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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

LC-MS-based metabolomic profiling of *Lepidium coronopus* water extract, anti-inflammatory and analgesic activities, and chemosystematic significance

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Abstract

The water extract of *Lepidium coronopus* (L.); Al-Shehbaz (syn. *Coronopus squamatus* (Forrsk.) Asch); (LCWE) was subjected to phytochemical investigation using HPLC-ESI-MS analysis. Thirteen flavonoid derivatives were detected. Nine kaempferol glycoside and acyl derivatives were identified, in addition to two quercetin and two isorhamnetin glycosides. Among the identified compounds, **8** and **12** (kaempferol-di-*O*-glucoside-sinapoyl acetate isomers) are newly identified natural products and were firstly identified in the current study using LC-MS technique. LCWE was evaluated for its anti-inflammatory potential in vivo. It showed significant inhibition of the carrageenan induced hind rat paw edema, showing potencies 78.5%, 78.5%, and 89.3% at 100, 200, and 300 mg/kg, respectively. Meanwhile, the effects of LCWE on PGE_2, TNF-α, and MPO production in the inflamed paw exudate were measured. Central and peripheral analgesic activities were evaluated by hot plate and writhing techniques. LCWE protected mice against acetic acid-induced writhing by 28.2%, 37.0%, and 54.2% at 100, 200, and 300 mg/kg. LCWE peripheral analgesia was stronger than central effect. LCWE also inhibited RANKL stimulated TRAP activity in RAW264 cells completely at 50 and 20 µg/mL without any significant cytotoxicity to RAW264 macrophages. The metabolomic profile of LCWE explained its biological activities. Furthermore, the identified flavonoid constituents have strong chemosystematic significance confirming the change of nomenclature from the genus *Coronopus* to the genus *Lepidium*.

Keywords LC-ESI-MS, *Lepidium coronopus* · Anti-inflammatory · Analgesic · Acylated flavonoids · Chemosystematics

Introduction

The genus *Lepidium* L. is the second largest genus after *Draba* L. in family *Brassicaceae* and consisting of approximately 231 species (Warwick et al. 2006). *Lepidium* is a cosmopolitan genus and distributed worldwide in temperate and subtropical regions, it is the most natural and readily distinguished of all genera of family *Brassicaceae* (*Cruciferae*) (338 genera) (Warwick et al. 2006). Several *Lepidium* species are edible such as *Lepidium draba* (hoary cress), *Lepidium meyenii* (maca), *Lepidium latifolium* (Dittander), *Lepidium sativum* (cress) (Facciola 1990), and *Lepidium ruderale* (roadside pepperweed) (Kunkel 1984). *Lepidium coronopus* is called "Harra" among farmers in Nile valley and Delta, this name reflects its acrid and pungent taste in Arabic. Leaves may be consumed raw or cooked (Tanaka 1976), it was also reported to be used with other boiled vegetables in traditional vegetable mixtures in Italy (Guarrera and Savo 2016). Due to its acrid

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taste, it requires long boiling to become more agreeable (Kunkel 1984). Moreover, the cooked root of *L. coronopus* is traditionally used to cure pains of the back and knees by the residents of Jerusalem (Lev 2007). Meanwhile, inflammation is a very common sign of many diseases and it can turn chronic such as osteoarthritis and asthma. In chronic inflammation, cell composition can change resulting in serious complications such as tumor formation (Lu et al. 2006). In this study, based on its traditional uses, we aim to investigate the anti-inflammatory and analgesic activities of *L. coronopus*; to evaluate its use as functional food or a component of nutraceutical preparations and to add economic incentives to this plant which have long been regarded as an agricultural weed. On the other hand, recent molecular phylogenetic studies resulted in many taxonomic changes that have been released for the *Brassicaceae* family (Warwick and Al-Shehbaz 2006). Among these changes, some genera whose reduction to synonymy is widely accepted as the genus *Coronopus* (now *Lepidium*). *Coronopus* Zinn., is among one of the most closely-related genera of *Lepidium* (Al-Shehbaz et al. 2006). Recently, all *Coronopus* species are synonymy of *Lepidium*, except one species; *Coronopus squamatus* (Forssk.) Asch., which have accepted name in *Lepidium* (*Lepidium coronopus* (L.) Al-Shehbaz) (Radulovic et al. 2008). This exception was originally described by Forsskål and Niebuhr (1775), who published the earliest generic name of *Coronopus* in fact *Lepidium*. The Flora of Egypt (Boulos 1999) and other sources such as (Greuter et al. 1986) have given the level of accepted species to *C. squamatus*. These sources also describe the genus *Coronopus* as consisting of approximately 10 species around the world (Radulovic et al. 2008) and as three species growing wild in Egypt as weed of cultivation (Boulos 1999). The flavonoid constituents of *L. coronopus*, previously reported as *C. squamatus*, have been investigated through our research group and six compounds were reported from aqueous alcoholic extract (Marzouk et al. 2010). Therefore, the similarities of the flavonoid composition of *L. coronopus* with that of other *Lepidium* provides a chemotaxonomic clue to change the nomenclature of the Egyptian plant from the genus *Coronopus* to *Lepidium*. For this reason, we carried out LC-ESI-MS analysis of LCWE to compare the profile of its flavonoid glycosides with plants from both *Lepidium* and *Coronopus* genera for chemosystematic purpose.

