histamine-induced contraction of tracheal muscles (Fujimoto et al., 1992).

Herein, a bio-guided fractionation for the defatted ethanolic extract (DEE) of the aerial parts of T. jasminoides was carried out to investigate the in vivo acute anti-inflammatory activity with the aim of determining the most active fraction. Isolation of the major compounds from the bioactive fraction as well as their anti-inflammatory activity has been studied. Furthermore, a qualitative analysis applying LC/ESI-MS technique was achieved for the most active fraction to identify other major compounds present in the bioactive fraction and give a clear phytochemical picture.

MATERIALS AND METHODS

Plant

Samples of T. jasminoides (Lindl.) Lem. used in this study were collected since May 2008 from El-Orman Botanical Garden, Giza, Egypt. Plant identity was kindly authenticated by Dr. Mohamed El Gebaly, Botany Taxonomist, Cairo University and Engineer Therese Labib, senior specialist of plant identification at El-Orman Botanical Garden, Giza, Egypt. A voucher sample (TS-002008), has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Chemicals and equipments

Inflammatory-grade carrageenan was purchased from FMC (Rockland, ME, USA). All extracts were dissolved in carboxymethylcellulose before being injected into the animals. Indomethacin was purchased from Sigma-aldrich, USA. TNF-α and IL-1β were quantified using enzyme- linked immunosorbent assay kits (Boster Biological Technology Co., Inc, CA, USA). All other chemicals were of the highest available commercial grade. Silva gel H (Merck, Darmstadt, Germany) for vacuum liquid chromatography (VLC), silica gel 60 (70 to 230 mesh ASTM, Fluka, Steinheim, Germany) and Sephadex LH 20 (Pharmacia, Stockholm, Sweden) were used for column chromatography. Thin-layer chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka) using the following solvent systems; S1 (n-hexane/ethyl acetate, 80:20 v/v), S2 (chloroform/methanol, 9:1) S3 (chloroform/methanol/formic acid, 90:10:2). The chromatograms were visualized under UV (at 254 and 366 nm) before and after exposure to ammonia vapour and spraying with AlCl₃, as well as after spraying with anisaldehyde/sulphuric acid reagent. Melting points (uncorrected) were determined on a Electrothermal 9100 (Ontario, Canada). Shimadzu-IR-435 Infrared spectrophotometer was used for measuring IR spectra. UV spectra were measured using a Shimadzu UV 240 (P/N 204-58000) spectrophotometer (Kyoto, Japan). 1H NMR (300 MHz) and 13C NMR (75 MHz) were measured on a Varian Mercury-VX-300 instrument (Palo Alto, CA, USA). The NMR spectra were recorded in CDCl₃ and DMSO-d₆ and chemical shifts were given in d (ppm) relative to TMS as internal standard.

Animals

Throughout the experiments, adult male Sprague–Dawley rats weighing 180 to 200 g were used for evaluating the anti-inflammatory...
activity and 25 to 30 g male albino mice for LD₅₀; all animals were supplied by Pharmacology Department, Faculty of Pharmacy, Ain Shams University. They were housed at a temperature of 23 ± 2°C with free access to water and standard food pellets. Rats were acclimatized in our animal facility for at least 1 week prior to the experiment. All animal procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care, and had been approved by the Ethics Committee of the National Research Centre (No. 9 to 031) in accordance with recommendations for the proper care and use of laboratory animals (NIH Publication No. 80 to 23; revised 1978).

**Extraction**

Two kilograms of the air-dried powdered aerial parts of *T. jasminoides* (Lindl.) Lem were exhaustively defatted with n-hexane (2.032 g). The defatted powder was dried then extracted with ethanol 90% (cold maceration) (2×4 L). The defatted ethanolic extract (DEE) was evaporated (60°C) under reduced pressure to yield chloroform (1.5 L), followed by ethyl acetate fraction (EAF) (13.3 g) and n-butanol fraction (BF) (2.032 g).

**Isolation of compounds from the bioactive EAF**

The bioactive EAF of the aerial parts of *T. jasminoides* was subjected to purification as follows.

Ten grams of the EAF were chromatographed on a VLC (7×12.5 cm), packed with 270 g silica gel 60. Gradient elution was performed starting with chloroform and increasing the polarity by 2.5% stepwise addition of ethyl acetate till 100%, followed by increasing the polarity with methanol in 5% increment till 50% CHCl₃ MeOH/EtOAc. Fractions were collected (200 ml each) and monitored by TLC using solvent systems S1-S3, the spots were visualized under UV light before and after exposure to ammonia vapour and by using aluminum chloride and p-anisaldehyde as spray reagents yielding collective fractions designated as fractions I-V. Fraction I (970 mg) was further purified on a column silica gel 60 (30 cm L × 3 cm D), eluted using 5 to 50% EtOAc/CH₂Cl₂, in fractions of 5 ml, to afford two main sub-fractions (Sub-fractions Ia and Ib). Sub-fraction Ia: (400 mg), purified on Si gel column (17 × 1 cm D) eluted with EtOAc:MeOH:H₂O (100:16:11) v/v, then purified over Rp-18 column with system MeOH: H₂O (50:50) v/v to yield compound C₁ (11 mg and compound C₄ (16 mg). Fraction II (200 mg) was chromatographed repeatedly on a sephadex LH-20 column (28×1.8 cm) using methanol: water (90:10 v/v) to give pale yellow powder of compound C₃ (97 mg) and 11 mg of yellow powder compound C₅. Fraction IV (0.6 g) chromatographed on a sephadex LH-20 column (28 × 1.8 cm) using methanol:water (80:20) v/v for elution, then chromatographed repeatedly over Rp-18 column using methanol:water (80:20) v/v for elution, to yield one major spot, dried and purified over Rp-18 column with methanol:water (50:50) v/v to give 210 mg of a white amorphous powder compound C₇. Each purified compound was subjected to detailed studies to elucidate its structure.

