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Original Article

# Phenolic Constituents, Anti-Inflammatory and Antidiabetic Activities of *Cyperus laevigatus* L.

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## ABSTRACT

**Background:** *Cyperus* species are well known traditional plants and used for several diseases around the world. **Aim of the Study:** Our study aimed to identification of the phenolic constituents in addition to evaluation of different extracts of *Cyperus laevigatus* L as antioxidant, anti-inflammatory and antidiabetic agents. **Materials and Methods:** The phenolic constituents were identified using spectroscopic techniques. The antioxidant activity was evaluated using *in vitro* DPPH assay. Total extract, methanol and EtOAc fractions were evaluated for their anti-inflammatory activity using RAW 264.7 macrophages assay. Antidiabetic activity of the total extract was examined biochemically and histopathologically using streptozotocin-induced diabetic rats. **Results:** A new flavone, chrysoeriol 7-*O*-β-(6‴-*O*-acetyl-β-D-glucopyranosyl)-(1→4) glucopyranoside (**1**), along with seven knowns (**2-8**) were isolated from *Cyperus laevigatus* L. The structures of isolated compounds were established depending upon 1D, 2D-NMR and HR-ESI-MS. The MeOH and EtOAc fractions exhibited significant antioxidant activity while the isolated flavonoids exhibited from moderate to weak antioxidant activity. The total extract, MeOH and EtOAc fractions exhibited significant anti-inflammatory activity using LPS-stimulated RAW 264.7 macrophages model by decreasing of NO accumulation by 76 – 66% and 84 – 67%, of the original accumulation values with increasing concentrations in comparison with the reference drug, dexamethasone. The total extract exhibited antidiabetic activity in streptozotocin-induced diabetic rats and this effect was manifested by decreasing serum levels of glucose, glucagon and NO. It also increased level of insulin and promoted paraoxonase activity. **Conclusion:** These results proved that this plant may be multiple sources for medicinal natural drugs especially for anti-inflammatory and antidiabetic.
**Key words**: *Cyperus laevigatus*, New Flavone, Antioxidant, Anti-Inflammatory, Antidiabetic.

## INTRODUCTION

Cyperaceae is a largest family in the monocotyledons that includes more than 100 genera. *Cyperus* genus is the largest genus in this family that contains more than 600 species.[1] *Cyperus* species are widely used as weeds in traditional medicines.[2] In 1983, Buolus reported that *Cyperus* species were traditionally used as an emollient to treat analgesic, diuretic, carminative and others.[3] Pharmacological studies on *Cyperus* sp. indicated a myriad of biological effects such as anti-inflammatory, hepatoprotective, gastroprotective, anti-malarial and anti-diabetic activities.[4-7] Several secondary metabolites were reported from *Cyperus* sp. Including quinones, flavonoids, sesquiterpenes, steroids and essential oils.[2-8-10] Herein we report the phenolic constituents of the aerial parts of *Cyperus laevigatus* (Family: Cyperaceae) as well as the pharmacological effects of the different extracts and isolated compounds.

## MATERIALS AND METHODS

### General Experimental procedures

Optical rotation was measured using JASCO P1020 polarimeter (JASCO International Co. Ltd., Tokyo, Japan). NMR spectra were recorded on JEOL AL-400 NMR spectrometer (JEOL Inc., Tokyo, Japan). HR-ESI-MS were recorded by a Shimadzu LC-MS-IT-TOF-MS spectrometer (Shimadzu Inc., Kyoto, Japan). An OMM 7070E Shimadzu visible recording model UV 200 and 240 spectrophotometers (Shimadzu Inc., Kyoto, Japan) were used for UV spectra.

### Plant material

Aerial parts of *Cyperus laevigatus* L., were collected from Baltim, Kafr Elsheikh, Egypt, in April 2013 and kindly identified by Asoc. Prof. Ahmed M. Abdel Gawad. A voucher specimen (PHG-P-CL179) was deposited in Ain Shams University herbarium, Cairo, Egypt.

