RESEARCH ARTICLE

Isolation of Novel Lupeol and Acetoside from Iraqi *Campsis grandiflora* Flowers and Evaluation of their Anti-inflammatory Effect on the Animal Model

Sarah S. Hasson[1], Ibrahim S. Abbas[1], Bahir A. R. Mshimesh[2*]

[1]*Department of Pharmacognocy and Medicinal Plants, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq*
[2]*Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq*

Received: 28th September, 2021; Revised: 20th October, 2021; Accepted: 20th November, 2021; Available Online: 25th December, 2021

ABSTRACT

Campsis grandiflora (Bignoniaceae) is a fast-growing deciduous climber, native to central and southern China. The dried flowers have been used as a carminative, blood tonic, and febrifuge in Chinese traditional medicine. This plant has an anti-inflammatory, anti-oxidant, anti-depressant, and anti-bacterial effect, beneficial in stagnant blood and endometriosis. In this study, the detection of lupeol and acetoside was performed using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC); while the isolation done by preparative layer and column chromatography using hexane and butanol extract of Iraqi *C. grandiflora* flowers. Spectral analysis by Fourier-transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance (NMR) was done for the two isolated phytochemicals. Furthermore, the anti-inflammatory activity of total ethanolic extract and one of the isolated compounds (acetoside) was assessed using an egg-albumin induced acute inflammation and formalin-induced subacute and chronic inflammation within mice model, compared with diclofenac. The results showed that the hexane and butanol extract of Iraqi *C. grandiflora* flowers contain lupeol and acetoside, respectively, and from our knowledge, this is the first novel study that suggested the presence of lupeol in the *C. grandiflora* flowers extract. Moreover, the total extract of this plant possesses a moderate anti-inflammatory activity against the acute inflammatory model and an excellent anti-inflammatory activity against the subacute and chronic inflammatory models. In conclusion, the anti-inflammatory effect of Iraqi *C. grandiflora* total extract attributed partly to caffeic acid derivatives, i.e., acetoside, and other phytochemicals such as terpenoids produce an additive or synergistic effect with acetoside.

Keywords: Acetoside, *Campsis grandiflora*, Egg-albumin, Formalin, Lupeol.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.4.35

How to cite this article: Hasson SS, Abbas IS, Mshimesh BAR. Isolation of Novel Lupeol and Acetoside from Iraqi *Campsis grandiflora* Flowers and Evaluation of their Anti-inflammatory Effect on the Animal Model. International Journal of Drug Delivery Technology. 2021;11(4):1329-1335.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Campsis grandiflora (Bignoniaceae) is a fast growing deciduous climber native to central and southern China; its flowers are hermaphrodite, curled, and bright orange-red. This plant blooms for about eight months, between the last week of March and the second week of October.[1] Flowering of *C. grandiflora* plant belongs to the cornucopia pattern, in which a large number of plant flowers are produced in each inflorescence for about several months, and this flowering pattern is considered generalized phenology of Bignoniaceae family.[2]

In Chinese traditional medicine, the dried flowers of this plant, also known as "aborticide" in chinese folk, have been used as a carminative, blood tonic, febrifuge, and depurative diuretic. Rheumatoid pains and menstrual problems exacerbated by blood stagnation, swelling breast after childbirth, rubella, bleeding rectum, and diabetes have been treated with a decoction of these flowers.[3,4] *C. grandiflora* was reported to have several active compounds e.g., polyphenols and terpenes. Apigenin and luteolin belong to the flavonoid type of polyphenols, while caffeic acid, acetoside, and leucosceptoside belong to the non-flavonoid type polyphenols were reported in the plant flowers.[3,5-7] Among the terpenes, oleanolic acid, ursolic acid, Arjunolic acid, Corosolic acid, etc., were reported in the plant flowers.[8] *C. grandiflora* flowers have several pharmacologic activities e.g. cellular protection and anti-oxidant effect,[3] anti-inflammatory effect,[9] anti-depressant effect,[3] with a beneficial role in stagnant blood, and endometriosis conditions.[5]

The objective of this study was detection and isolation of the lupeol and acetoside present in the Iraqi *C. grandiflora* flowers with the evaluation of the anti-inflammatory effect of total extract and isolated acetoside.

**Author for Correspondence: dr.bahirrazzaq@uomustansiriyah.edu.iq*

MATERIALS AND METHODS

Plant Material Collection

Flowers of *C. grandiflora* were collected from Baghdad, hay Aljamea`a in July 2020. A professional taxonomist authenticated the plant material at the College of Sciences, University of Baghdad. Flowers were separated from the rest of the plant and washed thoroughly. It was then dried under shade conditions at room temperature for seven days. After that, the dried flowers were ground in a mechanical grinder and finally stored in a glass container at 4°C.

Chemicals and Reagents

Lupeol and acetoside standards were purchased from Changdu Biopurify; hexane, ethanol, acetone, butanol, and formaldehyde solution 37% were obtained from Sigma Aldirch. Diclofenac sodium injection was purchased from Acino; Ovalbumin(powder) was supplied from Research Product International. Ethyl acetate, methanol, chloroform, and toluene were supplied from Alpha Chemika.