**Determination of median lethal dose (LD₅₀)**

The LD₅₀ of the DEE of the aerial parts of *T. jasminoides* was carried out according to Karber (1931).

**Screening for the acute anti-inflammatory activity**

The DEE and its sub-fractions (CF, EAF and BF) were investigated for their acute *in vivo* anti-inflammatory activities according to the carrageenan induced rat paw edema method (Winter et al., 1962). Six groups of 6 animals each of adult male albino rats were used. The rats were kept under the same hygienic conditions, well balanced diet and water supplied *ad libitum*. The first group received 1 ml saline orally (negative control). The second group was given indomethacin orally in a dose of 20 mg/kg body weight (positive control). The other four groups received the DEE, CF, EAF and BF fractions of the aerial parts each in a dose of 100 mg/kg body weight. One hour later, edema was induced by a sub plantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Three hours after the induction of inflammation, the rats were sacrificed. The right hind paw weight was measured immediately after carrageenan injection by water displacement using a plethysmometer (model 7140, Ugo Basile, Comerio, Italy). The paw weight was re-measured 1, 2 and 3 h after carrageenan injection. The mean response for increase in the paw edema after acute inflammation was calculated.

\[
\text{Edema (\%) = } \frac{\text{Weight of the right paw - Weight of the left paw}}{\text{Weight of the left paw}} \times 100
\]

The percentage of inhibition in the mean of the treated group in comparison with the control non-treated group was estimated and calculated according to the following equation:

\[
\text{Inhibition (\%) = } \frac{\text{Paw edema of control - Paw edema of treated}}{\text{Paw edema of control}} \times 100
\]

**In vivo anti inflammatory activity for the bioactive fractions and the major isolated compounds**

Carrageenan induced rat paw edema method was applied for the bioactive EAF and the major isolated compounds as mentioned earlier. The animals were equally divided into 8 groups (1 to 8), 6 animals per group. Using an intragastric tube, groups 1 and 2 were given the vehicle (0.5% carboxymethylcellulose). Animals in group 3 received indomethacin (10 mg/kg) as a standard anti-inflammatory drug suspended in aqueous carboxymethylcellulose (0.5% w/v), whereas remaining groups were treated by: EAF and the major isolated compounds: C₁, C₂, C₄ and C₇ at two dose levels (25 and 50 mg/kg). The dosing volume was kept constant (10 ml/kg) for all
and IL-1 eicosanoid-containing fluid and to stop further production of TNF-α containing 10 µM indomethacin was injected to aid removal of the inflammatory exudates. Right hind paws were removed. A volume of 0.1 ml of saline was used to facilitate drainage of the inflammatory exudates. For the purpose of this study, centrifugation at 1,800 g for 15 min. TNF-α and IL-1β levels in the collected inflammatory exudates, paws were centrifuged at 1,800 g for 15 min. TNF-α and IL-1β were quantified in the collected exudates using enzyme-linked immunosorbent assay kits (Boster Biological Technology Co., Inc, CA, USA) according to the manufacturer instructions. Both assays are based on the sandwich technique in which specific antibodies to TNF-α or IL-1β were precoated on to 96-well plate. The specific detection antibodies were biotinylated. The test samples and biotinylated detection antibodies were added sequentially followed by washing. Avidin-biotin Peroxidase Complex was added and unbound conjugates were washed. A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm using an ELISA microplate reader (ChroMate-4300, FL, USA). The intensity of the color is directly proportional to the concentration of TNF-α or IL-1β in the sample. All biological experiments were done in triplicates

### Measurement of TNF-α and IL-1β levels in the rat paw

Right hind paws were removed. A volume of 0.1 ml of saline containing 10 µM indomethacin was injected to aid removal of the inflammatory exudates. For the purpose of this study, centrifugation at 1,800 g for 15 min. TNF-α and IL-1β were quantified in the collected exudates using enzyme-linked immunosorbent assay kits (Boster Biological Technology Co., Inc, CA, USA) according to the manufacturer instructions. Both assays are based on the sandwich technique in which specific antibodies to TNF-α or IL-1β were precoated on to 96-well plate. The specific detection antibodies were biotinylated. The test samples and biotinylated detection antibodies were added sequentially followed by washing. Avidin-biotin Peroxidase Complex was added and unbound conjugates were washed. A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm using an ELISA microplate reader (ChroMate-4300, FL, USA). The intensity of the color is directly proportional to the concentration of TNF-α or IL-1β in the sample. All biological experiments were done in triplicates.

### Statistical analysis

Data were expressed as the means ± standard error of mean (SEM). The differences between groups were tested by one-way analyses of variance (ANOVA) followed by the Tukey post-hoc test. All statistical analyses were performed using Graph Pad Instat software version 3 (ISI_ software, CA, USA). The probability of P<0.05 was considered statistically significant.