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## Extraction and isolation

Air-dried powder aerial parts of *C. laevigatus* (2.0 kg) were extracted by 70% MeOH, filtered, and dried under vacuum to give dark black gum (85.0 gm). The dry extract was successively fractionated using *n*-hexane (12.0), $CH_2Cl_2$ (10.0), EtOAc (11.0) and MeOH (52.0), respectively. The MeOH fraction was subjected to polyamide CC and eluted with $H_2O$: EtOH in gradient afforded 8 major fractions (CL-1:CL-8). Fraction CL-3 (160.0 mg; 25% MeOH) was subjected to preparative paper chromatography that afforded two subfractions CL-3 (A, 112 mg; *Rf*: 0.4 AcOH) and (B; 43 mg; *Rf*: 0.65 AcOH). Subfractions CL-3-B was eluted on Sephadex LH-20 by 40% MeOH afforded compound **1** (8.3 mg). The other seven flavonoids (**2**-**8**) were purified using different chromatographic techniques such as preparative paper and Sephadex LH-20 column. Complete acid hydrolysis followed by PC investigation was preceded. [11]

## Spectroscopic data of new flavonoid (1)

Yellow amorphous solid (8 mg), {$[\alpha]_D^{25}$ −16° (c 0.01, MeOH)}, UV $\lambda_{max}$ nm (MeOH): 208, 266, 347; NaOAc: 213, 260, 348, 368; + Boric acid: 215, 258, 348; $AlCl_3$: 211, 279, 306.6, 355.4; + HCl: 216.2, 277.2, 300.8, 347.4; $H_3BO_3$: 209.2, 269.6, 336.6; +NaOAc: 221.8, 270.8, 334.8. HR-ESI-MS *m/z*: 689.6365 $[M+Na]^+$, ESI-MS *m/z* $[M-H]^-$: 666.1. $^1$H- (400 MHz); $^{13}$C- (100 MHz; DMSO-d6) NMR (see Table 1).

## Antioxidant activity of *C. laevigatus* extracts

The antioxidant activity of the different extracts of *C. laevigatus* aerial parts along with the isolated flavonoids **1-8** were evaluated in terms of DPPH radical-scavenging ability, as described before[12] in a comparising with a reference drug, ascorbic acid.

## Anti-inflammatory activity of different extracts of *C. laevigatus*

The anti-inflammatory of total extract, MeOH and EtOAc fractions was evaluated at different concentrations (12.5, 25, 50 and 100 µg/ml) using LPS-stimulated RAW264.7 macrophages model with a reference drug, dexamethasone as described previously.[13]

## Cell culture

Raw murine macrophages (RAW 264.7) were purchased from the American Type Culture collections and cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 U/mL penicillin G sodium, 100 U/mL streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained in humidified air containing 5% $CO_2$ at 37 °C (Cambrex BioScience, Copenhagen, Denmark).

## MTT cell viability assay

The mitochondrial-dependent reduction of MTT to formazan was used to measure cell respiration as an indicator of cell viability.[13] Cells ($0.5 \times 10^5$ cells/ well) in serum-free media were plated in a flat bottom 96-well microplate, and treated with 20 µL of different concentrations of the tested samples for 24 h at 37 °C, in a humidified 5% $CO_2$ atmosphere. After incubation, the media were removed and 40 µL MTT solution / well was added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µL of acidified isopropanol/well and the plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using 96 wells microplate ELISA reader.

## Inhibition of nitric oxide (NO) production

Raw murine macrophages (RAW 264.7) were seeded in 96-well plates at $0.5 \times 10^5$ cells / well for 2 h in RPMI without phenol red. The cells were stimulated with LPS with final concentrations of 10 µg/mL. The stimulated cells after 2 extra h were treated with serial concentrations of the tested samples, dexamethasone (50 µg/mL) or left with the LPS alone. Untreated cells were used as a negative control.[13]

Nitrite accumulation was used as an indicator of NO production using a microplate assay based on the Griess reaction. In each well of flat bottom 96 well-microplates, 40 µL of freshly prepared Griess reagent was mixed with 40 µL cell supernatant or different concentrations of sodium nitrite ranging from 0-100 µmol/L. The plate was incubated for 10 min in the dark and the absorbance of the mixture at 540 nm was determined using the microplate ELISA reader. The amount of nitrite in the media was calculated from NO standard curve.