Experimental

Materials

Plant material

The whole plant of *L. coronopus* was collected from Giza fields at Abo Mosalem village, along the canals on April 2014, during the fruiting stage of the plant. Voucher specimen (No. M/S 7820) was deposited in the herbarium of National Research Centre.

Extraction

The air-dried whole plant of *L. coronopus* was boiled with water to imitate its status of consumption as food. The solvent was evaporated under reduced pressure at 50 °C. An aliquot of the dried aqueous extract (20 mg) was dissolved in 20 mL HPLC grade solvent mixture of acetonitrile/methanol/water (1:1:2; *v*/*v*/*v*), filtered using membrane disc filter (0.45 µm), and then it was subjected to LC-MS analysis. The remaining amount of LCWE was kept refrigerated (4 °C) in tightly closed container until use for bioassay.

Animals

Mature male Wistar rats and Albino mice (150–200 g and 27–30 g, respectively) were utilized. Animals were procured from The Animal House Colony at the National Research Centre (NRC), Egypt. All animals were housed in hygienic cages in well-ventilated rooms with exhaust fans; received standard pellet diet and water were provided ad libitum. Animals were allowed to adapt to the laboratory environment for 1 week before experimentation. All animal procedures were performed after approval by the National Research Centre Medical Ethics Committee (16156) and in accordance with the recommendations of the proper care and use of laboratory animals.

Chemicals and kits

Carrageenan was obtained from Sigma Aldrich, Germany. Prostaglandin E_2 (PGE_2) and myeloperoxidase (MPO) assay ELISA kits were purchased from Kamiya Biomedical Company, USA. Assay kit for tumor necrosis factor alpha (TNF-α) was obtained from Abcam Company, USA.

Carrageenan induced inflammation in rats

The anti-inflammatory testing was performed in 25 male rats ($n = 5$), according to the previously described method of El-Desoky et al. (2018) and Ammar et al. (2013, 2016). Induction of paw edema was done in rats by subcutaneous (s.c.) injection of 0.1 mL of 1% (w/v) carrageenan in distilled water in the sub-plantar region of their left hind paws. A group of rats was left without any treatment but given a respective volume of distilled water, and was kept as control. LCWE was administered at doses of (100, 200, and 300 mg/kg, p.o.). Indomethacin (20 mg/kg, p.o.) was used as a reference drug. The paw volumes of rats were measured using plethysmometer (Ugo Basile, Italy), before and after

injection of 1% carrageenan at different time intervals (1, 2, and 3 h). Edema rate and inhibition rate of each group were calculated at the above-mentioned time intervals according to El-Desoky et al. (2018) as follows:

$$\text{Edema rate}(\%) = (\text{Vt} - \text{Vo})/\text{Vo}$$

$$\text{Inhibition rate}(\%) = (\text{Ec} - \text{Et})/\text{Ec}$$

where Vo is the volume before carrageenan injection (mL), Vt is the volume at *t* hour after carrageenan injection (mL), Ec is the edema rate of control group, Et is the edema rate of treated group.

Animals were sacrificed 3 h after carrageenan injection and left hind paws were cut. Saline containing 10 µM indomethacin (0.1 mL) was injected to prevent further PG production. The paws were lacerated with a scalpel, suspended off the bottom of polypropylene tubes to facilitate drainage of the inflammatory exudates. Paw exudate samples were centrifuged at 1800*g* for 15 min (Smith et al. 1998). PGE_2, TNF-α, and MPO were quantified in the collected exudates using commercial (ELISA) kits, according to the manufacturer manual.

The total amount of PGE_2 expressed in the edematous fluid was calculated as follows:

$$PGE_2(\text{ng/paw}) = [\text{ng } PGE_2 \text{in sample} \times \text{paw edema vol. (mL)}]/\text{sample vol. } (= 0.1\text{ml}).$$

Peripheral analgesic activity in mice (writhing test)

Acetic acid-induced writhing assay was done as described by Abdel-Rahman et al. (2015), by an intraperitoneal injection of acetic acid (0.7% aqueous solution) in a dose of 10 mL/kg b.wt, 30 min before drug administration. The number of writhes per animal was recorded for 20 min after acetic acid injection. Percent protection was calculated based on the following ratio:

$$\text{Protection}\% = (\text{Control mean} - \text{Treated mean})/\text{Control mean} \times 100$$

Twenty-five mice were divided into 5 groups of 5 animals each. Group 1 was kept as normal control. LCWE was administered at doses of 100, 200, and 300 mg/kg, p.o. to mice of groups 2, 3, and 4, respectively. Mice of group 5 (reference group) were orally treated with acetyl salicylic acid in a dose of 150 mg/kg b.wt.