### Apparatus and chromatographic conditions

LC analysis was performed using Agilent 1100 (Helwell Packard) HPLC system coupled to a UV detector and an Esquire 3000 plus ion trap mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) equipped with an electrospray ionization (ESI). Gradient elution of analytes was achieved using water and acetonitrile, both containing 0.1% HCOOH at a constant flow rate of 600 µl min⁻¹, was used. Chromatographic separation of the EAF was performed on RP-C₁₈ column; 250×4 mm ID, 5 µm (Merck, Darmstadt, Germany) at 30°C, connected to a guard column; 10 × 4 mm ID, 5 µm (Phenomenex, Torrance, CA). The mobile phase consisting of acetonitrile/0.1% formic acid (solvent system A) and water/0.1% formic acid (solvent system B) was eluted with a gradient system as solution A-B: by 0 min (100% A), 2 min (95% A), 5 min (70% A), 8 min (66% A), 11 min (45% A) and 17 to 20 min (100% A) v/v. The flow rate was set at 0.1 µl min⁻¹ and the sample injection volume was 15 µl. The UV detection wavelength was set at 190 to 600 nm. Mass spectroscopic conditions were used as follows: the extract was ionized in the negative ESI interface of the mass spectrometer. The temperature and the voltage of the heated capillary were 300°C and 25 V, respectively. The sheath gas-nitrogen (61/min); (-) 4KV was used. Mass range was set to 200 to 1000 m/z.

### Sample preparation

Two milligrams of the EAF were dissolved in 5 ml of methanol in a volumetric flask and ultrasonicated. Purification was performed using solid phase extraction (SPE) cartridges (LiChrolut RP-18; Merck), which were activated and pre-conditioned using 3 × 1 ml of methanol followed by 3 × 1 ml of water. Purification was then carried out by applying the dissolved extract onto the SPE cartridge, followed by elution with H₂O: MeOH (1:4). The purified fraction was then separately subjected to LC/ESI-MS analysis. Identification of the phenolic compounds was carried out by comparing MS¹ and MS² of each peak with the reference data. For more confirmation selective ion monitoring (SIM) technique was applied.

### RESULTS

The LD₉₀ of the DEE of the aerial parts was safe up to 5000 mg/kg body weight. The data (Table 1) showed that the EAF of the aerial parts was the most bioactive fraction revealed by its high potency (76.92%) at the given dose of 100 mg/kg body weight compared to indomethacin (20 mg/kg body weight). Consequently, the bioactive EAF was chosen for phytochemical study and seven compounds (1 to 7) (Figure 1) were isolated. The compounds were identified based on their spectral data analyses. Moreover, the aim of this study was to measure

<table>
<thead>
<tr>
<th>Group</th>
<th>% Edema (Mean ± S.E)</th>
<th>Inhibition (%)</th>
<th>Potency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (un-treated)(1 ml saline)</td>
<td>59.7±1.8</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>DEE (100 mg/kg body weight)</td>
<td>36.4±1.3*</td>
<td>39.02</td>
<td>63.82</td>
</tr>
<tr>
<td>EAF (100 mg/kg body weight)</td>
<td>31.5±1.1*</td>
<td>47.23</td>
<td>76.92</td>
</tr>
<tr>
<td>CF (100 mg/kg body weight)</td>
<td>41.9±1.6*</td>
<td>29.81</td>
<td>48.75</td>
</tr>
<tr>
<td>BF(100 mg/kg body weight)</td>
<td>68±1.9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin (20 mg/kg body weight)</td>
<td>23.2±0.4*</td>
<td>61.14</td>
<td>100</td>
</tr>
</tbody>
</table>

*Significantly different from control group at P<0.01. DEE: Defatted ethanolic extract, EAF: ethyl acetate fraction, CF: chloroform fraction, BF: n-Butanol fractions. Data are presented as mean ± SEM. n=6.
the activity of the EAF alongside with the isolated compounds at lower doses (50 and 25 mg/kg body weight) to determine the least dose responsible for the activity in addition to determining their mechanism of action.

**Compound 1**

White amorphous powder (MeOH, 190 mg), M.P: 167-170°C, EI-MS: m/z (rel. int. %): 387.1(95%), 357 (60%), 339 (50%), 329 (100%), 249 (20%), 193 (18%), (49%), 195 (100%), IR: 3412, 1770, 1590, 1515, and 1465 cm⁻¹. ¹H-NMR δ ppm (400 MHz, DMSO), δH 6.58 (s, 1H, 8'-OH), 3.04, 2.95 (d, each 1H, J = 12 Hz, H-7'), δH 2.77 (dd, 1H, J = 12.4, 3.2 Hz, Ha-7), 2.53 (m, 1H, Hb-7), δH 4.00 (dd, 1H, J = 7.2, 6.4 Hz, Ha-9), δH 3.98 (dd, 1H, J = 7.2, 9.2 Hz, Hb-9), 3.72, 3.65 and 3.63 Hz (each s, 3H, 4-OCH₃, 3-OCH₃, 3`-OCH₃), δH 6.85 (d, 1H, J = 1.7 Hz, H-2'), 6.82 (dd, 1H, J = 1.7, 8 Hz, H-6'), 6.80 (d, 1H, J = 8 Hz, H-5'), a singlet signal at δH 6.67 (2H, H-2, H-6). ¹³C-NMR δ ppm (100 MHz, DMSO); δc 178.4 (C-9), 70.1 (C-8'), 55.8 (3-OCH₃), 55.8 (3`-OCH₃), 42.7 (C-8).