## Antidiabetic activity of total alcoholic extract of *C. laevigatus*

### Chemicals and animals

Streptozotocin (**S**TZ) was purchased from Sigma Chemical Co. St. Louis, MO, USA. The study was conducted on 60 adult albino rats (200-210 g) (National Research Centre (NRC), Dokki, Giza, Egypt). Rats were performed in accordance with the Ethics Committee of the NRC. Rats were divided into 4 groups (15 rats in each) as follow: control group, rats received intragastric *C. laevigatus* MeOH fraction (50 mg /kg b.w. day) dissolved in distilled water, Diabetic group, diabetes were induced by single subcutaneous injection of streptozotocin (50 mg/kg b.w.) The animals were considered diabetic if fasting glucose level was 200 mg/dL after 48 hours of the injection, treated group, diabetic rats received intragastric *C. laevigatus* total extract (50 mg/kg b. w. day).[14]

### Biochemical Analysis

Serum glucose was performed according to the method of Passing, 1983.[15] Serum glucagon and insulin were performed according to previous reported methods.[15,16] NO was determined according to the reported method[17] where nitrite, stable end product of nitric oxide radical, is mostly used as indicator for the production of NO. The activity of paraoxonase was measured spectrophotometrically in supernatants using phenyl acetate as the substrate. In this assay, aryl esterase/paraoxonase catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The rate of phenol formation is measured by monitoring the increase in absorbance at 270 nm at 25 °C. Absorbance at 270 nm was taken every 15 s for 120 s using UV Spectrophotometer.[18]

### Histopathological evaluation

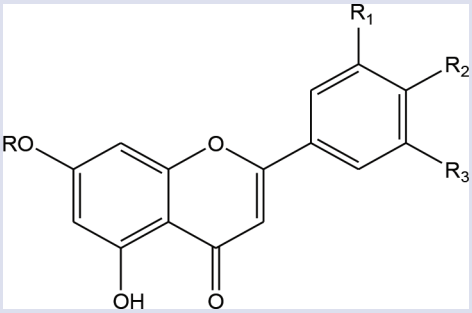
The pancreatic tissues were dissected out immediately, fixed in 10% normal formalin dehydrated in series of alcohol and then to xylene each for 1 h followed by embedding in wax at 60°C. Paraffin blocks of the tissues were sectioned to 5 µm thickness. The sections were then stained with hematoxylin and eosin for histopathological evaluation.[19]

### Statistical analysis

All data were expressed as mean ± standard error. Data were analyzed using one-way ANOVA using SPSS (Version 16). Duncan's new multiple-range test was used to assess differences between means. A significant difference was considered at the level of $P < 0.05$.

# RESULTS AND DISCUSSION

A new acylated flavone diglucoside namely, chrysoeriol 7-O-β-(6‴-O-acetyl-β-D-glucopyranosyl)-(1→4) glucopyranoside (**1**) and seven knowns, apigenin (**2**), apigenin 7-*O*-β-glucopyranoside (**3**), luteolin (**4**), luteolin 7-*O*-β-glucopyranoside (**5**), chrysoeriol (**6**), chrysoeriol 7-*O*-*β*-glucopyranoside (**7**), and tricin (**8**)[20,21] were isolated for the first time from the aerial parts of *C. laevigatus* (Figure 1A). The structures



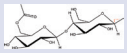

| No | R | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| 1 |  | $OCH_3$ | OH | H |
| 2 | H | H | OH | H |
| 3 | *β-glu* | H | OH | H |
| 4 | H | OH | OH | H |
| 5 | *β-glu* | OH | OH | H |
| 6 | H | $OCH_3$ | OH | H |
| 7 | *β-glu* | $OCH_3$ | OH | H |
| 8 | H | $OCH_3$ | H | $OCH_3$ |