Central analgesic activity in rats (hot plate test)

The experiment was carried out as described by Turner (1965) using hot-plate apparatus. The plate temperature was maintained at 53 ± 0.5 °C. Twenty-five male rats were divided into 5 groups of 5 animals each. The reaction time of the rat to the thermal stimulus was the time interval between placing the animal in the hot plate and when it licked its hind paw or jumped. Reaction time was measured prior to extract and drug treatment (0 min). The reaction time was again measured at 30 min and repeated at 60 and 90 min post-treatment. Group 1 was kept as normal control. LCWE was administered at doses of 100, 200, and 300 mg/kg, p.o. to rats of groups 2, 3, and 4, respectively. Rats of group 5 (reference group) were orally treated with acetyl salicylic acid in a dose of 150 mg/kg b.wt. Response latency or reaction time was the time between placing the animal on the plate and its reaction. The cut-off time of 60 set was used to avoid tissue damage (Abdel-Rahman et al. 2015). Latency was converted to percent of maximum possible effect (%MPE) according to the following formula:

$$\%\ \text{MPE} = (\text{Post drug latency} - \text{pre drug latency}/\text{cut} - \text{off latency} - \text{pre drug latency}) \times 100$$

TRAP activity

The murine RAW264 cell line was obtained from the RIKEN Cell Bank (Tsukuba) and maintained in MEMα medium (SIGMA) containing 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific) and 1× penicillin/streptomycin (Wako) under a humidified atmosphere containing 5% CO_2 at 37 °C. In the TRAP assay, RAW264 cells were seeded on a 96-well plate (1000 cells/well), cultured for 1 day, and were then treated with sRANKL (a soluble form of RANKL; Santa Cruz Biotechnology; 50 ng/mL) and samples (10 µg/mL; DMSO, control) for 4 days. The cells were then washed with phosphate-buffered saline (PBS) and lysed with TRAP buffer (50 mM sodium tartrate, 50 mM sodium acetate, 150 mM KCl, 0.1% Triton X-100, 1 mM sodium ascorbate, and 0.1 mM $FeCl_3$, pH 5.7; 100 µL/well) on ice for 10 min. The resulting cell extract (20 µL) was added to 100 µL of TRAP buffer containing 2.5 mM *p*-nitrophenyl phosphate (Thermo Fisher Scientific) and incubated at 37 °C for 4 h. To stop the reaction, 50 µL of 0.9 M NaOH was added to the reaction mixture and the reaction product (*p*-nitrophenolate) was measured as the absorbance at 405 nm. The TRAP activity was expressed as the percent of RANKL-treated group.

Acid hydrolysis and paper chromatography

100 mg of the aqueous extract was hydrolyzed with 10 mL HCl 2 N at 100 °C for 2 h. The acidic solution was fractioned with 10 mL ethyl acetate after cooling. Ethyl acetate layer was dried with anhydrous Na_2SO_4 then evaporated. The hydrolyzed extract was subjected to one dimension

Table 1 Peak assignment, molecular weight (MW), molecular ion $[M-H]^-$, mass ion fragments, and tentative identification of compounds detected in aqueous extract of *L. coronopus* by LC-ESI (−ve)-MS

Peak	t_R (min)	MW	$[M-H]^-$	*m/z* fragments	Tentative identification
1	19.46	310	309	247, 191, 176	Unknown
2	20.63	772	771	609, 285	Kaempferol-di-*O*-glucoside-*O*-glucoside
3	21.63	788	787	625, 463,301	Quercetin-tri-*O*-glucoside
4	22.96	772	771	60,94,47,285	Kaempferol-tri-*O*-glucoside
5	25.55	626	625	463, 301	Quercetin- di-*O*-glucoside
6	27.81	610	609	447, 285	Kaempferol-di-*O*-glucoside
7	28.39	640	639	477, 315	Isorhamentin-di-*O*-glucoside
8	34.57	858	857	609, 285, 533, 223, 207, 192	Kaempferol-di-*O*-glucoside-sinapoyl acetate
9	35.65	964	963	609, 285	Kaempferol-*O*-hydroxyferuloylglucoside-di-*O*-glucoside
10	35.9	978	977	815, 609, 285, 223	Kaempferol-*O*-sinapoyldiglucoside-*O*-glucoside
11	36.91	948	947	623, 609, 285	Kaempferol-*O*-feruloylglucoside-di-*O*-glucoside
12	38.41	858	857	609, 533, 285, 223, 207, 192	Kaempferol-di-*O*-glucoside-sinapoyl acetate
13	39.58	448	447	285	Kaempferol-7-*O*-glucoside[a]
14	40.08	478	477	315	Isorhamentin-7-*O*-glucoside[a]
15	46.09	452	451	223, 145, 117	Unknown

[a]Marzouk et al. (2010)

paper chromatography (PC) Whatman No. 1 (Whatman Ltd., Maidstone, Kent, England) using solvent systems; 50% AcOH (H_2O: AcOH, 1:1) and BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer) to detect the aglycones. Also, the aqueous layer was carefully neutralized, then subjected to PC investigation using BBPW (Benzene: *n*-BuOH: pyridine: H_2O; 1:5:3:3; upper layer) to detect the sugars (Mabry et al. 1970). Flavonoid aglycones (Fluka AG, Buchs SG; Switzerland) and sugar samples (E. Merck, Darmstadt, Germany) were used as authentic references.