**Compound 2**

White amorphous powder (MeOH, 120 mg), M.P: 170-175°C, EI-MS: m/z (rel. int. %): 373.3 (95%), 355.1 (97%), 339 (50%), 327 (100%), 235 (22%), 223 (20%). IR: 3414, 1765, 1589, 1511, and 1458 cm⁻¹. ¹H-NMR δ ppm (400 MHz, DMSO); δH 6.59 (s, 1H, 8'-OH), 3.10, 2.96 (d, each 1H, J = 12 Hz, H-7'), 2.51 (dd, 1H, J = 12.4, 3.2 Hz, H₈-7), 2.51 (m, 1H, H₄-7), at δH 4.1 (dd, 1H, J = 7.2, 6.4 Hz, H₈-9), 3.99 (dd, 1H, J = 7.2, 9.2 Hz, H₄-9), 3.69 and 3.63 Hz (s, 3H, 3-OCH₃, 3`-OCH₃), 6.82 (d, 1H, J = 1.7 Hz, H-2'), 6.8 (dd, 1H, J = 1.7, 8 Hz, H-6'), 6.77 (d, 1H, J = 8 Hz, H-5'), singlet signal at δH 6.68 (2H, H-2, H-6). ¹³C-NMR δ ppm (100 MHz, DMSO); δc 178.4 (C-9'), 70.1 (C-8'), 55.8 (3-OCH₃), 55.8 (3`-OCH₃), 42.7 (C-8).

**Compound 3**

Seven milligrams of pale yellow powder, M.P. 343-346°C, UV λ_max nm (MeOH): 268, 298(sh.), 338; NaOMe: 276, 300(sh.), 348, 392; AlCl₃: 276, 300, 348, 384; AlCl₃/HCl: 278, 298(sh.), 342, 382; NaOAC: 278, 336, 386; NaOAC/H₂BO₃: 276, 298, 338. ¹H-NMR δ ppm (300 MHz, DMSO); δH 7.91 (1H, br.d, H-6'), 6.94 (1H, d, J =9 Hz, H-5'), 6.91 (1H, d, J =9 Hz, H-5'), 6.46 (1H, s, H-3), 6.45 (1H, d, J=2.1 Hz, H-8), 6.18 (1H, d, J= 9 Hz, H-6).

**Compound 4**

Thirty-five milligrams of yellow powder, M.P. 223-225°C, UV λ_max nm (MeOH): 267, 290(sh.), 345; NaOMe: 274, 324 (sh.), 406; AlCl₃: 278, 296(sh.), 346, 420; AlCl₃/HCl: 276, 296(sh.), 358, 390; NaOAC: 276, 320(sh.), 404; NaOAC/H₂BO₃: 276, 294(sh.), 370. ¹H-NMR δ ppm (300
**Table 2.** The effect of EAF and the isolated compounds on rat paw volume in carrageenan-induced rat paw edema model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw volume (ml) 1 h</th>
<th>% Edema inhibition</th>
<th>Paw volume (ml) 2 h</th>
<th>% Edema inhibition</th>
<th>Paw volume (ml) 3 h</th>
<th>% Edema inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.66 ± 0.07</td>
<td>-</td>
<td>0.68 ± 0.05</td>
<td>-</td>
<td>0.65 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.718 ± 0.144</td>
<td>-</td>
<td>0.705 ± 0.10</td>
<td>-</td>
<td>0.814 ± 0.10</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.695 ± 0.04</td>
<td>40.0</td>
<td>0.59 ± 0.08</td>
<td>50.77</td>
<td>0.56 ± 0.04</td>
<td>68.49</td>
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<tr>
<td>EAF (25 mg/kg)</td>
<td>0.711 ± 0.02</td>
<td>11.43</td>
<td>0.65 ± 0.04</td>
<td>23.85</td>
<td>0.65 ± 0.02</td>
<td>43.84</td>
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<tr>
<td>EAF (50 mg/kg)</td>
<td>0.708 ± 0.03</td>
<td>17.14</td>
<td>0.63 ± 0.05</td>
<td>31.54</td>
<td>0.59 ± 0.03</td>
<td>61.19</td>
</tr>
<tr>
<td>C₁ (25 mg/kg)</td>
<td>0.715 ± 0.07</td>
<td>5.71</td>
<td>0.67 ± 0.04</td>
<td>11.54</td>
<td>0.69 ± 0.09</td>
<td>34.70</td>
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<tr>
<td>C₁ (50 mg/kg)</td>
<td>0.713 ± 0.03</td>
<td>8.57</td>
<td>0.64 ± 0.05</td>
<td>25.38</td>
<td>0.6 ± 0.05</td>
<td>50.92</td>
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<td>C₂ (25 mg/kg)</td>
<td>0.703 ± 0.09</td>
<td>25.712</td>
<td>0.63 ± 0.06</td>
<td>32.31</td>
<td>0.63 ± 0.12</td>
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<td>C₂ (50 mg/kg)</td>
<td>0.705 ± 0.03</td>
<td>22.86</td>
<td>0.58 ± 0.04</td>
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<td>0.56 ± 0.04</td>
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<tr>
<td>C₅ (25 mg/kg)</td>
<td>0.713 ± 0.06</td>
<td>8.57</td>
<td>0.66 ± 0.05</td>
<td>18.46</td>
<td>0.71 ± 0.05</td>
<td>26.03</td>
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<tr>
<td>C₅ (50 mg/kg)</td>
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<td>5.71</td>
<td>0.65 ± 0.07</td>
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<tr>
<td>C₇ (25 mg/kg)</td>
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<td>5.71</td>
<td>0.67 ± 0.05</td>
<td>11.54</td>
<td>0.71 ± 0.05</td>
<td>29.22</td>
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<tr>
<td>C₇ (50 mg/kg)</td>
<td>0.713 ± 0.03</td>
<td>8.57</td>
<td>0.66 ± 0.03</td>
<td>16.15</td>
<td>0.67 ± 0.02</td>
<td>40.18</td>
</tr>
</tbody>
</table>