**Figure 1A:** Isolated flavonoids from *C. laevigatus*.

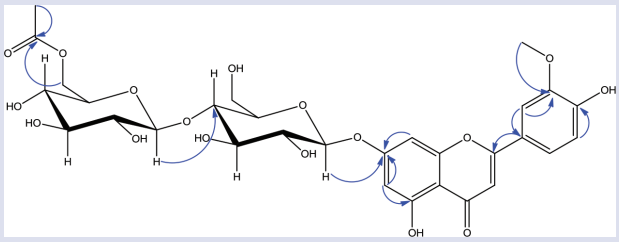


**Figure 1B:** Significant HMBC (→ ) correlations of the new flavonoid(1)

**Table 1:** $^{1}$H- (400 MHz) and $^{13}$C-NMR (100 MHz) of compound 1 (DMSO-d6).

| Position | $^{1}$H-NMR | $^{13}$C-NMR |
|---|---|---|
| • Aglycon | | |
| 2 | --- | 164.9 |
| 3 | 6.71 s | 104.8 |
| 4 | --- | 182.3 |
| 5 | --- | 161.5 |
| 6 | 6.41 d (*J* = 2.0 Hz) | 99.9 |
| 7 | ------- | 163.4 |
| 8 | 6.77 d (*J* = 2.0 Hz) | 94.9 |
| 9 | --- | 157.3 |
| 10 | --- | 105.6 |
| 1’ | --- | 121.5 |
| 2’ | 7.34 s | 113.8 |
| 3’ | --- | 148.6 |
| 4’ | --- | 146.2 |
| 5’ | 6.9 d (*J* = 8.0 Hz) | 116.4 |
| 6’ | 7.42 d (*J* = 8.0 Hz) | 119.5 |
| $OCH_3$ | 3.91 s | 56.1 |
| • 7-O-Glc | | |
| 1” | 5.11 d (*J* = 7.2 Hz) | 99.8 |
| 2” | 3.21 – 3.65 | 73.2 |
| 3” | | 73.5 |
| 4” | | 79.5 |
| 5” | | 74.2 |
| 6” | | 62.3 |
| • $^{4}$Glc-O- Glc | | |
| 1”’ | 5.08 d (*J* = 7.2 Hz) | 100.0 |
| 2”’ | 3.16 – 4.11 | 75.3 |
| 3”’ | | 76.6 |
| 4”’ | | 72.1 |
| 5”’ | | 73.5 |
| 6”’ | | 63.3 |
| $OCH_3$ | 3.91 s | 56.7 |
| • $^{6```}$Acetate | | |
| CO | --- | 172.3 |
| $COOCH_3$ | 2.15 br s | 21.1 |

*All assignments are based on 1D and 2D measurements (HMBC, HSQC, COSY).

of isolated compounds were established using spectroscopic methods, including UV, 1D, 2D-NMR and HR-ESI-MS.

UV spectroscopic data of compound (**1**) indicated a flavone with free 5 and 4’-hydroxyl groups.[11] HR-ESI-MS spectrum showed a molecular ion peak $[M+Na]^+$ at *m/z* 689.6365, corresponding to the molecular formula of $C_{30}H_{34}O_{17}$. The $^{1}$H-NMR spectral data (Table 1) showed a downfield shift of H-6 at $\delta_H$ 6.41 (d, *J* = 2.0), H-8 at 6.77 (d, *J* = 2.0 Hz) suggested the presence of C-7-*O*-substitution. A characteristic signal of methoxy group at $\delta_H$ 3.91 s was detected. Additionally, the spectrum showed two anomeric protons at $\delta_H$ 5.11 (d, *J* = 7.2) and at $\delta_H$ 5.08 (d, *J* = 7.2). The signals appeared at $\delta_H$ 3.16 – 4.11 were attributed to the protons of the remaining sugar. A characteristic proton signal of one methyl for acetyl group was observed at $\delta_H$ 2.15 s. $^{13}$C-NMR spectrum showed 30 carbon resonances (Table 1), two of which were carbonyls which appeared at $\delta_C$ 182.3 for C-4 and at $\delta_C$ 172.3 for characteristic for an acetoxy group. Dept-145 experiment suggested the presence of two methylene, sixteen methine signals, one metyl of methoxy group (at $\delta_C$ 56.1); ome methyl for acetoxy group (at $\delta_C$ 21.1) and ten quaternary carbon signals. The C-7-*O*-sabstitution was suggested by the downfield shift carbon signal of C-7 at $\delta_C$ 163.4. Ten carbon signals for two glucosyl moieties were showed at $\delta_C$ 73.2 (C-2”), 73.5 (C-3”), 79.5 (C-4”), 74.2 (C-5”), 62.3 (C-6”), 75.3 (C-2”’), 76.6 (C-3”’), 72.1 (C-4”’), 73.5 (C-5”’), 63.3 (C-6”’), two anomeric carbons at $\delta_C$ 99.8 (C-1”), $\delta_C$ 100.0 (C-1”’). The assignment of the protonated carbons was established by HSQC experiment. The 7-*O*-glucosidic linkage was confirmed by HMBC correlation of the anomeric proton at $\delta_H$ 5.11 (d, *J* = 7.20, H-1”) and C-7 at 163.4 ($J^3$). The very clear downfield shieft of C-4” at $\delta_C$ 79.5 in addition to the HMBC correlation of the anomeric proton at $\delta_H$ 5.08 (d, *J* = 7.20, 1”’) with the C-4” ($J^3$) deduced the 4” → 1”’ diglucoside linkage [22]. Also the downfield shieft of the C-6”’ by approximately 1 ppm at $\delta_C$ 63.3 suggested the acetylated C-6”’ that supported by HMBC correlation of H-6”’ at $\delta_H$ 3.16