LC-ESI-MS analysis

LC-ESI-MS analysis: HPLC (Waters Alliance 2695) and MS spectrometry (Waters 3100). The mobile phase was freshly prepared by filtering through membrane disc filter (0.45 µm) then degassed by sonication. For gradient elution, the mobile phase consists of solvent A (0.1% formic acid (FA) in H_2O) and solvent B (0.1% FA in CH_3CN/MeOH (1:1; *v*/*v*)). The linear gradient profile was as follows: 95% A (5 min), 95–90% A (10 min), 90–50% A (55 min), 50–95% A (65 min), and 95% A (70 min). The injection volume was 10 µL. The flow rate (0.6 mL/min) was split 1:1 before the MS interface with negative ion mode parameters (source temperature 150 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, cone voltage 50 eV, capillary voltage 3 kV, and desolvation gas flow 600 L/h). Spectra were recorded in the ESI negative mode between 50 and 1000 *m/z*. The peaks and spectra were processed using the Maslynx 4.1 software. Chemical compounds were tentatively identified by comparing their mass fragmentation pattern with literature (Table 1).

Statistical analysis

Results were analyzed using a one-way ANOVA followed by a Dunnett's multiple comparison test using SPSS statistics 17.0 (Chicago, USA), and expressed as means ± standard error.

Results and discussion

LC-ESI-MS analysis

As shown in Table 1 (Fig. 1), compound **2** showed a deprotonated molecular ion peak at *m/z* 771. The fragment at *m/z* 609 is due to the loss of mono hexose unite $[M-H-162]^-$, and that at *m/z* 285 $[M-H-162-324]^-$, of kaempferol aglycone, is due to the loss of two other hexose units attached to the same hydroxyl group. Therefore, compound **2** was tentatively assigned as kaempferol-di-*O*-glucoside-*O*-glucoside (Francescato et al. 2013). Compound **3** showed a molecular ion peak at *m/z* 787. The fragment ions at *m/z*: 625 $[M-H-162]^-$, 463 $[M-H-2^- \times 162]^-$, 301$[M-H-3 \times 162]^-$ are corresponding to the successive loss of three hexoside moieties attached to

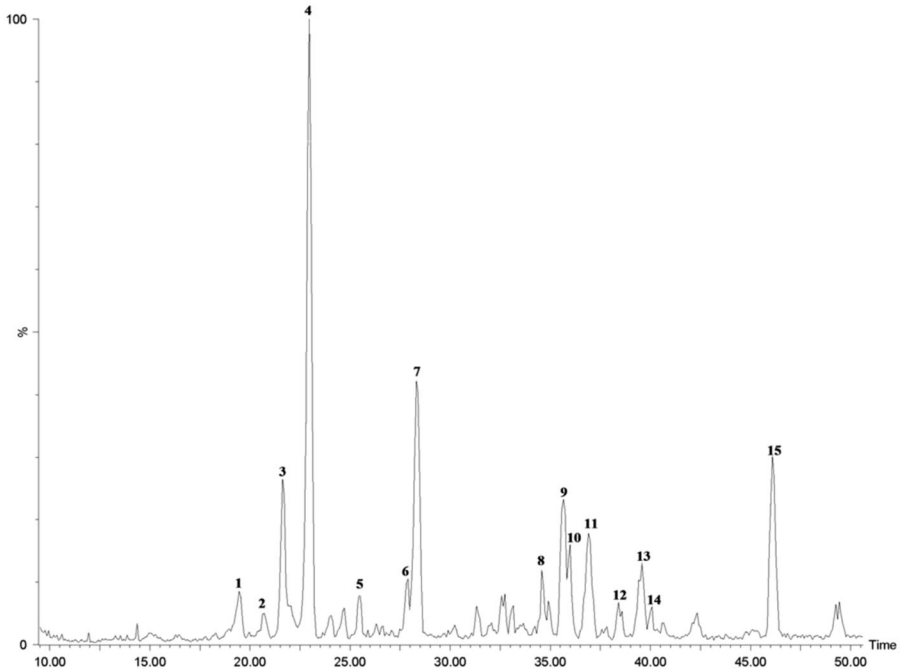

Fig. 1 LC-ESI-MS chromatogram of phenolic compounds in *Lepidium coronopus* water extract