**Compound 5**

Ninety seven milligrams of yellow powder, M.P. 315-317°C, UV λmax nm (MeOH): 256, 301 sh, 372; NaOMe: 247 sh, 330, 406; AlCl₃: 269, 457; AlCl₃/ HCl: 267, 303 sh, 352 sh, 429; NaOAC: 268, 329 sh, 390; NaOAC/ H₂BO₃: 259, 386. ¹H-NMR δ ppm (300 MHz, DMSO) δ₉ 7.37 (1H, br.d, H-6'), 7.32 (1H, br.s, H-2'), 6.91 (1H, d, J = 8.7, H-5'), 6.53 (1H, s, H-3), 6.44 (1H, d, J = 2 Hz, H-8), 6.89 (1H, d, J = 11.2, H-5'), 7.72 (1H, br.s, H-2') and 7.64 (1H, br.d, H-6').

**Compound 6**

Fourteen milligrams of yellow powder, M.P. 255-258°C, Rₜ=0.4 in S₃, it gave +ve Molisch's test indicating its glycosidic nature. UVλmax nm (MeOH): 258, 268sh, 345; NaOMe: 268, 386; AlCl₃: 274, 298sh, 404; AlCl₃/HCl: 268, 296sh, 356sh, 388; NaOAC: 268, 348, 398; NaOAC/H₂BO₃: 266, 370. ¹H-NMR δ ppm (300 MHz, DMSO) aglycone: δ₉ δ 3.86 (1H, br.d, H-6), 7.39 (1H, br.s, H-2'), 6.93 (1H, d, J = 8.7, H-5'), 6.89 (1H, s, H-3), 6.92 (1H, d, J = 2.1 Hz, H-8), 6.77 (1H, d, J = 8 Hz, H-6); Sugar: δ 3.96 (1H, d, J = 10 Hz, H-1'), 3.51 (1H, d, J = 10 Hz, H-4'). ¹³C-NMR δ ppm (75 MHz, DMSO); δ 183.8 (C-4), 166.5 (C-2), 164.5 (C-7), 162.6 (C-5), 158.7 (C-9), 150.9 (C-4'), 146.3 (C-3'), 122.8 (C-1'), 120.3 (C-6'), 116.9 (C-5'), 116.6 (C-2'), 107.1 (C-10), 104.04 (C-3), 101.4(C-1'''), 101.03 (C-6), 95.9 (C-8).

**Compound 7**

210 mg of white powder, M.P. 167-170°C, Rₜ=0.38 in S₃, it gave +ve Molisch’s test indicating its glycosidic nature. EI-MS: m/z (rel. int. %): 549.5 (97%), 387.5 (100%), 357 (60%), 339 (50%), 329 (80%), 249 (20%), 193 (18%); IR: 3414, 1768, 1592, 1512, and 1460 cm⁻¹. ¹HNMR δ ppm (400 MHz, DMSO) aglycone: δ 6.65 (s, 1H, 8'-OH), 3.01, 2.87 (d, each 1H, J = 13.5 Hz, H-7'), 2.83 (dd, 1H, J = 12.4, 3.2 Hz, H-5'), 2.64 (m, 1H, H-7), 3.97 (dd, 1H, J = 8.4, 6.4 Hz, H-9), 3.94 (dd, 1H, J = 8.4, 9.2 Hz, H-9), δ 3.71, 3.69 and 3.63 Hz (each s, 3H, 4-OCH₃, 3-OCH₃, 3'-OCH₃), δ 3.67 (d, 1H, J = 1.7 Hz, H-2'), 6.84 (dd, 1H, J = 1.7, 8 Hz, H-6'), 6.81 (d, 1H, J = 8 Hz, H-5'), δ 6.68 (2H, H-2, H-6), sugar: δ 4.84 (d, 1H, J = 6 Hz). ¹³C-NMR δ ppm (100 MHz, DMSO); δ 177.8 (C-9'), 101.1 (C-1'''), 75.3 (C-8'), 55.6 (4-OCH₃), 55.4 (3-OCH₃), 53.3 (3'-OCH₃), 42.7 (C-8).

As shown in Table 2, intraplanter injection of carrageenan into rats resulted in severe discernible inflammation and significant increase in the mean volume of the challenged paw compared to that of the untreated paws (after 2 to 3 h of carrageenan injection). Pre-treatment of rats with EAF and the major isolated compounds: C₁, C₂, C₅ and C₇, at dose levels of 25 and 50 mg/kg did not significantly inhibit the carrageenan-induced increase in the edema volume of rat paws after 1 and 2 h of carrageenan injection, except for C₂ at 50 mg/kg (56.92% inhibition). Nevertheless, their
Table 3. Effect of oral administration of EAF and the isolated compounds on TNF-α level in carrageenan-induced rat paw edema model.