Preparation of Extract for the Detection and Isolation of Phytochemicals

Powdered plant (100 g) was extracted by soxhlet apparatus with *n*-hexane (1200 mL) till exhaustion. The extract was dried using a rotary evaporator, weighted, and labeled as hexane extract (HE). The defatted plant material was further extracted with ethanol 80% (1200 mL). The extract was concentrated using a rotary evaporator and dry at room temperature, weighted, and labeled as ethanol extract (EE). The ethanolic extract was dissolved in (40 mL) of distilled water and partitioned with *n*-butanol (5×100 mL). The upper layer was collected, dried by rotary evaporator, then labeled as Butanol Extract (BE) while the lower layer was discarded.[10]

TLC Analysis

An aliquot of hexane extract dissolved in about 2 mL of hexane then applied on analytical TLC plate against standard lupeol, and developed in the following mobile phases (MP):[11-14]

- MP1: Aceton:hexane (1:3)
- MP2: Hexane:ethyl acetate (7:2)
- MP3: Toluene:chloroform:ethyl acetate (5:4:1)
- MP4: Chloroform:acetone (9:1)

The plates were sprayed with anisaldehyde-sulphuric acid reagent allowed for the detection of distinct spots. Moreover, an aliquot of butanol extract dissolved in 2 mL of absolute methanol, then applied on analytical TLC plate against standard acetoside, and developed in the following mobile phases.[15-18]

- MP5: Methanol :ethyl acetate:water (16.5:100:13.5)
- MP6: Ethyl acetate:water:formic acid: acetic acid (100:18:10:10)
- MP7: Methanol: ethyl acetate:water (17:90:10)
- MP8: Methanol :ethyl acetate:water (15:77: 8)

The detection of separated spots was done under uv light (254, 366 nm).

HPLC Analysis

One milligram from hexane extract and standard lupeol was dissolved separately in HPLC grade methanol (1-mL) using a mobile phase consisting of acetonitrile:methanol (70:30, v/v). The flow rate was 1-mL/min, and the detector was monitored at 210 nm.[19] Moreover, (1 mg) from butanol extract and standard acetoside was dissolved separately in HPLC grade methanol (1-mL) using a mobile phase 20% aqueous phosphoric acid solution (0.1%) and 80% acetonitrile:methanol (2:3, v/v) at 330 nm.[20]

Isolation of Phytochemicals

Hexane extract (1.5 gram) was dissolved in hexane and conducted on preparative layer chromatography (PLC) plates against standard lupeol[21] and developed in MP2 mobile phase.[13] The detection was done by spraying the plates' side with anisaldehyde-sulphuric acid reagent followed by heating. The bands at $R_f = 0.5$ were scraped off and the scrapped silica was then eluted with warmed hexane. The purity of the C1 compound was confirmed by analytical TLC using the MP4 mobile phase. Furthermore, The C2 compound was isolated from butanol extract by column chromatography (CC). The stationary phase used in CC was silica gel mixed with MP7 mobile phase[16] making a slurry. The silica gel slurry was packed in a glass column, then (2.9) gram of butanol extract was dissolved in methanol and gradually added to the slurry. The eluent (MP7) poured continuously on the column, each fraction collected from the column was examined by analytical TLC against standard acetoside using the same mobile phase. Fractions containing C2 spot were combined, dried, dissolved in absolute methanol, and purified by PLC plates using the MP5 mobile phase.[14] The bands at Rf = 0.4 appear under UV light (254,366 nm) were scrapped off, then eluted with methanol. The purity of the C2 was confirmed by analytical TLC using the MP6 mobile phase.

Spectral Analysis of the Isolated Compounds

Each isolated compound was subjected to different spectral analysis, e.g., FTIR and NMR.

The Anti-inflammatory Assay

Preparation of Extract for the Anti-inflammatory Assay

Powdered plant material (100 g) was extracted with 1200 mL of 80% ethanol by soxhlet apparatus. The ethanolic extract was dried by rotary evaporator, weighted, labeled as total extract (TE) and stored at 4°C.[27]

Pilot Study for Dose Determination

Ten male mice were randomly divided into five groups, two animals each

- The first group (negative control): received N/S 10 mL/kg i.p.[22]
- The second group (positive control) : received diclofenac 5 mg/kg in N/S i.p.
- The third group (low dose extract group) : received 100 mg/kg TE in N/S i.p.
- The fourth group (medium dose extract group): received 200 mg/kg TE in N/S i.p.

- The fifth group (High dose extract group): received 300 mg/kg TE in N/S i.p.