**Table 2: Antioxidant activity of *C. laevigatus* extracts and isolated flavonoids (1-8).**

| Tested sample | DPPH assay $IC_{50}$ (µg/ml) |
|---|---|
| n-hexane fraction | 76.98±2.69 |
| EtOAc fraction | 57.58±1.93 |
| MeOH fraction | 24.28±0.67 |
| Compd 1 | 65.93±1.57 |
| Compd 2 | 28.78±1.21 |
| Compd 3 | 34.12±0.98 |
| Compd 4 | 26.54±1.03 |
| Compd 5 | 31.19±1.14 |
| Compd 6 | 33.76±0.87 |
| Compd 7 | 52.62±0.65 |
| Compd 8 | 58.44±1.74 |
| Ascorbic acid | 1.85±0.12 |

m with the acetyl carbonyl group at $\delta_C$ 172.3 ($J^3$). Moreover, a correlation between the methyl proton signal at $\delta_H$ 2.15 s and the carbonyl group at $\delta_C$ 172.3 ($J^2$) was observed. Also the HMBC correlation between the methyl proton at $\delta_H$ 3.91 s and C-3' at $\delta_C$ 148.6 ($J^3$) indicated methoxylation of C-3' (Figure 1B). The $\beta$ orientation of the glucosidic linkage was confirmed was by the large coupling constant of the anomeric proton (7.2 ppm).[22,23] The structure of this compound was confirmed by mass fragments; at *m/z* 367.1056 characteristic to 6'''-*O*-acetyl-$\beta$-*D*-diglucopyranoside; at *m/z* 301.1413 characteristic to chrysoeriol and at *m/z* 461.2 in the negative mode ESI-MS characteristic to chrysoeriol-7-*O*-glucoside. The acid hydrolysis of **1** afforded an aglycone and a sugar moiety, which was composed of chrysoeriol and glucose. From the above mentioned data, the structure of **1** was characterized as chrysoeriol 7-O-β-(6'''-O-acetyl-β-D-glucopyranosyl)-(1→4) glucopyranoside **(1)**

## Antioxidant activity

The total extract and MeOH fractions showed moderate DPPH radical scavenging activity with $IC_{50}$ 23.39±0.72 and 24.28±0.56, respectively but EtOAc, and *n*-hexane showed weak activity with $IC_{50}$ 57.89±0.71, and 76.98±0.73, respectively. Also the isolated flavonoids exhibited from moderate to weak antioxidant activity by the order of compound **4>2>5>6>3>7>8>1** (Table 2). The moderate antioxidant activity of both the total extract and MeOH fraction was attributed to the high flavonoid content. It was reported that flavonoids possess antioxidant activity especially luteolin derivatives and methoxylated flavonoids.[24] The DPPH reaction mechanism with the compounds that exhibited activity depending upon the free OH groups in B-ring, so the flavons exhibited more activity that the flavons 7-O-glycosides.[25]

## Anti-inflammatory activity

Cytotoxicity results confirmed that the tested extracts are safe on RAW264.7 macrophages with different concentrations (12.5, 25, 50 and 100 µg/ml). The total extract (12.5, 25, 50, and 100 µg/ml) and MeOH fraction (12.5, 25, and 50 µg/ml) decreased NO% accumulation as the concentration increased reaching 76 - 66% and 84 - 67%, respectively. Also, the EtOAc fraction (12.5, 25, and 50 mg) decreased the NO% accumulation with concentration increased with values ranging from 77 - 66%. The results of the anti-inflammatory assay suggested that the total, MeOH and EtOAc extracts exhibited potent activity at different concentrations and the results were comparable with the reference drug,