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α level conc. (pg/ml) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.69 ± 0.63</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>84.90 a ± 3.77</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>12.60 b ± 1.30</td>
</tr>
<tr>
<td>EAF (25 mg/kg)</td>
<td>26.15 ab ± 3.44</td>
</tr>
<tr>
<td>EAF (50 mg/kg)</td>
<td>11.35 b ± 1.44</td>
</tr>
<tr>
<td>C1 (25 mg/kg)</td>
<td>23.44 ab ± 2.86</td>
</tr>
<tr>
<td>C1 (50 mg/kg)</td>
<td>6.98 b ± 1.57</td>
</tr>
<tr>
<td>C2 (25 mg/kg)</td>
<td>22.60 ab ± 3.77</td>
</tr>
<tr>
<td>C2 (50 mg/kg)</td>
<td>6.98 b ± 1.80</td>
</tr>
<tr>
<td>C5 (25 mg/kg)</td>
<td>55.73 ab ± 8.13</td>
</tr>
<tr>
<td>C5 (50 mg/kg)</td>
<td>22.40 ab ± 2.95</td>
</tr>
<tr>
<td>C7 (25 mg/kg)</td>
<td>44.90 ab ± 4.39</td>
</tr>
<tr>
<td>C7 (50 mg/kg)</td>
<td>15.31 ab ± 1.25</td>
</tr>
</tbody>
</table>

Table 4. Effect of oral administration of EAF and the major isolated compounds on IL-1β concentration in carrageenan-induced rat paw edema model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1β concentration (pg/ml) Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.24 ± 2.91</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>118.14 a ± 11.12</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>52.91 b ± 5.2</td>
</tr>
<tr>
<td>EAF (25 mg/kg)</td>
<td>20.2 b ± 1.87</td>
</tr>
<tr>
<td>EAF (50 mg/kg)</td>
<td>6.76 b ± 0.63</td>
</tr>
<tr>
<td>C1 (25 mg/kg)</td>
<td>33.57 b ± 3.08</td>
</tr>
<tr>
<td>C1 (50 mg/kg)</td>
<td>24.84 b ± 2.36</td>
</tr>
<tr>
<td>C2 (25 mg/kg)</td>
<td>77.27 b ± 7.65</td>
</tr>
<tr>
<td>C2 (50 mg/kg)</td>
<td>39.65 b ± 3.87</td>
</tr>
<tr>
<td>C5 (25 mg/kg)</td>
<td>27.97 b ± 2.56</td>
</tr>
<tr>
<td>C5 (50 mg/kg)</td>
<td>9.56 b ± 0.87</td>
</tr>
<tr>
<td>C7 (25 mg/kg)</td>
<td>32.37 b ± 2.89</td>
</tr>
<tr>
<td>C7 (50 mg/kg)</td>
<td>12.76 b ± 1.17</td>
</tr>
</tbody>
</table>

EAF: Ethyl acetate fraction; C1-C7: The major isolated compounds from the ethyl acetate fraction tested at two dose levels (25 and 50 mg); Carrageenan: untreated group. Indomethacin (10 mg/kg): treated group. Data are expressed as mean ± SEM of six rats; n=6. *p<0.001 compared to control group. **p<0.001 compared to carrageenan-treated group.

Inhibitory effect on carrageenan-induced edema was significantly improved after 3 h at the two tested doses (in a dose dependent manner) except for the compounds C5 and C7 exhibited weak activity at the two tested doses as regards to the standard drug. As illustrated in Tables 3 and 4, injection of carrageenan into the rat hind paw induced a significant increase in the hind paw TNF-α and IL-1β concentrations, 3 h after injection, 84.9 and 118.14 pg/ml, respectively, compared to the control (4.69 and 29.24 pg/ml), respectively. Treatment of rats with EAF and the major isolated compounds at both dose levels caused a significant reduction of increased TNF-α generation by carrageenan (P<0.05). The two lignan compounds C1 and C2 showed the highest decrease in the TNF-α level at the two tested doses; 25 and 50 mg/kg by 23 and 6.8 pg/ml, respectively as regards to C1 and 22 and 6.9 pg/ml, respectively as regards to C2. The EAF showed a lower activity than the two compounds at the
two tested doses 25 and 50 mg/kg by decreasing TNF-α level to 26 and 11 pg/ml, respectively. On the other hand, C₅ and C₇ were less active than the EAF and C₁ and C₂ at the two tested doses being more potent at the higher dose (50 mg/kg). A similar pattern of activity was obtained with IL-1β concentration. Although C₁ and C₂ were the most active regarding TNF-α inhibition; this activity was reversed in case of measuring the level of IL-1β. Where C₃ and C₇ showed a decrease in the IL-1β level more than C₁ and C₂; C₅ decreased IL-1β level by 27.97 and 9.76 pg/ml at 25 and 50 mg/kg, respectively, while C₇ caused a decrease by 32.37 and 12.76 pg/ml at 25 and 50 mg/kg, respectively. Also, the EAF was the most active by decreasing IL-1β level to 20.2 and 6.76 pg/ml at 25 and 50 mg/kg, respectively. The increase of both TNF-α and IL-1β levels was also significantly prevented by indomethacin (10 mg/kg, i.p., P<0.05). It is noteworthy to mention that the EAF, C₁, C₅ and C₇ decreased the IL-1β levels more than that of indomethacin at the two tested doses.