Sixteen minutes later, the paw thickness was measured using a digital vernier caliper. Following that, 20 µL of 1% formalin was injected into the plantar area of the right hind paw in order to stimulate inflammation.[23] The paw thickness was measured at 60 minutes interval for 4 hours period,[26] and the ability of the drug to inhibit the formed oedema was represented as a percentage of inhibition of paw oedema, and this was estimated using the formula below: [25]

$$\% \text{ of inhibition} = \left[1 - \frac{V_T}{V_C}\right] \times 100$$

V_T: mean odema of positive control or treatment groups, V_C: mean odema of negative control group

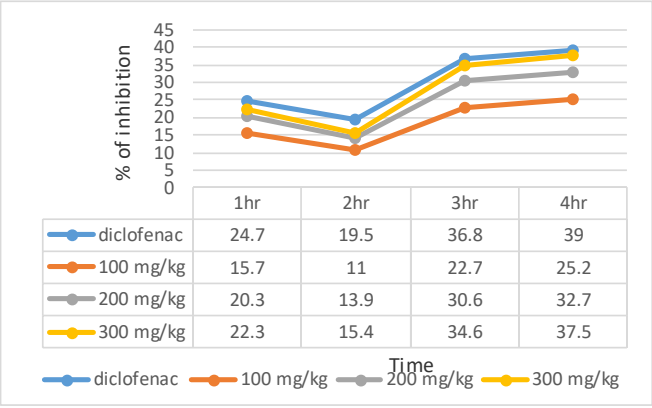
The pilot study results (Figure 1) revealed that the high dose extract group exhibited high anti-inflammatory activity among other extract groups. So 300 mg/kg of total *C. grandiflora* flower extract was considered an effective dose in the main experiment.

Study Design

Thirty-two male mice have been divided into four groups, eight animals each.

- The first group (Negative control): Received N/S 10 mL/kg i.p.[22]
- The second group (Positive control) : Received diclofenac 5 mg/kg dissolved in N/S i.p.[24]
- The third group (Total extract group) : Received 300 mg/kg total extract dissolved in N/S i.p.
- The fourth group (Acetoside group): Received 25 mg/kg of the isolated acetoside in N/S i.p.[26]

Sixteen minutes later, the paw thickness was measured using a digital vernier caliper. The induction of acute inflammation has been done by injecting 0.1 mL of egg albumin (ovalbumin 1% w/v in normal saline[29]) in the plantar surface of the right hind paw. The paw thickness was measured after 1 to 4 hours.[24] The induction of sub-acute and chronic inflammation has been done by injecting 20 µL of formalin[23] (1% v/v in N/S) in the plantar area of the right hind paw. The paw thickness was measured at 1.5, 24, and 48 hours in sub-acute inflammatory model,[28] and measured after six consecutive days in chronic inflammatory model.[29]



	1hr	2hr	3hr	4hr
diclofenac	24.7	19.5	36.8	39
100 mg/kg	15.7	11	22.7	25.2
200 mg/kg	20.3	13.9	30.6	32.7
300 mg/kg	22.3	15.4	34.6	37.5

Figure 1: Pilot study for Iraqi *C.grandiflora* dose determination.

Statistical Analysis

Analysis of data was done using the Statistical Packages for Social Sciences- version 16 (SPSS-16.0). Moreover, the anti-inflammatory assay's significance in different means was established using a t-test and one-way analysis of variance (ANOVA) followed by the post-hoc Tukey test.

RESULTS AND DISCUSSION

TLC Results

This is the first study that suggested the presence of lupeol in the hexane extract of *C. grandiflora* flowers. This result is a consequence of matching the R_f values of both lupeol and compound 1(C1) spots in four mobile phases. Phenyl propanoid such as acetoside was previously isolated from *C. grandiflora* flower extract; and the results of TLC analysis was indicated the existence of acetoside in the Iraqi *C. grandiflora* butanol extract, as shown in Table 1 and Figure 2.

HPLC Results

The results revealed the presence of lupeol in hexane extract in which the peak with a retention time 4.34 minutes in the hexane extract, was matched the peak of standard lupeol with a retention time of 4.68 minutes. Moreover, the peak at

Table 1: TLC results

Mobile phase	*The R_f value of C1 in hexane extract*	*The R_f value of standard lupeol*
MP1	0.54	0.56
MP2	0.48	0.49
MP3	0.61	0.62
MP4	0.81	0.81
Mobile phase	The R_f value of C2 in butanol extract	The R_f value of standard acetoside
MP5	0.50	0.53
MP6	0.75	0.77
MP7	0.55	0.55
MP8	0.83	0.83

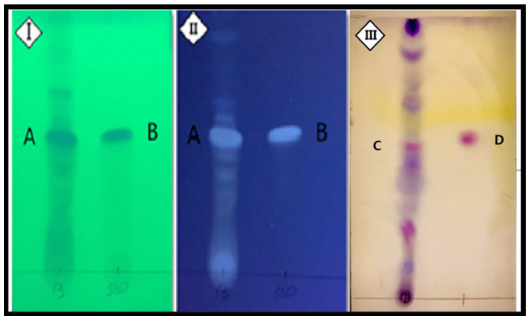


Figure 2: Analytical TLC for Iraqi *C.grandiflora* flowers extract: (I) TLC for butanol extract and acetoside standard under 254 nm, (II) TLC for butanol extract and acetoside standard under 366 nm, (III) TLC for the hexane extract and lupeol standard (A) C2 spot, (B) standard acetoside spot, (C) C1 spot, (D) standard lupeol spot

7.404 minutes in the HPLC chromatogram of butanol extract matched the peak of standard acetoside at 7.04 minutes and this revealed the existence of acetoside in butanol extract of Iraqi *C. grandiflora* flowers.

