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Research Article

UPLC-ESI/MS/MS Profiling and Anti-Inflammatory Activity of *Gleditsia caspica*

Hagar Ashraf [a], Ashaimaa Y. Moussa [a], Amany A. Seleem[b], Omayma A. Eldahshan[a,c], Abdel Nasser B. Singab [*a,c]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*
[b]*Department of Toxicology and Narcotics National Research Center, Cairo, Egypt*
[c]*Drug Discovery Center Research and Development, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*

ABSTRACT

The Caspian Locust is the common name of *Gleditsia caspica, a tree* cultivated in the southern and western Caspian Sea in Russia and Iran. Herein, a qualitative characterization of the chemical constituents of *Gleditsia caspica* leaves extract using ultra-performance liquid chromatography coupled to electrospray ionization tandem mass fragmentation (UPLC-ESI/MS/MS) analysis was performed revealing the presence of nine compounds: six flavonoids, one triterpenoid, one phytosterol, and one long chain ester. Six compounds were newly reported from *Gleditsia caspica* and two for the first time from genus *Gleditsia*. Moreover, the *in-vivo* anti-inflammatory activities of two different doses; 50 and 100 mg/kg of the aqueous-alcoholic extract of *Gleditsia caspica* leaves were evaluated in the carrageenan-induced paw edema model. The percentage of edema inhibition was calculated after 1, 2, 3, and 4 h of the administration showing a significant reduction in edema thickness in a dose and time-dependent manner compared to the control drug, indomethacin. These results put forward *Gleditsia caspica* extract as a potential natural anti-inflammatory agent with minimal side effects.

Keywords: *Gleditsia caspica; flavonoids; Fabaceae; glycosides; inflammation.*

***Correspondence** | Abdel Nasser B. Singab; Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt. Email: dean@pharma.asu.edu.eg

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1. INTRODUCTION

Gleditsia caspica Desf. (Caspian Locust), subfamily Caesalpinioideae, is one of fourteen species belonging to family Fabaceae. Genus *Gleditsia* has been widely used in traditional medicine since 2000 years ago **[1]**. The majority of *Gleditsia* species diversity is found in Eastern Asia, Southern Caucasus, North, and South America **[2]** where they have been used in treating productive cough, asthma, suppuration, headache, apoplexy, carbuncle, swelling, and scabies. While phytochemical studies revealed the presence of sterols **[3]**, triterpenes **[3-5]**, phenolics **[6, 7]**, alkaloids **[8, 9]** and saponins **[10, 11]** as the most characteristic constituents of the fruits and thorns**,** *Gleditsia triacanthos* was the only species whose leaves were studied and revealed the presence of flavonoids and the alkaloid triacanthine **[8, 12, 13]**.

The fruit extract of *Gleditsia caspica* revealed the presence of eleven novel saponins (caspicaoside A-K) and three oleanane type triterpenoidal saponins (caspicaoside L-N) **[14-16]**. Saponins isolated from *Gleditsia caspica* fruit extract (caspicaoside A-D) and (caspicaoside L-N) were reported to exhibit various cytotoxic activities on different cancer cell lines **[14]**; moreover, they were proved to possess anti-mutagenic activity **[17]**. Accordingly, there is a vacancy of information regarding the chemical constituents of the *Gleditsia* leaves and their biological activity. Genus *Gleditsia* possessed a variety of biological activities such as anti-inflammatory **[18]**, cytotoxic **[19-21]**, antiallergic **[22, 23]**, antihyperlipidemic **[24]**, analgesic **[25]**, antimicrobial **[6, 26],** antioxidant **[8, 12, 13]** and antimutagenic activities **[3, 17]**, yet few studies tested the *in-vivo* bioactivity against inflammation **[18, 22, 27, 28]**.

More than 70% of the anti-inflammatory drugs are synthetic despite having potentially adverse effects **[29]**. Non-steroidal anti-inflammatory drugs (NSAIDS) as salicylates, pyrazolones, indole derivatives, and quinolone derivatives are the most widely used anti-inflammatory molecules **[30]**. Recently, many NSAIDs have been used as topical preparations in the treatment of some inflammatory disorders. Photoallergic contact dermatitis has been reported from propionic acid derivatives and photosensitivity from piroxicam **[31, 32]**. Moreover, contact urticaria has been reported from oxyphenbutazone **[33]**. On the other hand, topical anti-inflammatory synthetic corticosteroids possess a variety of adverse effects such as skin atrophy and telangiectasia, that's why long-term use of topical steroids is limited **[34]**. Topical synthetic retinoids are a class of anti-inflammatory drugs used mainly in acne treatment and can lead to toxic contact dermatitis **[35]**.