different hydroxyl groups of quercetin nucleus. Thus, compound **3** was tentatively identified to be quercetin-tri-*O*-glucoside (Apel et al. 2017). By the same manner, the deprotonated molecular ion peak of compound **4** appeared at *m/z* 771, and the successive loss of three hexoside moieties showed fragment ions at *m/z*: 609, 447, 285. Therefore, compound **4** was tentatively identified to be kaempferol-tri-*O*-glucoside. Also, compound **6** showed two fragments at *m/z*: 447 and 285 due to the loss of two hexoside moieties, so **6** was identified as kaempferol-di-*O*-glucoside (El-Hagrassy et al. 2017). The quasi-molecular ion of compound **5** appeared at *m/z* 625. The fragment ion at *m/z* 463 is due to the loss of hexose unit; while that at *m/z* 301, quercetin aglycone, is due to the loss of other hexose moiety. So compound **5** was identified as quercetin-di-*O*-glucoside (Francescato et al. 2013). Two isorhamnetin derivatives were identified; one of them attached to two hexose units and appeared at *m/z* 639; while the other is attached to one hexose unit and appeared at *m/z* 477. Therefore, compounds **7** and **14** were tentatively identified to be the di-*O*-glucoside and the -*O*- glucoside derivatives of isorhamnetin (El-Hagrassy et al. 2017). Sinapic acid derivatives are characteristic constituents in the family *Brassicaceae* (Nićiforović et al. 2014). It shows a wide range of biological activities including anti-inflammatory, antimicrobial, anticancer, anti-anxiety, and antioxidant activities (Nićiforović et al. 2014). From the plant under investigation, three sinapic acid derivatives were identified (compounds **8**, **10**, and **12**). Compounds **8** and **12** are monoacylated kaempferol-di-*O*-glucosides, they showed the same quasi-molecular ion peak at *m/z* 857 ($[M-H]^-$). The fragment ion at *m/z* 609 is due to the loss of sinapoyl acetate ($[M-H-248]^-$); while the aglycone fragment of kaempferol at *m/z* 285 ($[M-H-248-2\times162]^-$) is due to the loss of two hexose units after cleavage of the sinapoyl acetate group. The appearance of fragment ion at *m/z* 533 ($[M-H-2\times162]^-$) confirmed the attachment of the two hexose units and sinapoyl acetate group to two different hydroxyl groups. Sinapic acid fragment ion appeared at *m/z* 223. Therefore, compounds **8** and **12** are two isomers of kaempferol-di-*O*- glucoside-sinapoyl acetate. To the best of our knowledge, compounds **8** and **12** are newly identified natural products. Further studies are needed for their isolation and full characterization. Compound **9** is a monoacylated kaempferol-*O*-glucoside-di-*O*-glucoside. Its pseudo-molecular ion peak appeared at *m/z* 963 and fragmented further to *m/z* 609 ($[M-H-162-192]^-$) by losing 354 amu (the loss of hexose and hydroxyferulic acid moieties); which leads to the tentative identification of **9** as kaempferol-*O*-hydroxyferuloylglucoside-di-*O*-glucoside (Harbaum et al. 2007). On the other hand, compound **10** is a monoacylated kaempferol-di-*O*-glucoside-*O*-glucoside. Its molecular anion peak appeared at *m/z* 977 ($[M-H]^-$). The fragment ion at *m/z* 815 is caused by the loss of hexose unit ($[M-H-162]^-$); while that at *m/z* 609 is due to the loss of sinapoyl moiety ($[M-H-162-206]^-$) followed by losing of other two hexose units leads to the formation of kaempferol fragment ion at *m/z* 285 ($[M-H-162-206-2\times162]^-$). This order of fragmentation confirmed that, the sinapoyldihexoside group is connected to the same hydroxyl group; while the third hexose unite is attached to another hydroxyl group. Therefore,

compound **10** was tentatively identified as kaempferol-*O*-sinapoyldiglucoside-*O*-glucoside (Harbaum et al. 2007). Also, compound **11** is a monoacylated kaempferol-tri-*O*-hexoside. Its deprotonated molecular ion appeared at *m/z* 947 ([M-H]$^-$). The fragment ion at *m/z* 609 ([M-H-338]$^-$) is due to the loss of feruloylhexoside moiety and that at *m/z* 285 ([M-H-338-2 × 162]$^-$) is due to the loss of two hexoside units, attached to the same hydroxyl group, after cleavage of feruloylhexoside moiety. Appearance of fragment ion at *m/z* 623 ([M-H-2 × 162]$^-$) confirmed the attachment of the two hexose units and feruloylhexoside group to two different hydroxyl groups. Based on these evidences, compound **11** was tentatively identified to be kaempferol-feruloylglucoside-di-*O*-glucoside (Harbaum et al. 2007). Compound **13** showed a pseudo molecular ion peak at *m/z* 447 ([M-H]$^-$). It produced a fragment ion at *m/z* 285 due to the loss of hexoside moiety [M-H-162]$^-$. Consequently, compound **13** was identified as kaempferol-7-*O*-glucoside.

Acid hydrolysis

The PC of the ethyl acetate extract gave three yellow spots under UV, indicating the flavonol nucleolus, and has R_f as kaempferol, quercetin, and isorhamnetin aglycones. Glucose, arabinose, and rhamnose were detected as sugar moieties in the aqueous extract. Complete hydrolysis indicated that all glycosides were in *O*-glycoside form.

Inhibition of carrageenan-induced paw edema

The anti-inflammatory effect of LCWE was tested in carrageenan-induced rat paw edema model. A biphasic inflammatory response resulted following sub-planter injection of carrageenan in rats. The first phase is associated with the release of several mediators such as histamine, serotonin, and TNF-α. However, the second phase is correlated with the enhancement of prostaglandins oxygen derived free radicals and inducible cyclooxygenase release. This second phase is sensitive to most clinically effective anti-inflammatory and anti-edematous agents (Calixto et al. 2004). Marked increase in the paw volume was recorded 3 h after carrageenan injection (56.1% compared to baseline) (Fig. 2).