Isolation of Phytochemicals

Hexane extract of *C. grandiflora* flowers was applied on PLC plates to give 40 mg (2.66 % w/w) of C1. Furthermore, butanol extract was subjected to silica gel CC, affording forty fractions. Fractions that contained the C2 spots, i.e.fraction 5 to 22, were combined, dried, and purified on PLC plates to give 100 mg of C2 (3.44 % w/w), as shown in Figure 3.

Spectral Analysis of the Isolated Compounds

Fourier Transform Infrared Spectrometry (FTIR) Results

The wavenumbers of the isolated compound(C1)showed a characteristic broad peak at 3360 to 3443 cm^{-1} that proved the O-H group with peaks at 3014 and 1637 cm^{-1}, which established the presence of sp^2 hybridized carbons. Meanwhile, The wavenumbers of the isolated compound (C2) showed a characteristic broad peak at 3271 to 3562 cm^{-1} that approved the O-H groups with a peak at 1699 cm^{-1} which confirmed the presence of the carbonyl group. Moreover, the aromatic carbons were confirmed by a stretching vibration at 1626 and 1498 cm^{-1}. These results were verified by comparing the wavenumbers with standard lupeol and acetoside and with the reported literature,[30,31] as in Figure 4.

Nuclear Magnatic Resonance (NMR) Results

In the ^{1}HNMR spectrum of isolated C1, the chemical shifts started at 0.66 ppm, corresponding to the hydrogens of saturated hydrocarbons. The allylic protons were next, with a chemical shift of 1.88 ppm. At 3.12, a proton adjacent to the inductive effect can be seen. Finally, the vinylic protons were shown at 4.55 and 4.69 ppm, as shown in Figure (5-I). The chemical shifts in the ^{13}CNMR spectrum of the isolated C1 started at a range of 14.2 to 54.75 ppm which reflected carbons of saturated hydrocarbons. Then, a chemical shift of 76.66 ppm was represented the carbon that adjacent to the hydroxyl group. Finally, the chemical shifts at 109.53 and 150.06 ppm were attributed to the vinylic carbons of the C1, as shown in Figure (5-II).

In the ^{1}HNMR spectrum of isolated C2, the chemical shifts started at 0.98 ppm, representing the hydrogens of saturated hydrocarbons. The allylic protons were observed at 2.52 ppm, and protons adjacent to the inductive effect can be seen at a range of 3.62 to 5.54 ppm. Moreover, the vinylic protons were shown at 6.24 and 7.50 ppm, and aromatic protons appeared at a chemical shift of 6.5 to 7.04 ppm. Finally, at 9.2 and 9.61 ppm, the phenolic hydroxyl protons of the C2 were observed, as shown in Figure 6-I.

While in the ^{13}CNMR spectrum of this compound (Figure 6-II), the chemical shifts started at 18.03 ppm, reflecting carbons of saturated hydrocarbons. Then, a chemical shift of 60.60 to 113.4 ppm has represented the carbons adjacent to a heteroatom. The vinylic carbons of the C2 were observed at 114.58 and

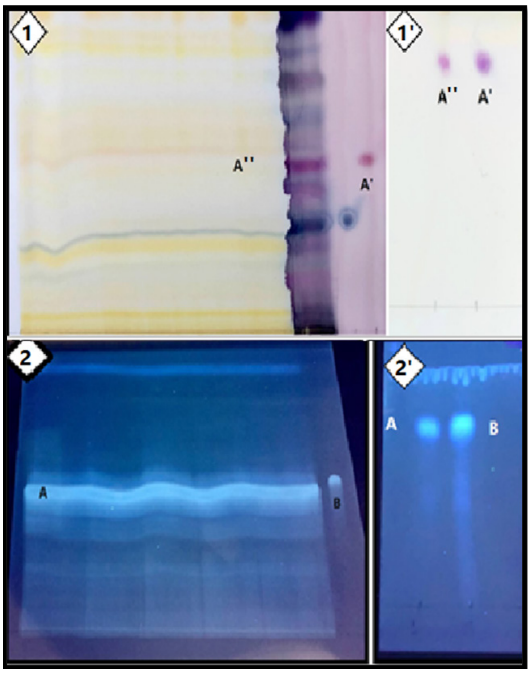


Figure 3: Isolation and purity confirmation of C1 and C2 compounds: (1) PLC plate for isolation of C1, (1') TLC plate for purity confirmation of C1, (2) PLC plate for isolation of C2, (2') TLC plate for purity confirmation of C2 (A'')C1 spot, (A') standard lupeol spot, (A) C2 spot, (B) standard acetoside spot.