Medicinal herbs have great potential to act as therapeutic agents against inflammation mainly because of their antioxidant and cytoprotective properties **[36-38]**. Biological investigations of *Gleditsia* species revealed that both thorns and fruits extracts of *Gleditsia sinensis* possessed anti-inflammatory activities with different mechanisms of actions **[18, 22, 27, 28]**. Herein, we aimed to characterize for the first time the chemical profile of *Gleditsia caspica* leaves using UPLC-ESI/MS/MS; moreover, the *in-vivo* anti-inflammatory activity in carrageenan-induced paw edema model was probed to present a potential new natural topical anti-inflammatory agent with the least side effects.

1. MATERIALS AND METHODS

2.1 Plant material

Fresh leaves of *Gleditsia caspica* (Fabaceae) were collected from the plant growing in El-Orman botanical garden, Ministry of Agriculture, Giza, in December (2016). They were kindly authenticated by Ms. Therese Labib, a botanical specialist and consultant at Orman and Qubba Botanical Gardens. A voucher specimen of the authenticated plant was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University under the registration code (PHG-P-GC-212).

2.2 Extract preparation

The intact air-dried plant material was ground into powder, macerated in distilled methanol 70% for 3 days, and filtered. The process was repeated four times before the hydro-alcoholic extract was concentrated using a rotary evaporator. The filtrate was completely evaporated *in vacuo* at 45 ^{o}C till dryness to yield a solid dark brown residue (250 g).

2.3 UPLC-ESI/MS/MS profiling

Ultra-performance liquid chromatographic (UPLC) analysis coupled with an ESI mass spectrometer detector allowed simultaneous isolation of the compounds and determination of the isolated peaks molecular weights. Samples (100 µg/mL) were dissolved in high-performance liquid chromatography (HPLC) analytical grade methanol, filtered using a membrane disc filter (0.2 µm), and subjected to UPLC-ESI-MS/MS analysis. Samples (10 µL) were injected into the UPLC instrument, Waters® equipped with a reversed-phase C-18 column (ACQUITY UPLC - BEH C18 1.7 µm particle size -2.1×50 mm). The mobile phase was filtered using a 0.2 µm membrane disc filter and sonicated before injection. The Elution flow rate was adjusted to 0.2 mL/min; the gradient was composed of two eluents: water acidified with 0.1% formic acid and methanol acidified with 0.1% formic acid. Both negative and positive ion modes were used on a triquadrupole (XEVO) mass spectrometry, Waters® Corporation, Milford, MA01757 U.S.A. 30 eV cone voltage, 3 kV capillary voltage at 150 °C, 440 °C desolvation temperature were applied. Detection of mass spectra was in the ESI range *m/z* 100–1000 using the software Maslynx 4.1 and tentatively identified by comparing the retention time (Rt) peaks in the mass spectrum and their fragmentation pattern with reported data.

2.4. *In-vivo* anti-inflammatory evaluation

The anti-inflammatory activity of the hydro-alcoholic extract of *Gleditsia caspica* leaves was evaluated in the carrageenan-induced paw edema model according to the method described before **[39]**. Twenty four adult male albino rats, weighing between 130-150 g were maintained under normal laboratory conditions at room temperature of 25-30 °C, 60-65% relative humidity, and provided with standard diet and water. The study was conducted according to regulations of the Institutional Animal Ethical Committee of the National Research Centre which gave its consent following the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (registration no. 90123) **[40]**. Four groups, each of six animals, were treated as follow: group I: received 1 mL of saline serving as control, group II: received 50 mg/kg of the *Gleditsia caspica* extract, group III: received 100 mg/kg of the *Gleditsia caspica* extract, and group IV: received 20 mg/kg of the reference indomethacin. One hour later, paw swelling was induced by sub plantar injection of 0.1 mL of 1% carrageenan solution in saline into the right hind paw of all groups. The edema thickness was calculated by measuring the hind paw thickness immediately before sub plantar injection and at 1, 2, 3, and 4 h after drug administration. The percentage of inhibitory activity at each time point was calculated according to the following equation **[41]**.

Percent of inhibition= X-Y/X ×100

Where "X" represents the animal's paw diameter in the control group and "Y" represents the diameter of the animal's paw administered with the tested materials.