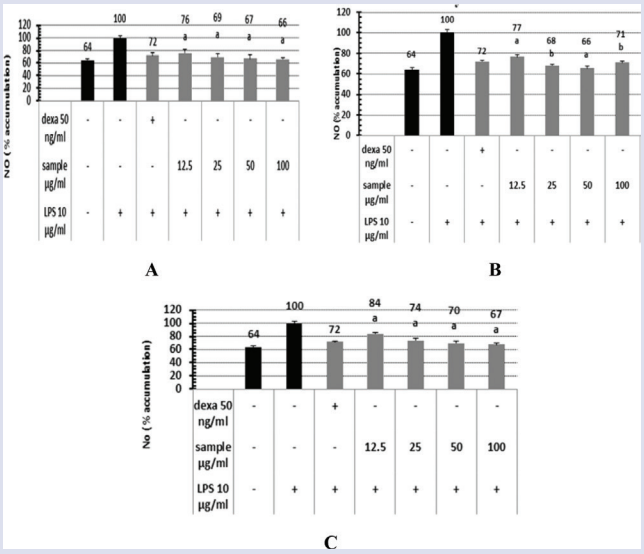



**Figure 2:** NO% accumulation in response to; B) total extract; C) MeOH fraction and D) EtOAC fraction of *C. laevigatus*. The results were compared with the results of LPS stimulated cells, non-treated cells and dexamethasone (50 µg/ml) treated cells. [a] P ≤ 0.005, [b] P ≤ 0.0005 compared to LPS stimulated cells.

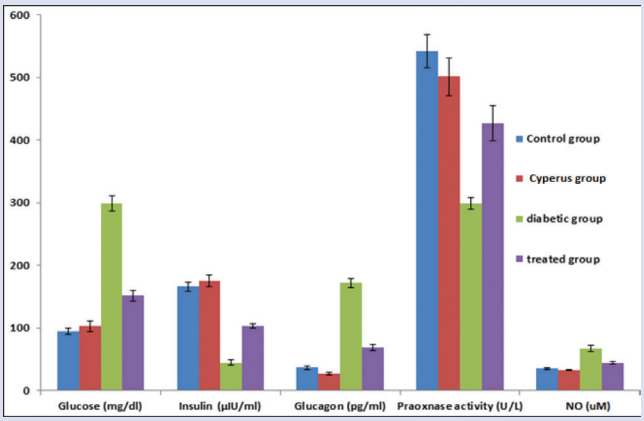



**Figure 3:** Mean serum glucose, glucagon and NO levels were significantly high and serum insulin and serum praoxnase activity level were significantly low in diabetic group compared to normal group. Mean serum glucose, glucagon and NO levels were significantly low and serum insulin and serum praoxnase activity level were significantly high in treated group compared to diabetic group.

dexamethasone (Figure 2). A strong correlation has been always noticed between chemical constituents and herbal biological activities. By the same analogy, the anti-inflammatory activity of *C. laevigatus* can be correlated to its chemical constituents. The phytochemical study of this plant elucidated that it is rich with bioactive compounds. Luteolin, its methoxylated and glycosides derivatives inhibited the NO production and iNOS expression in LPS-stimulated BV-2 microglial cells.[26]

## Antidiabetic activity of total extract

*Cyperus laevigatus* aerial parts total extract did not exhibit any obvious toxic symptoms or mortality in rats up to 5000 mg/kg b.w. after 14 days.

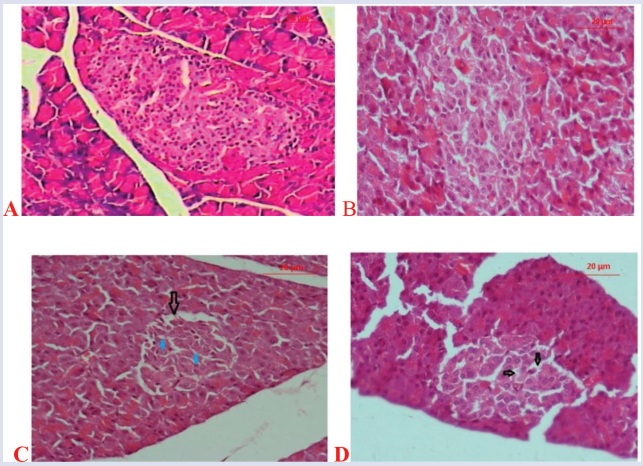



**Figure 4:** Section of pancreas of A) control group shows the normal structure of exocrine (dense-staining acinar cells) and endocrine pancreas (light-staining islet of Langerhans), B) rat treated with *C. laevigatus* extract (50 mg/kg b.w./day) shows the normal structure of exocrine and endocrine pancreas, C): diabetic rat shows decrease in pancreatic islet size, atrophy and vacuolation, and connective tissue invasion in the parenchyma of pancreas islet (black arrow) is shown. A reduction in the pancreatic b-cell (blue arrow) numbers compared to the control group, D): diabetic rat received intragastric *C. laevigatus* extract (50 mg/kg b.w./day) shows normal structure of the pancreas. Few degenerative cells (black arrow) are seen in the islet (H & E stain, Scale Bar: 20 µm).