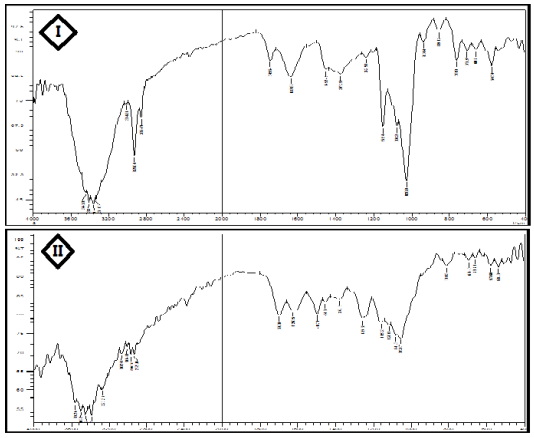


Figure 4: The IR spectrum of the isolated compounds: (I) C1 compound, (II) C2 compound.

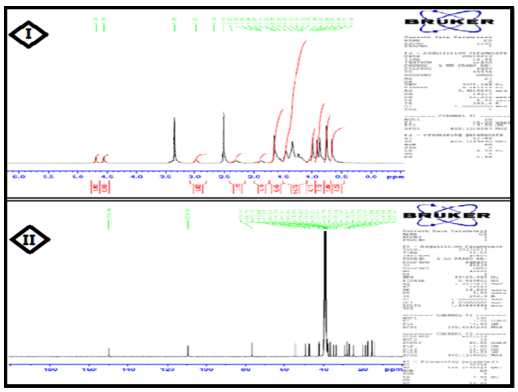


Figure 5: The NMR spectrum of C1: (I) ^{1}HNMR, (II) ^{13}CNMR

Table 2: The effect of different treatment groups on egg albumin-induced acute inflammation

	Mean change in paw thickness (mm) % of inhibition							
Treatment groups	*1 hour*	*2 hours*	*3 hours*	*4 hours*	*1 hours*	*2 hours*	*3 hours*	*4 hours*
Negative control (N/S group)	3.26 ± 0.075	3.48 ± 0.039	3.53 ±0.041	3.63 ±0.037	-	-	-	-
Positive control (Diclofenac 5 mg/kg)	2.60 ± 0.050^{*a}	2.53 ± 0.059^{*a}	2.45 ± 0.094^{*a}	2.32 ± 0.079^{*a}	20.3	27.3	30.3	36.1
Total extract group (300 mg/kg)	2.90 ± 0.026^{*b}	2.81 ± 0.078^{*b}	2.75 ± 0.073^{*b}	2.65 ± 0.050^{*b}	11.1	19.4	22.3	27.2
Acetoside group (25 mg/kg)	3.05 ± 0.084 ^{b}	2.92 ± 0.095^{*b}	2.88 ± 0.076^{*b}	2.76 ± 0.062^{*b}	6.5	16.5	18.4	24.1

Each value represents the mean ± SEM for 8 animals; *:indicate significant difference($p \leq 0.05$) as compared with negative control;non-identical superscript letters (a,b) indicate statistically significant difference ($p \leq 0.05$) among groups.

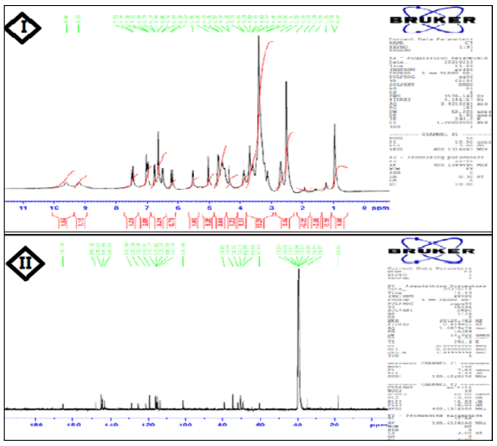


Figure 6: The NMR spectrum of C2: (I)^{1}HNMR, (II) ^{13}CNMR

143.42 ppm since the latter was adjacent to the inductive effect. Finally, aromatic carbons and the carbonyl group can be seen at 115.3 to 148.32 and 165.56 ppm, respectively. These results were confirmed by comparing the chemical shifts of the C1 and C2 with the reported literature.[30,32]

The different chromatographic techniques and various spectral analysis proved that the compound 1 and 2 were lupeol and acetoside, respectively.

The Anti-inflammatory Assay

Acute Inflammatory Model

The freshly prepared egg-albumin solution has been used as an odematogenic agent since it is widely used for evaluating the anti-inflammatory activity of drugs.[33,34] Egg-albumin stimulates the release of serotonin, histamine, or bradykinin, resulting in local edema and subsequently leading to acute inflammation.[34] Table 2 showed no significant difference between the total extract and the isolated acetoside groups, and the positive control group significantly differed from these groups all over the experiment.

Subacute and Chronic Inflammatory Models

Both models have used formalin as a phlogistic agent since it produces more continuous inflammation than other agents.[35] In subacute inflammatory models, all treatment groups (Table 3) were significantly differ ($p \leq 0.05$) as compared with negative control. In addition to that, there is no significant difference in the paw thickness at 1.5 and 24 hours post formalin injection of the total extract and acetoside groups. Meanwhile, at 48 hours post formalin injection, there was a significant difference between these groups. Moreover, the total extract group was non-significantly different from positive control at 48 hours post-induction of sub-acute inflammation.