LCWE (100, 200, and 300 mg/kg) resulted in significant inhibition of rat paw edema, showing potencies of 78.5%, 78.5%, and 89.3%, respectively after 1 h of edema induction (Supplementary Data, Table S1). Pre-treatment with LCWE (100, 200, and 300 mg/kg) resulted in significant suppression of carrageenan-induced edema by about 39.2%, 40.7%, and 42.4%, respectively after 3 h of edema induction (Supplementary Data, Table S1). Likewise, indomethacin caused significant inhibition of edema volume by about 64.9%. As a response to inflammation the

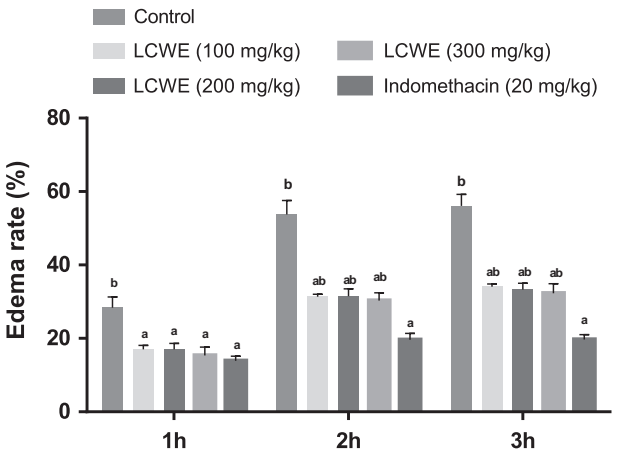

Fig. 2 Acute inflammatory activity of *L. coronopus* water extract (LCWE), $n = 5$. "a"—$P < 0.05$: statistically significant from control (LSD followed by Dunnett's test). "b"—$P < 0.05$: statistically significant from indomethacin (LSD followed by Dunnett's test)

chemical mediator prostaglandin E_2, a product of arachidonic acid metabolism increased, along with the increase in tumor necrosis factor alpha (TNF-α). TNF-α is the proinflammatory cytokine that has many biological effects, as increasing the expression of other cytokines, chemokines, and adhesion molecules, as well as activation of neutrophils (Wright et al. 2010). Activation of neutrophils is known to generate and release a number of tissue-damaging factors including reactive oxygen species such as superoxide anion, as well as enzymes such as myeloperoxidase (MPO) and proteases. Myeloperoxidase reacts rapidly with nitric oxide and peroxynitrite so that at sites of inflammation myeloperoxidase has a major impact on oxidative injury caused by neutrophils (Kettle and Winterbourn 1997). As shown in Fig. 3, the increase in paw volume after carrageenan injection was accompanied with an elevation in PGE_2 production in the paw exudate. Administration of LCWE resulted in significant decrease in PGE_2 level. Moreover, pretreatment of rats with LCWE (100, 200, 300 mg/kg), 1 h before carrageenan resulted in significant decrease in TNF-α and MPO levels, compared to control group (Fig. 3).

Effect on acetic acid induced writhing

Examination of the peripheral anti-nociceptive effect of LCWE was performed using acetic acid-induced abdominal constrictions in mice (writhing effect). This animal model is an efficient analgesic model for screening agents against visceral inflammatory pain. It is a visceral pain model widely used to screen potential analgesic substances (Abdel-Rahman et al. 2015). The obtained data, shown in Fig. 4, revealed that LCWE possesses peripheral analgesic effect at the three examined dose levels as compared with the control group. The protection % arranged was 28.2, 37.0, 54.2, and