2.5. Statistical analysis

Anti-inflammatory data were expressed as mean ± standard deviation and statistical analysis was performed using the one-way analysis of variance (ANOVA), followed by posthoc Tukey's test for multiple comparisons. Values with $P<0.05$ were considered statistically significant.

3. RESULTS

3.1 Metabolites profiling of *Gleditsia caspica* extract

This study aimed to characterize the Phyto-constituents **(Table 1)** within the bioactive *Gleditsia caspica* extract using the UPLC-ESI/MS/MS approach, which revealed the presence of six flavonoids, one triterpenoid, one phytosterol, and one long chain ester.

Table 1. Peaks assignments using UPLC-ESI/MS/MS of metabolites in *Gleditsia caspica* extract

Peak no.	Identified Compound	Retention time (min)	Molecular ion (m/z)	Fragment ions (m/z)	Reference
GC1	Kaempferol -O-rhamnosyl hexoside -O- rhamnoside	7.27	$[M-H]^-$ at *m/z* 739	448, 431, 285, 119	[42]
GC2	Eriodictyol-7-O- hexoside	8.53	$[M-H]^-$ at *m/z* 449 $[M+H]^+$ at *m/z* 451	287, 289, 151, 153	[43]
GC3	Apigenin C-hexoside	9.28	$[M-H]^-$ at *m/z* 431 $[M+H]^+$ at *m/z* 433	313, 311, 283	[44]
GC4	Naringenin-7-O- hexoside-deoxy-hexoside	9.86	$[M-H]^-$ at *m/z* 579	271, 151	[45, 46]
GC5	Naringenin-*7-O*-hexoside	25.32	$[M+Na]^+$ at *m/z* 457 $[M+H]^+$ at *m/z* 435	163	[47]
GC6	Dihydrokeampferol	11.04	$[M-H]^-$ at *m/z* 287	151, 135	[48]
GC7	Lupeol	25.86	$[M+H]^+$ at *m/z* 427	392, 109, 94, 91, 57	[49]
GC8	Stigmasterol	26.6	$[M+H]^+$ of *m/z* 413 $[M-H_2O+H]^+$ at *m/z* 395	269, 215	[50, 51]
GC9	2(R)-26-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]oxy}t-2,3-dihydroxypropyl ester	27.83	$[M-H]^-$ of *m/z* 621	501	[20]

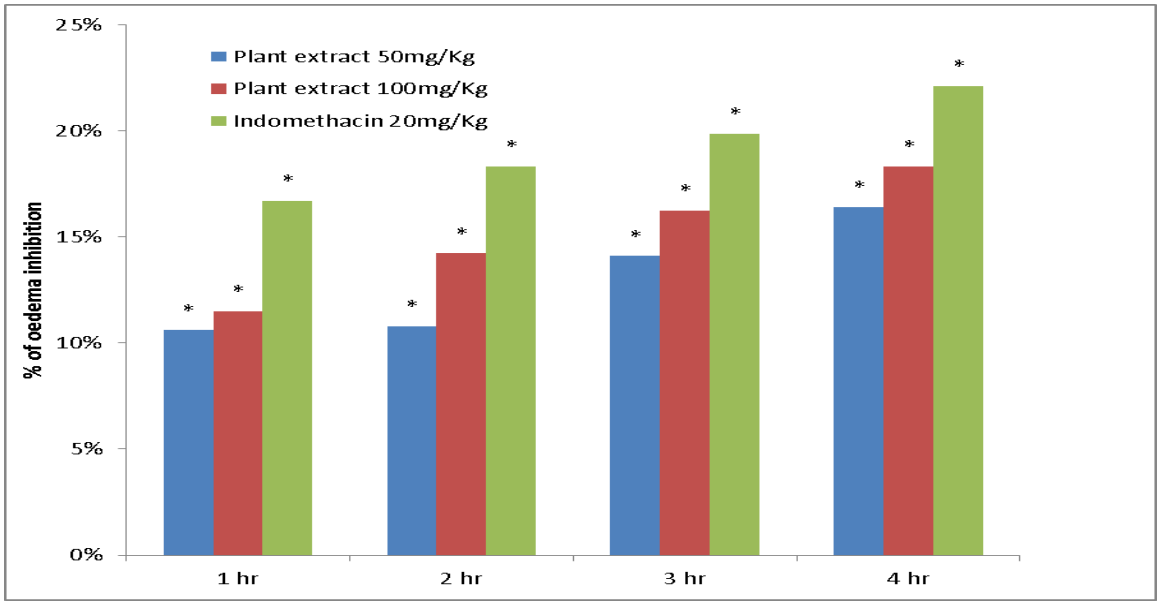

Fig. 1. Effect of *Gleditsia caspica* extract on rat skin edema thickness in two different doses after 1, 2, 3, and 4 h of the samples administration. *The difference is significant at $P<0.05$