These findings explained as the anti-inflammatory effect of Iraqi *C. grandiflora* total extract was increased during the time, then became prominent and proximal to the positive control at 48 hours post formalin injection.

In chronic inflammatory model, the treatment with *C. grandiflora* total extract, acetoside, and positive control was significantly amilorate formalin-induced chronic inflammation compared with negative control ($p \leq 0.05$), as illustrated in Table 4. Both diclofenac and total extract produced a comparable effect on formalin-induced paw edema in which no significant difference was detected between these groups. Moreover, there was a significant difference ($p \leq 0.05$) in paw thickness of the acetoside group after 6 days of treatment compared to diclofenac and total extract groups; this means that the anti-inflammatory effect of acetoside was lower than that of both groups.

The different animal models showed that Iraqi *C. grandiflora* total extract possesses a moderate anti-inflammatory activity against the acute inflammatory model and an excellent anti-inflammatory activity against the subacute and chronic inflammatory models, compared with diclofenac. Diclofenac sodium was chosen for this study as reference drug since it is commonly used in clinical practice to treat inflammatory and postoperative conditions and chronic cancer pain.[36]Moreover, these results were in agreement with another study in which the *C.grandiflora* total ethanolic extract has topical anti-inflammatory activity through inhibition of arachidonic acid and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ear edema in the animal model, compared with prednisolone.[22]

The findings of the *in vivo* anti-inflammatory tests suggested that the anti-inflammatory effect of Iraqi *C.grandiflora* total extract attributed partly to caffeic acid derivatives, i.e., acetoside, and there are other phytochemicals such as terpenoids produces an additive or synergistic effect with acetoside and consequently produced this excellent activity. Many reports suggested a strong anti-inflammatory

Table 3: The effect of different treatment groups on formalin-induced subacute inflammation

	Mean change in paw thickness (mm) % of inhibition					
Treatment groups	*1.5 hours*	*24 hours*	*48 hours*	*1.5 hours*	*24 hours*	*48 hours*
Negative control (N/S group)	3.32 ± 0.059	3.02 ± 0.072	2.97 ± 0.070	-	-	-
Positive control (Diclofenac 5 mg/kg)	2.57 ± 0.036^{*a}	2.28 ± 0.058^{*a}	2.23 ± 0.026^{*a}	22.6	24.3	24.8
Total extract group (300 mg/kg)	2.88 ± 0.044^{*b}	2.58 ± 0.061^{*b}	2.35 ± 0.050^{*a}	13.2	14.5	21.1
Acetoside group (25 mg/kg)	2.98 ± 0.074^{*b}	2.67 ± 0.079^{*b}	2.48 ± 0.029^{*b}	10.2	11.6	16.5

Each value represents the mean±SEM for 8 animals ; *:indicate significant difference($p \leq 0.05$) as compared with negative control ; non-identical superscript letters (a,b) indicate statistically significant difference ($p \leq 0.05$) among groups.

Table 4: The effect of different treatment groups on formalin-induced chronic inflammation

Treatment groups	*Mean change in paw thickness at day 7*	*% of inhibition*
Negative control (N/S group)	3.03 ± 0.084	-
Positive control (Diclofenac 5mg/kg)	2.33 ± 0.041^{*a}	23.1
Total extract group (300 mg/kg)	2.41 ± 0.035^{*a}	20.6
Acetoside group (25 mg/kg)	2.53 ± 0.037^{*b}	16.5

Each value represents the mean±SEM for 8 animals ; *:indicate significant difference($p \leq 0.05$) as compared with negative control ; non-identical superscript letters (a,b) indicate statistically significant difference ($p \leq 0.05$) among groups.

effect of these compounds and, as a result, argued with this explanation.[39,40]

CONCLUSION

Lupeol was isolated for the first time from *C. grandiflora* plant. This compound and the other isolated compound (acetoside) were confirmed with different spectroscopic techniques, i.e., FTIR and NMR. The total extract of the Iraqi *C. grandiflora* flowers possess a powerful anti-inflammatory activity compared with diclofenac. Moreover, the isolated acetoside exhibited an anti-inflammatory effect lower than that of other treatment groups, and other compounds are acting by synergism with this compound resulting in the excellent anti-inflammatory activity of this plant.

ACKNOWLEDGMENT

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad, Iraq, for its support in the present work.