Based on the aforementioned results, it was of interest to analyze the EAF using LC/ESI-MS analysis applying "selective ion monitoring (SIM)" technique to identify other compounds, which were not isolated, that might also contribute to the observed bioactivity and identify the major fingerprint ions. Figure 2 showed the ion chromatogram of the separated phenolics from EAF obtained from the LC-MS, their relative abundance as well as their retention times. Only the compounds of interest according to the available literature were selected for further identification (MS²). Table 5 revealed the detection of the phenolic compounds of interest and the results of MS² for each compound.

**DISCUSSION**

Recently, the popularity of remedies consisting of natural products found in foods, roots and herbs has increased in both the domestic and global healthcare markets. The
Table 5. Results of MS² of the identified phenolic compounds from the EAF of the aerial parts of *Trachelospermum jasminoides* (Lindl.)Lem.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>Rt</th>
<th>[M-H]</th>
<th>MS²</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apigenin 7-β-D-glucoside</td>
<td>9.51</td>
<td>431.3</td>
<td>431.3 (100%), 269</td>
<td>C_{21}H_{20}O_{10}</td>
</tr>
<tr>
<td>2</td>
<td>Diadzin</td>
<td>9.81</td>
<td>415.3</td>
<td>415.3 (100%), 253</td>
<td>C_{12}H_{20}O_{10}</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>11.47</td>
<td>301.2</td>
<td>301.2, 155 (100%)</td>
<td>C_{16}H_{10}O_{7}</td>
</tr>
<tr>
<td>4</td>
<td>Luteolin</td>
<td>13.08</td>
<td>285.2</td>
<td>285.2 (100%), 217, 199, 175, 155, 133, 107</td>
<td>C_{15}H_{10}O_{6}</td>
</tr>
<tr>
<td>5</td>
<td>Apigenin</td>
<td>13.91</td>
<td>269.2</td>
<td>269.2 (100%), 151</td>
<td>C_{15}H_{10}O_{5}</td>
</tr>
<tr>
<td>6</td>
<td>Chrysoeriol</td>
<td>14.54</td>
<td>299.2</td>
<td>299.2 (100%), 285</td>
<td>C_{16}H_{12}O_{6}</td>
</tr>
<tr>
<td>7</td>
<td>Arctigenin</td>
<td>15.65</td>
<td>371.1</td>
<td>371.1, 356 (100%), 312, 295, 209</td>
<td>C_{21}H_{24}O_{6}</td>
</tr>
<tr>
<td>8</td>
<td>Matairesinol</td>
<td>15.90</td>
<td>357.1</td>
<td>357.1, 342, 313 (100%), 298, 209</td>
<td>C_{20}H_{22}O_{6}</td>
</tr>
<tr>
<td>9</td>
<td>Trachelogenin</td>
<td>16.06</td>
<td>387.1</td>
<td>387.1, 357, 339, 329 (100%), 249, 193</td>
<td>C_{21}H_{24}O_{7}</td>
</tr>
<tr>
<td>10</td>
<td>Nor-Trachelogenin</td>
<td>16.76</td>
<td>373.3</td>
<td>373.3, 355, 327 (100%), 235, 223</td>
<td>C_{20}H_{22}O_{7}</td>
</tr>
</tbody>
</table>

EAF: Ethyl acetate fraction Rt: Retention time; [M-H] = molecular ion peak; MS² = MS/MS.

Attention of pharmacologist throughout the world has been focused on finding out more safe and potent anti-inflammatory drug. Biological results revealed that, the LD₅₀ of the tested extract was safe and non-toxic (Buck et al., 1976). The EAF of the aerial parts was the most bioactive fraction evidenced by its high potency (76.92% relative to indomethacin) at a given dose of 100 mg/kg body weight relative to the other tested extracts and fractions and compared to indomethacin (20 mg/kg body weight). Based on the given results, seven compounds have been isolated from the bioactive EAF; three lignans: trachelogenin (C₁), nor-trachelogenin (C₂) and tracheloside (C₇), in addition to four flavonoids; apigenin (C₃), luteolin (C₄), quercetin (C₅) and luteolin-7-O-β-D-glucopyranoside (C₆). Identification of the isolated compounds was achieved by analysis of their physico-chemical properties and spectral data (1D & 2D NMR) and by comparison with reported data (Tan et al., 2005; Nishibe, 1994).

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation (Vinegar et al., 1969). It is a biphasic response; the first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak within 3 h. The second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. Indomethacin is a cyclooxygenase inhibitor, the EAF and the major isolated tested compounds showed a significant anti-inflammatory activity against carrageenan induced paw edema which is comparable to indomethacin. In that view, it might have the same mode of action to inhibit the cyclooxygenase enzyme (COX) (Kumari et al., 2012). It was also reported that the ethanol extract of *T. jasminoides* L. showed potent inhibitory activities against both COX-1 and PLA₂ (Lai et al., 2003). Moreover, COX-2 inhibitory activity of flavonoids and lignans has been reported (D’Mello et al., 2011; Zhao et al., 2009).