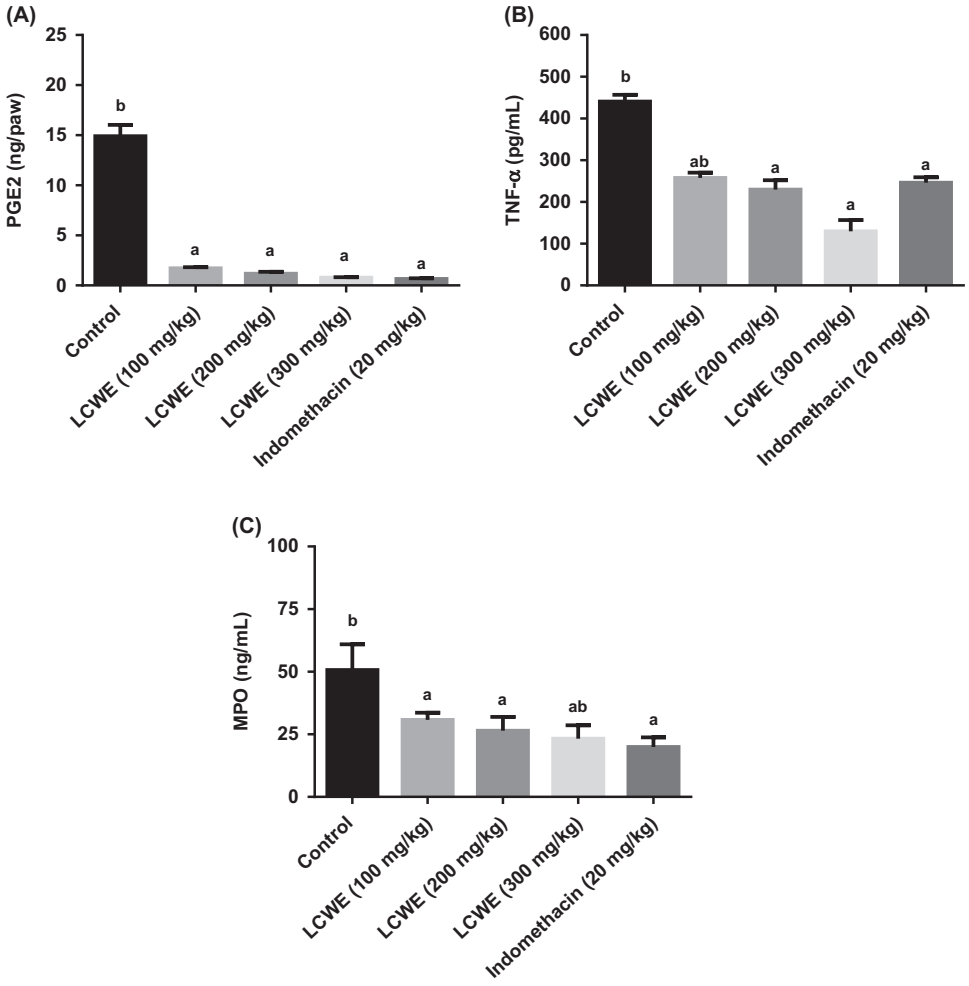

Fig. 3 Effect of *L. coronopus* water extract (LCWE) on **a** PGE2, **b** TNF-α, and **c** MPO levels in paw exudate, $n = 5$. "a"—$P < 0.05$: statistically significant from control (LSD followed by Dunnett's test). "b"—$P < 0.05$: statistically significant from indomethacin (LSD followed by Dunnett's test)

51.2, for *L. coronopus* (100, 200, 300 mg/kg) and acetyl salicylic acid (150 mg/kg), respectively.

Effect on hot-plate latency

The hot plate test is specified to test the central anti-nociception and to measure complex responses to inflammation and nociception (Santa-Cecília et al. 2011). In the current study, lower doses of LCWE failed to increase the reaction time after 30 and 30 min of administration compared to the control group. However, hot plate latency increased significantly after 90 min in animals treated with LCWE at the three examined dose levels, with significant increase in %MPE in a dose-dependent manner (Fig. 5) (Table S4). Acetyl salicylic acid (150 mg/kg) was the most potent between all treated-groups.

TRAP activity

LCWE was tested at three dose levels, 50, 20, and 10 µg/mL. The extract was found to completely inhibit TRAP activity in RANKL stimulated RAW264 macrophages at 50 and 20 µg/mL and inhibited 72% of TRAP activity at 10 µg/mL.

Metabolomic profiling of the extract under investigation could clearly explain the results of biological investigation. The high contents of phenolic constituents in the form of flavonols and flavonol tri, di, and monoglycosides, together with their acylated derivatives are known to have potent anti-inflammatory effect via slowing down the metabolism of arachidonic acid and histamine release. Flavonoids can inhibit lipoxygenase, cyclooxygenase, and phospholipase A2 and consequently can inhibit macro and micro trauma induced lysosomal enzyme secretion and arachidonic acid release from membranes (Gabor 1986). On the other hand polar metabolites identified in the extracts are not likely to cross the blood brain barrier, which explains why *L. coronopus* aqueous extract revealed limited central analgesic effect compared to its peripheral analgesia (Craig and Stitzel 2004). Meanwhile, the symptoms of acetic acid induced writhing such as extension of the forelimbs and body elongation are believed to be influenced by arachidonic acid

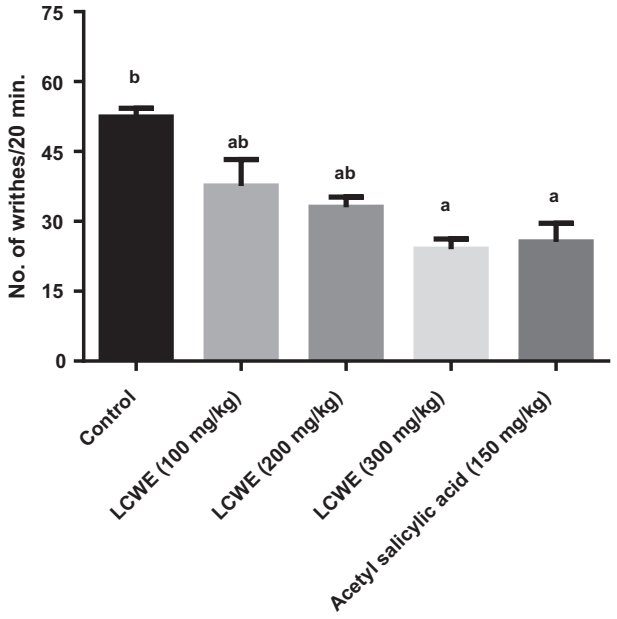

Fig. 4 Peripheral analgesic activity of *L. coronopus* water extract (LCWE). Values represent the mean ± S.E. of five mice for each group. "a"—$P < 0.05$: statistically significant from control (LSD followed by Dunnett's test). "b"—$P < 0.05$: statistically significant from acetyl salicylic acid (LSD followed by Dunnett's test)