Table 2. Acute *in-vivo* anti-inflammatory activity of plant extracts and indomethacin (reference drug) in carrageenan-induced paw edema model

Group	Zero time	Paw diameter (mm) 1 h	2 h	3 h	4 h
		Edema inhibition (%)			
Control (Carrageenan +1 mL saline)	3.46±0.03	4.61±0.09*	4.64±0.09*	4.68±0.1*	4.75±0.1*
Plant extract 50 mg/kg	3.46±0.07	4.12±0.1* 10.6%	4.14±0.1* 10.78%	4.02±0.1* 14.1%	3.97±0.08* 16.42%
Plant extract 100 mg/kg	3.47±0.03	4.08±0.1* 11.5%	3.98±0.06* 14.22%	3.92±0.1* 16.24%	3.88±0.07* 18.32%
Indomethacin 20 mg/kg	3.51±0.05	3.84±0.03* 16.7%	3.79±0.03* 18.32%	3.75±0.04* 19.87%	3.70±0.06* 22.11%

Data represented as mean ± SD of six independent experiments. Analysis was done using the one way analysis of variance (ANOVA), followed by post-hoc Tukey's test. *The difference is significant at $P<0.05$.

3.2. Evaluation of the *in-vivo* anti-inflammatory activities

One of the most frequently used methods to evaluate the anti-inflammatory effects of natural products is Carrageenan-induced paw edema. Carrageenan was injected and induced inflammation in rat hind paw and increased paw diameter to 4.75 mm. *Gleditsia caspica* extract reduced hind paw inflammation time and dose-dependently. Results were summarized in (**table 2 and Fig. 1)**.

3. DISCUSSION

In this study, the characterization of the Phyto-constituents of *Gleditsia caspica* extract using the UPLC-ESI-LC/MS/MS approach was carried out to identify the chemical components within the plant associated with this biological activity. Kaempferol -O-rhamnosyl hexoside -O-rhamnoside; reported for the first time from genus *Gleditsia* was detected in peak (GC1) having its molecular ion $[M-H]^-$ at *m/z* 739 with fragments at *m/z* 448 $[M-291-H]^-$ typical to kaempferol-3-hexoside and *m/z* 431 $[M-308-H]^-$ typical to kaempferol-7-deoxy-hexoside with MS/MS fragment ions at *m/z* 119 produced by the retro-Diels-Alder (RDA) cleavage of the C ring of kaempferol. Such fragments confirmed the presence of kaempferol -O-rhamnosyl hexoside -O- rhamnoside that was previously detected **[42]**. Peak (GC2) represented the flavonoid eriodictyol-7-O-hexoside; previously isolated from the fruits of the same species **[48]**. Its parent ion peak $[M-H]^-$ at *m/z* 449 and $[M+H]^+$ at *m/z* 451 were obvious; additionally, it has two ion peaks at *m/z* 287 and *m/z* 289 correspondings to the aglycone part with a molecular mass of 288 Da with MS/MS fragment ions at *m/z* 151 in the negative mode and m/z 153 in the positive mode corresponding to the characteristic (RDA) cleavage between different chemical bonds of the ring C **[43]**. Peak (GC3) was shown to be apigenin flavone glycoside previously isolated from *Gleditsia triacanthos* as vitexin and iso-vitexin **[8]**. It revealed molecular ion peaks of $[M-H]^-$ at *m/z* 431 and $[M+H]^+$ at *m/z* 433, in which MS/MS spectrum showed two predominant fragments at *m/z* 313