Biochemical markers in the present work exhibited that the diabetic group treated with *C. laevigatus* extract showed a decrease in the glucose, glucagon, and NO serum levels and promote serum insulin and paraoxonase levels. Zhang *et al*. 2010[27] reported that flavonoids exhibited antidiabetic activity by decreasing fasting blood glucose (FBG) and glucagon serum levels while increasing insulin serum levels (Figure 3). Histological examination of the pancreas of the control and extract treated rats indicated normal architecture (Figure 4-A; B). The islets of Langerhans found in the pancreatic tissue were round in shape with normal cell lining. On the other hand, the acini were arranged in a well-organized manner. The interlobular ducts were surrounded with the supporting tissue. These results were consistent with Jarral *et al*. 2013[28] that found that beta-cells comprise the major of islets' cells of rat's pancreas.

In the diabetic rats, the pancreas showed a decrease in the pancreatic islet size, atrophy, vacuolation, and connective tissue invasion in the parenchyma of the pancreas islets. The sections revealed a reduced pancreatic $\beta$-cell numbers compared to the control group (Figure 4-C). STZ-induced diabetes may be due to the selective destroying of pancreatic $\beta$-cells, which is responsible for the insulin production from endocrine cells.[29] The pancreas of the diabetic rats treated with *C. laevigatus* extract showed dramatic suppression of all abnormal histological changes as compared to the diabetic group (Figure 4-D).

The pancreas of the diabetic rats treated with *C. laevigatus* extract showed dramatic suppression of all abnormal histological changes. Regarding the mechanism by which *C. laevigatus* can improve $\beta$-cells, researchers found that flavonoids, flavonoid glycosides and phenolic acids exhibited a strong contribution as antioxidant agents that can regenerate the changes in the morphology of $\beta$-cells.[24-30-31]

## CONCLUSION

A new flavonoid, chrysoeriol 7-O-β-(6‴-O-acetyl-β-D-glucopyranosyl)-(1→4) glucopyranoside (1), and seven knowns (2-8) were isolated and identified from aerial parts of *C. laevigatus* alcoholic extract. The different extracts and isolated compounds exhibited from moderate to low antioxidant activity. The total acoholic extract, MeOH and EtOAC fractions exhibited significant anti-inflammatory activity using by decreasing of NO accumulation in comparison with dexamethasone as a reference drug using LPS-stimulated RAW 264.7 macrophages model. The MeOH fraction exhibited antidiabetic activity by decreasing levels of glucose, glucagon and NO along with increasing level of insulin and promoted paraoxonase activity in streptozotocin-induced diabetic rats.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interests.

## ABBREVIATION USED

**DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **RAW 264.7:** Mouse RAW-264.7 Cell lines; **1D, 2D-NMR:** one and two dimensional nuclear magnetic resonance; **NO:** Nitric Oxide; **PC:** Paper Chromatography; **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide.

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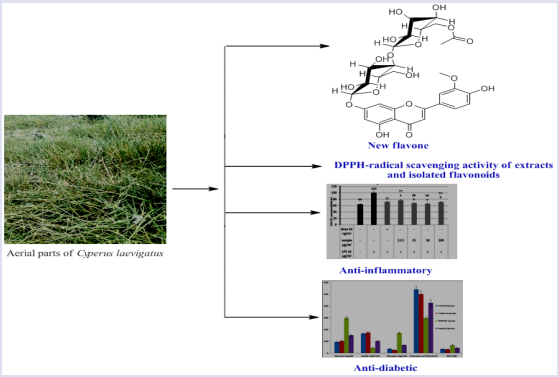
## GRAPHICAL ABSTRACT




## SUMMARY

- Isolation and identification of a new flavonoid along with 7 knowns fron aerial parts of *C. laevigatus*.
- Evaluation of antioxidant of the isolated flavonoids.
- Evaluation of anti-inflammatory of the different solvent successive fractions.
- Study of andtidiabetic activity of the total alcoholic extract.

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