REFERENCES

1. Seema C, Archana S. Flower biology and compatibility system of Campsis grandiflora (Bignoniaceae) in Uttar Pradesh, India. The Int. j. plant reprod. biol. 2014;6(1):61-74.
2. Elias TS, Gelband H. Morphology and Anatomy of Floral and Extrafloral Nectaries in Campsis (Bignoniaceae). Am J Bot. 1976;63(10):1349-1353.
3. Yu HC, Wu J, Zhang HX, Zhang HS, Qiao TT, Zhang JX, *et al*. Antidepressant-like and anti-oxidative efficacy of Campsis grandiflora flower. J Pharm Pharmacol. 2015;67(12):1705-1715.
4. Chu C, Xia L, Bai LP, Li Q, Li P, Chen HB, *et al*. Authentication of the 31 species of toxic and potent Chinese materia medica by light microscopy, part 3: Two species of T/PCMM from flowers and their common adulterants. Microsc Res Tech. 2009;72(6):454-463.
5. Oku H, Iwaoka E, Shinga M, Yamamoto E, Iinuma M, Ishiguro K. Effect of the Dried Flowers of Campsis grandiflora on Stagnant Blood Syndrome. Nat Prod Commun. 2019;14(9):1934578X 1987344.
6. Han XH, Oh JH, Hong SS, Lee C, Park JI, Lee MK, *et al*. Novel iridoids from the flowers of campsis grandiflora. Arch Pharm Res. 2012;35(2):327-332.
7. Li ZN, Sun PP, Zhang L. Campsisgrandiflora as a new host species harbouring two novel 16SrI subgroups of phytoplasmas. For Pathol. 2020;50(4):1-7.
8. Kim DH, Han KM, Chung IS, Kim DK, Kim SH, Kwon BM, *et al*. Triterpenoids from the flower of Campsis grandiflora K. Schum. as human Acyl-CoA: Cholesterol acyltransferase inhibitors. Arch Pharm Res. 2005;28(5):550-556.
9. Cui XY, Kim JH, Zhao X, Chen BQ, Lee BC, Pyo HB, *et al*. Antioxidative and acute anti-inflammatory effects of Campsis grandiflora flower. J Ethnopharmacol. 2006;103(2): 223-228.
10. Azeez RAK, Abaas IS, Kadhim EJ. Isolation and Characterization of Isorhamnetin and Kaempferol from Elaeagnus Angustifolia (F:Elaeagnaceae) Cultivated in Iraq. Al Mustansiriyah J Pharm Sci. 2019;19:12-21.
11. Tawfeeq TA, Jasim GA, Nasser AA. Isolation of umbelliferone from leaves of Conocarpus erectus L. cultivated in Iraq. Al Mustansiriyah J Pharm Sci. 2020;20(4).
12. Daeseleire E, Vanoosthuyze K, Van Peteghem C. Application of high-performance thin-layer chromatography and gas