correspondings to the glycosyl ring fracture, *m/z* 283 corresponding to the aglycone part and a characteristic fragment at *m/z* 311 [M-H-120] **[44]**. Peak (GC4) was recognized as naringenin-7-O-glycoside (disaccharide derivative); reported for the first time from genus *Gleditsia* and produced a $[M-H]^-$ at *m/z* 579 typical to disaccharide derivatives of naringenin and the MS/MS spectrum showed two characteristic daughter ion peaks for naringenin at *m/z* 271 and 151 **[45, 46]**. Furthermore, peak (GC6) was characterized as dihydrokeampferol; previously isolated from *Gleditsia sinensis* **[6]** owing to its molecular ion peak $[M-H]^-$ at *m/z* 287 and the fragment ions at *m/z* 151 and *m/z* 135 **[48]**. Lupeol; detected for the first time from genus *Gleditsia* was identified at peak (GC7) that produced a $[M+H]^+$ at *m/z* 427 and was confirmed by the characteristic fragments at *m/z* 392, *m/z* 109, *m/z* 94, *m/z* 91 and *m/z* 57 as reported previously **[49]**. Stigmasterol; previously isolated from *Gleditsia sinensis* was recognized in peak (GC8) by the molecular ion $[M+H]^+$ of *m/z* 413 with $[M-H_2O+H]^+$ at *m/z* 395 **[3]**. Its MS/MS spectrum showed a fragment ion peak at *m/z* 269 that was attributed to the loss of the side chain with two hydrogen atoms from the steroidal nucleus, which is characteristic to steroids with unsaturated side chain and a characteristic fragment at *m/z* 215 **[50, 51]**. Naringenin-*7-O*-hexoside; previously isolated from the fruits of *Gleditsia caspica* **[47]** has been detected in *Gleditsia caspica* leaves extract in peak (GC6) that showed a molecular ion peak of $[M+H]^+$ at *m/z* 435 and $[M+Na]^+$ at *m/z* 457 with MS/MS fragment at *m/z* 163 correspondings to the hexose moiety. A newly isolated long-chain ester; 2(R)-26-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]oxy}t-2,3-dihydroxy propyl ester **[20]** was identified as the first report from this species in peak (GC9) with deprotonated molecule $[M-H]^-$ of *m/z* 621 and MS/MS fragment at *m/z* 501 ($[M-120]^-$) that could arise from the cleavage of one ester bond.

This study was also conducted to evaluate the *in-vivo* anti-inflammatory activity of *Gleditsia caspica* extract. As aforementioned, the carrageenan-induced paw edema model is one of the major models used in the assessment of the anti-inflammatory effects of natural products. After one hour of *Gleditsia caspica* administration, there wasn't a marked reduction in the rat hind paw edema compared to the reference group. During the follow-up period, an increase in the percentage of the edema inhibition was noticed. Paw diameter reduction was increased by increasing the dose from 50 mg/kg to 100 mg/kg, and the effect of the extract at 100 mg/kg dose was comparable to that of the reference drug indomethacin. The reduction in paw diameter recorded 18.32% and 22.11% for the extract (100 mg/kg) and reference drug (indomethacin), respectively, after four hours of carrageenan administration. Accordingly, it could be concluded that the hydro-alcoholic extract of *Gleditsia caspica* exhibited time and dose-dependent anti-inflammatory activity. Flavonoids are key constituents involved in the anti-inflammatory activities within the plants. In this study, six flavonoids were tentatively identified within *Gleditsia caspica* extract. Biological investigations have reported different anti-inflammatory mechanisms of eriodictyol **[52]**, apigenin **[53]**, kaempferol **[54]**, and naringenin **[55]** and their glycosides. It could be concluded that *Gleditsia caspica* extract is a potential bioactive candidate that could be incorporated in topical anti-inflammatory preparations owing to its flavonoids components.

CONCLUSION

UPLC-ESI/MS/MS analysis of *Gleditsia caspica* extract revealed its richness in flavonoids, newly reported compounds from this genus, and from this species. Antiinflammatory assays revealed that the hydro-alcoholic extract

of *Gleditsia caspica* possessed dose and time dependant activity. These results suggested the possible use of *Gleditsia caspica* extract in topical anti-inflammatory preparations with minimal side effects.

DECLARATIONS

Ethics approval and consent to participate

The protocol of the experiment was approved by the Institutional Animal Ethical Committee in the National Research Center (registration no. 90123) **[40]**.

Consent to publish

Not applicable.

Availability of data and materials

All data generated or analyzed during this study were included in the main manuscript.

Competing interests

The authors declare that no competing interests exist.

Funding Statement

No funding source was received.

Authors' contributions

The manuscript was drafted and written by Hagar Ashraf. Dr. Ashaimaa Y. Moussa, Prof. Omayma A. Eldahshan, and Prof. Abdel Nasser B. Singab have provided comments and revised the manuscript. All authors have read and approved the final manuscript.

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LIST OF ABBREVIATIONS

UPLC, Ultra performance liquid chromatography; ESI, Electrospray ionization; MS, Mass spectrometry; eV, Electron volt; kV, Kilovolt; L/h, Liter per hour; Rt, Retention time; ANOVA, Analysis of variance.

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