One of the major side effects of indomethacin is that it causes gastric erosion (Maciel et al., 2004; Yoshikawa et al., 1993; whittle, 1977). In our study, the isolated compounds that exhibited anti inflammatory activity by different mechanisms are reported for their gastroprotective and anti ulcer activities; flavonoids display gastroprotective effects acting as anti-secretory, cytoprotective and antioxidant agents. Flavonoids also act in healing of gastric ulcers, that is, they can be new alternatives for suppression or modulation of peptic ulcers associated with *H. pylori* (Farzaei et al., 2013). Other studies (Zayachkivska et al., 2005) reported that plant-originated flavonoid substances are highly gastroprotective due to enhancement of the expression of NOS and release of NO and neuropeptides, such as calcitonin gene related peptide (CGRP) released from sensory afferent nerves increasing gastric micro-circulation. Moreover, it was reported that some lignan derivatives possess anti-ulcerogenic activity (Gurbuz et al., 2004).

Many diseases as arteriosclerosis, chronic hepatitis and pulmonary fibrosis, involve the overproduction of inflammatory mediators (Isomaki and Punnone, 1997; Libby et al., 2002; Tilg et al., 1992; Coker et al., 1998) and by inhibiting their production might serve to prevent or suppress a variety of inflammatory diseases. Pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α, have...
The EAF and the major tested compounds dose-dependently decreased the production of the most prominent pro-inflammatory cytokines; IL-1β and TNF-α. Furthermore, our findings provide evidence of the bioactivity of the EAF and the tested compounds in inflammatory diseases and suggest that they may exert anti-inflammatory effect by inhibiting the pro-inflammatory mediators. Our results are in accordance with the previously reported results for the mechanism of action of trachelogenin, nor-trachelogenin and tracheloside, apigenin, quercetin and luteolin for exerting their anti-inflammatory activities through modulation of the cytokine system (Jia et al., 2014; Seo et al., 2014; Zhu et al., 2013; Harrison and Cooper, 2008; Chen et al., 2004; Ueda, 2004; Xagorari et al., 2001; Habtemariam et al., 2000).

As a result of the renewed focus on natural remedies, efficient identification and analysis of active compounds is a growing area of method development. LC analysis of the EAF resulted in the identification of 10 phenolic compounds. The compounds were identified by their molecular ion peaks in negative mode and by comparing the reported data (Sanchez et al., 2003; Eklund et al., 2008; El-Helal et al., 2010).

The peak number 3, Rt. 11.47 min had absorption maxima at 365 nm, indicating its flavonol nature (Table 5). MS fragmentation ions at m/z 301 in negative representing [M-H], and MS/MS fragmentation matched that of authentic standard quercetin (m/z 179 and 151) (El-Helal et al., 2010). Peaks 4, Rt. 13.08 min and 6, Rt. 14.54 min showed a base peak at m/z 285 and 299, respectively. The MS/MS fragmentation of the compound represented by peak 4 matched that of luteolin (m/z 217, 199, 175, 155, 133, 107). MS/MS of the compound represented by peak 6 showed the base peak at m/z 285 with the loss of 14 amu corresponding to loss of a CH₃ group. This was in accordance with the fragmentation of luteolin-3’-O-methyl ether (chryseriol) (El-Helal et al., 2010). Peak 7, Rt 15.65 min with molecular ion peak at m/z 371 corresponds to [M-H]⁻, MS/MS of the compound showed m/z 356 with the loss of 15 amu corresponding to loss of a methyl group, m/z 312 indicated a loss of 44 amu loss of CO₂ characteristic for the butyrolactone moiety. The loss of an OH group could be indicated from the presence of m/z 295 [312-17]. The presence of a peak 209 indicates the loss 86 amu. This fragmentation was in accordance with that of Arctigenin (Eklund et al., 2008). Peak 8, Rt. 15.9 min with a molecular ion peak m/z 357.1 corresponding to [M-H] showed the same fragmentation pattern as that of Arctigenin except for the lack of a methyl group. This is in accordance with that of matairesinol (Eklund et al., 2008). Peak 9, Rt. 16.06 min and molecular ion m/z 387.1 [M-H]⁻, its MS/MS pattern showed m/z 357 with the loss of 30 amu, corresponding to the loss of 2 methyls, m/z 339 corresponds to loss of H₂O from 357, 329 (100%), 249, 193. This fragmentation is in accordance with that of trachelogenin (Eklund et al., 2008). Peak 10, Rt. 16.76 min showed a molecular ion peak at m/z 373.3 corresponding to [M-H] with the same fragmentation pattern as trachelogenin devoid of a methyl group. This was in accordance with that of Nor-trachelogenin (Eklund et al., 2008).

By the aid of LC-ESI/MS analysis, the picture has been completed confirming the presence of these phenolic compounds which were isolated from the EAF in addition to other compounds.

**Conclusion**

Applying bio-guided fractionation, seven compounds in the bioactive EAF of the aerial parts of *T. jasminoides* cultivated in Egypt were isolated; three lignans and four flavonoids. The bioactive fraction together with the major isolated compounds; trachelogenin, nor-trachelogenin, tracheloside and quercetin revealed significant anti-inflammatory effect by inhibiting pro-inflammatory cytokines. Therefore, an extract that could be used as an anti-inflammatory remedy that may serve as model for anti-inflammatory drug development was proposed. Another important point is that the potent anti-inflammatory activity together with the anti ulcerogenic
effect of the phenolic compounds from *Trachelospermum jasminoides* should be further evaluated to develop safe agents to introduce in modern therapy.

**Conflict of interest**

The authors declare no conflict of interest.

**REFERENCES**