chromatography-mass spectrometry to the detection of new anabolic steroids used as growth promoters in cattle fattening. J Chromatogr A. 1994;674(1-2):247-253.
13. Abdul-jalil TZ, Hussein AA, Saour KY. Extraction and Isolation of β-sitosterol from Iraqi Wild Lycium barbarum by Different Techniques (Probe and Bath Ultrasound, HPTLC and PHPLC). Iraqi J Pharm Sci. 2017;26(2):75-84.
14. Al Habsi AAS, Hossain MA. Isolation, structure characterization and prediction of antioxidant activity of two new compounds from the leaves of Dodonaea viscosa native to the Sultanate of Oman. Egypt J Basic Appl Sci [Internet]. 2018;5(2):157-64. Available from: https://doi.org/10.1016/j.ejbas.2018.04.004
15. Jaber BM, Jasim SF. Phytochemical Study of Stigmasterol and β -sitosterol in Viola odorata Plant Cultivated in Iraq. Iraqi J Biotechnol. 2014;13(2):86-94.
16. Frum Y, Viljoen AM, Van Heerden FR. Verbascoside and luteolin-5-O-β-d-glucoside isolated from Halleria lucida L. exhibit antagonistic anti-oxidant properties in vitro. South African J Bot. 2007;73(4):583-587.
17. Alrubaae OS, Jasim GA, Nasser AA. Cytotoxicityassay of ethyl acetate extract and isolated rutin and quercttin of Iraqi cultivated artemisia abrotanum L. Int J Pharm Res. 2020;12(1):952-959.
18. Khalaf HA, Abbas IS, Tawfeeq AA, Mahdi MF. Determination, Isolation, and Identification of Aucubin and Verbascoside in the Leaves of Iraqi Plantago Lancoleta L. Using Different Detecting Methods. Int J Pharm Pharm Sci. 2018;11(2):74-80.
19. Jesionek W, Majer-Dziedzic B, Choma IM. Separation, Identification, and Investigation of Antioxidant Ability of Plant Extract Components Using TLC, LC-MS, and TLC-DPPH•. J Liq Chromatogr Relat Technol. 2015;38(11):1147-1153.
20. Shah W, Kekare MB, Vaidya V. Development and validation of high performance liquid chromatographic method for the simultaneous determination of B-sitosterol and lupeol in Vernonia cinerea Linn. Int J Pharma Bio Sci. 2010;1(3):1-5.
21. Xie J, Tan F, Zhu J, Yue C, Li Q. Separation, purification and quantification of verbascoside from Penstemon barbatus (Cav.) Roth. Food Chem. 2012;135(4):2536-2541.
22. Azeez RA, Abaas IS, Kadhim EJ. Isolation and characterization of β-sitosterol from elaeagnus angustifolia cultivated in iraq. Asian J Pharm Clin Res. 2018;11(11):442-446.
23. Laaboudi W, Ghanam J, Aissam H, Merzouki M, Benlemlih M. Anti-Inflammatory and Analgesic Activities of Olive Tree Extract. 2016;8(7):17-1.
24. Wanjari M, Kumar P, Umathe SN. Anti-inflammatory effect of ethanolic extract of Ficus bengalensis Linn. in carrageenan induced paw edema in rats. Pharmacogn J [Internet]. 2011;3(23):96-99. Available from: http://dx.doi.org/10.5530/pj.2011.23.15
25. Aouey B, Samet AM, Fetoui H, Simmonds MSJ, Bouaziz M. Anti-oxidant, anti-inflammatory, analgesic and antipyretic activities of grapevine leaf extract (Vitis vinifera) in mice and identification of its active constituents by LC–MS/MS analyses. Biomed Pharmacother [Internet]. 2016;84:1088-1098. Available from: http://dx.doi.org/10.1016/j.biopha.2016.10.033
26. Wani SH. Phytochemical investigation and Anti-inflammatory activity of aqueous extract of leaves of Clerodendrum serratum Linn. 2020;(January 2016).
27. Kumar T, Jain V. Activities of Bridelia retusa Methanolic Fruit. Sci World J. 2014;2014:1–12.
28. Wahba HM, Abouzid SF, Sleem AA, Apers S, Pieters L, Shahat AA. Chemical and biological investigation of some Clerodendrum species cultivated in Egypt. Pharm Biol. 2011;49(1):66-72.
29. Akindele AJ, Oladimeji-Salami JA, Usuwah BA. Antinociceptive and Anti-Inflammatory Activities of Telfairia occidentalis Hydroethanolic Leaf Extract (Cucurbitaceae). J Med Food. 2015;18(10):1157-1163.
30. Sulaiman AA, Hussain SA, Jum 'ma KM, Sigar AH. Evaluation of the Anti-Inflammatory Effect of Pioglitazone in Experimental Models of Inflammation in Rats. Iraqi J Pharm Sci. 2009;18(6):1-6.
31. Ravirala R. Research Journal of Pharmaceutical, Biological and Chemical Sciences Analgesic and Anti-inflammatory effect of Different Extracts of Flemingia. 2013;4(3):1412-1423.
32. Olivier DK, Shikanga EA, Combrinck S, Krause RWM, Regnier T, Dlamini TP. Phenylethanoid glycosides from Lippia javanica. South African J Bot [Internet]. 2010;76(1):58-63. Available from: http://dx.doi.org/10.1016/j.sajb.2009.07.002
33. Silva ATM e, Magalhães CG, Duarte LP, Mussel W da N, Ruiz ALTG, Shiozawa L, *et al*. Lupeol and its esters: NMR, powder XRD data and in vitro evaluation of cancer cell growth. Brazilian J Pharm Sci. 2018;53(3):1-0.
34. Viswanatha GL, Kumar HY, Venkataranganna M V., Prasad NBL, Shylaja H. Anticonvulsant Activity Guided Isolation of Acteoside from Methanolic Root Extracts of Colebrookea oppositifolia Smith. J Biol Act Prod from Nat. 2019;9(5): 328-334.
35. Jigam AA, Akanya HO, Ogbadoyi EO, Dauda BEN, Evans EC. In vivo antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of Lippia multiflora mold. J Med Plants Res. 2009;3(3):148-154.
36. Sofidiya MO, Odukoya OA, Adedapo AA, Mbagwu HOC, Afolayan AJ, Familoni OB. Investigation of the anti-inflammatory and antinociceptive activities of Hymenocardia acida Tul. (Hymenocardiaceae). African J Biotechnol. 2010;9(49):8454-1849.
37. Goulart AC. Study of the inflammatory process induced by injection of carrageenan or formalin in the rat temporomandibular joint Estudo do processo inflamatório induzido pela injeção de carragenina ou de formalina na articulação temporomandibular de ratos. 2005;19(2):99-105.
38. Navarrete A, Sánchez-Mendoza ME, Arrieta J, Cruz-Antonio L, Oliva I, Castañeda-Hernández G. Gastroprotection and effect of the simultaneous administration of Cuachalalate (Amphipterygium adstringens) on the pharmacokinetics and anti-inflammatory activity of diclofenac in rats. J Pharm Pharmacol. 2005;57(12):1629-1636.
39. Díaz AM, Abad MJ, Fernández L, Silván AM, De Santos J, Bermejo P. Phenylpropanoid glycosides from Scrophularia scorodonia: In vitro anti-inflammatory activity. Life Sci. 2004;74(20):2515-2526.
40. Beserra FP, Vieira AJ, Gushiken LF, de Souza EO, Hussni MF, *et al*. Lupeol, a dietary triterpene, enhances wound healing in streptozotocin-induced hyperglycemic rats with modulatory effects on inflammation, oxidative stress, and angiogenesis. Oxidative medicine and cellular longevity. 2019 May 9;2019.