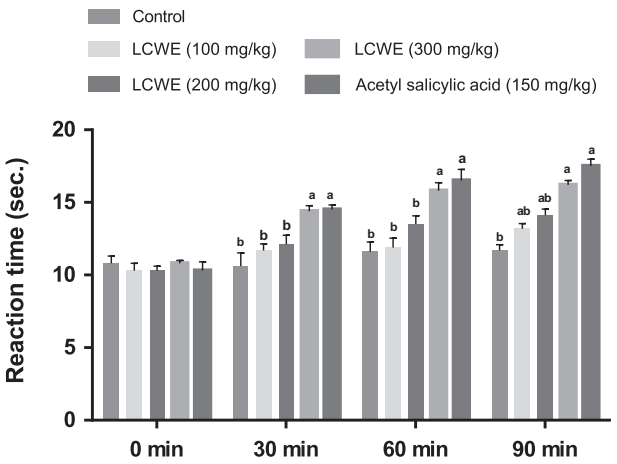

Fig. 5 Central analgesic activity of *L. coronopus* water extract (LCWE). Values represent the mean ± S.E. of five rats for each group. "a"—$P < 0.05$: statistically significant from control (LSD followed by Dunnett's test). "b"—$P < 0.05$: statistically significant from acetyl salicylic acid (LSD followed by Dunnett's test). MPE refers to the maximal possible effect

pathway (Ribeiro et al. 2000), which can be significantly inhibited by flavonoids (Gabor 1986). Several reports on the inhibitory effect of flavonols on RANKL induced osteoclastogenesis have been released supporting the results of TRAP activity inhibition of LCWE in RANKL induced RAW264 macrophages (Tsuji et al. 2009; Pang et al. 2006).

Chemosystematic significance

The flavonoid profile of *L. coronopus* (*C. squamatus*) revealed the presence of mono-, di-, and tri-*O*-glucosides of flavonols (kaempferol, quercetin, and/or isorhamnetin) as well as acylated tri-*O*-glucosides of kaempferol. In addition to being detected in *L. coronopus* (*C. squamatus*), they have been isolated from different *Lepidium* species. The mono-*O*-glucoside flavonols; 7-*O*-glucoside of kaempferol and isorhamnetin (peaks **13** and **14**) were previously reported from *L. sativum* (Agarwal and Verma 2011) as well as the di-*O*-glucosides of kaempferol and quercetin (peaks **5** and **6**) were also detected in the same species as glucosylation of OH groups at position **3** and **7** (Agarwal and Verma 2011). Additionally, the tri-*O*-glucoside flavonols (peaks **2**–**4** and **7**) have been reported in three *Lepidium* species; 3-*O*-sophoroside-7-*O*-glucoside of kaempferol and isorhamnetin were isolated from the seeds of *Lepidium apetalum* (Han et al. 2015; Shi et al. 2015) and the aerial part of *L. latifolium* (Xiang et al. 2018) and also 3-*O*-(2″,6″ di-*O*-glucoside)-glucoside of kaempferol and quercetin from *L. apetalum* seeds (Han et al. 2015; Shi et al. 2015). Kaempferol acyl glucosides (peaks **10** and **11**) were also reported in *L. latifolium* (Xiang et al. 2018) and represented as 3-*O*-sinapoyl or feruloyl sophoroside-7-*O*-glucoside derivative. Consequently, the presence of these flavonoids seems to suggest a very close relationship between *Coronopus* and *Lepidium*, added more support for the recent change to the genus classification. *Coronopus didymus* which belongs to the genus *Coronopus* has been investigated from a flavonoid point of view, where chrysoeriol, chrysoeriol 4′-*O*-glucoside and chrysoeriol 4′-*O*-(6″-acetyl)-glucoside were detected (Prabhakar et al. 2002). The presence of such compounds (methylated flavones) seems to differentiate the flavonoid profile of *L. coronopus* from that of the genus *Coronopus*. The similarity between *L. coronopus* (*C. squamatus*) and other *Lepidium* genera are not only from the flavonoids point of view, but also through the glucosinolate contents (Radulovic et al. 2008). Morphologically, they are also very similar, where their vegetative parts with simple trichomes and pinnatifid leaves and their fruit structure with thickened, reticulate, rugose, to tuberculate, indehiscent fruit valves and they both have two ovules per ovary (Al-Shehbaz et al. 2002; Radulovic et al. 2008).

Conclusions

In conclusion, our study demonstrated the anti-inflammatory effect of *L. coronopus*. The inhibition of pro-inflammatory enzyme, myeloperoxidase, activity and the decrease in TNF-α and PGE_2 concentrations appears to be a justification for its action. *L. coronopus* water extract

exhibited analgesic activities, both peripherally and centrally, however, the peripheral effect was more prominent than central analgesic effect. Furthermore, the identified flavonoid constituents were found to explain the studied biological effects and had strong chemosystematic significance confirming the change of nomenclature from the genus *Coronopus* to the genus *Lepidium*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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