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Research Article

Total Phenolic, Total Flavonoid Content, Two Isolates and Bioactivity of *Lavandula pubescens* Decne

Mousa O[1,2], Gouda B[1*], Salama M[2] , El-Eraky W[3], Kassem H[2]

[1]*Pharmacognosy Department, Faculty of Pharmacy, Ahram Canadian University, Egypt*
[2]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt*
[3]*Pharmacology Department, National Research Center, Cairo, Egypt*

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ABSTRACT

The phenolics and flavonoids content of *Lavandula pubescens* Decne. cultivated in Egypt were determined. The phenolic content of the ethanolic extract of the flowering aerial parts of the plant was measured by using Folin-Ciocalteu assay colourimetric method. The total phenolic content was expressed as 234.17±0.37 µg GAE/mg of sample. The total flavonoids of the extract were measured spectrophotometrically by using aluminium chloride colorimetric assay. The percentage of flavonoids was calculated as 134.36±0.067 µg quercetin/ mg of sample. Two compounds were isolated from the ethyl acetate fraction and identified on basis of their ^{1}H-NMR and ^{13}C-NMR as acacetin-7-O- glucoside and gallic acid. Significant biological assessment of the three tested concentrations of the extract was obtained applying the carrageenan-induced rat paw oedema and hot plate method as models for acute anti-inflammatory and analgesic effects respectively. A remarkable hepatoprotective activity of the extract as regards to silymarin, against paracetamol induced acute liver damage in rat was observed. A considerable decrease in the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was recorded. Liver histopathology of the methanolic extract treated rats exhibited significant protection against paracetamol intoxication.

Keywords: *Lavandula pubescens* Decne., acacetin-7-O- glucoside, gallic acid, acute anti-inflammatory, analgesic, hepatoprotective activity.

INTRODUCTION

The genus *Lavandula* is endemic across the Mediterranean Basin to India and includes about 30 species. The genus comprises annuals, herbaceous plants, shrubs and many members of the genus are cultivated as ornamental plants for garden. The genus exhibits insecticidal, anxiolytic, antioxidant, anti-inflammatory, anticonvulsant, immunomodulatory, sedative, local anaesthetic and spasmolytic activities[1]. *Lavandula pubescens* Decne. is known as "fahita" and has been traditionally used in Yemeni folk medicine as carminative, insect repellent and antiseptic[2]. Quercetin, apigenin, kaempferol glucosides were reported in *Lavandula angustifolia* aerial parts by using high performance liquid chromatography-mass spectrometry analysis[3]. RP-DAD-HPLC analysis of ethanolic extract, obtained from fresh leaves of *Lavandula multifida*, revealed the prescence of carvacrol, vitexin, and 7- or 8-glucoside derivatives of hypolaetin, scutellarein, luteolin, isoscutellarein, apigenin, and chrysoeriol[4]. The extracts and essential oil of the leaves of *Lavandula angustifolia* Mill. demonstrated anti-inflammatory and analgesic activities using Carrageenan test, formalin and acetic acid-induced writhing tests[5]. A recent study[6] displayed a broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria of the methanolic extract of the flowering aerial parts of *Lavandula pubescens* using agar diffusion method. *Lavandula stoechas* essential oil exhibited potential hepato- and nephroprotective effects against malathion-induced oxidative stress in mice[7]. In the current study, the content of the total phenolic compounds and flavonoids of the ethanolic extract of *L. pubescens* flowering aerial parts were measured in order to correlate them with the biological assessment. Two isolates were obtained and identified as acacetin-7-O- glucoside and gallic acid from the ethyl acetate fraction. Owing to the reported antimicrobial activity[6] of *L. pubescens*, the methanolic extract was assessed for anti-inflammatory, analgesic and hepatoprotective activities to find credence of the traditional usage of the plant in anti-inflammatory and hepatoprotective disorders.

MATERIALS AND METHODS

Plant material

The plant material of *Lavandula pubescens* Decne. used in this study was collected during the flowering stage during the years 2011-2013 from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. The plant was kindly authenticated by Dr. Mohamed El-gebaly, Senior specialist of plant identification and voucher specimens are kept at the

**Author for Correspondence:*

Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo, Egypt.

Preparation of extracts

For isolation purpose, two kilograms of the air-dried flowering aerial part were exhaustively extracted with methanol 70%. The methanolic extract was evaporated under reduced pressure to dryness (66.15 g). The dry residue was then suspended in water and partitioned successively with *n*-hexane followed by methylene chloride then by ethyl acetate followed by *n*-butanol. The solvents were evaporated under reduced pressure to give *n*-hexane extract (34.18 g), methylene chloride extract (5.93 g), ethyl acetate extract (5.41 g) and *n*-butanol extract (5 g). The ethyl acetate fraction was utilized for the isolation purpose.

For determination of the total phenolic and flavonoid content, ethanol (90%) extract was prepared from powdered flowering aerial parts (5 grams) by exhaustive percolation at ambient temperature, followed by evaporation of the solvent and the dried extract was kept for further investigation.

For the biological evaluation, the methanol extract was prepared by exhaustive cold maceration of the dried powdered flowering aerial part of the plant (500 g) in methanol (70%). The solvent was evaporated to dryness (15 g) and the dried extract saved for further investigation.

Material and Apparatus for Phytochemical Investigation

Solvents and authentics used in this study were of analytical grades and all were purchased from E-Merck (Darmstadt, Germany).

Estimation of total phenolic and total flavonoid content

The total phenolic content was determined using Folin-Ciocalteu colourimetric method[8] and expressed as µg gallic acid content of equivalent (GAE/mg) dry extract. The total flavonoid content of the extract was determined following a colorimetric method[9] and values were expressed as µg quercitin content of equivalent (QE/mg) dry extract. For each concentration, three replicates were carried out and the average of the obtained absorbance was plotted versus the concentration.

Folin-Ciocalteu was utilized for determination of total polyphenolic content obtained from Loba-Chemie (Mumbai,india). Reagents for spectrophotometric determinations[10]: Anhydrous $AlCl_3$ (13.334 g) was dissolved in one liter distilled water and was used for colorimetric assay of flavonoids (0.1 M $AlCl_3$).

UV-visible spectrophotometer

Jenway double beam spectrophotometer was used for recording UV spectra and measuring the absorbance in UV and visible range.

Stationary phases for chromatography

Thin layer chromatography (TLC) was carried out on precoated silica gel GF 254 (20× 20 cm, Fluka). Various stationary phases were used for column chromatography (CC), Sephadex LH 20 (Pharmacia Fine Chemicals AB Uppsala, Sweden), Silica gel H 60 without gypsum for vacuum liquid chromatography (VLC) (E-Merck, Germany), Silica gel 60 for column chromatography (70-230 mesh, Fluka, Sigma-Aldrich chemicals-Germany) and Silica gel RP-18 (70-230 mesh, Fluka, Sigma-Aldrich chemicals-Germany).

Solvents systems and spray reagents

Solvent systems **S_1:** Chloroform: Methanol (95: 5 v/v), **S_2:** Chloroform: Methanol (90:10 v/v) and **S_3:** Chloroform: Methanol (85:15 v/v) were utilized for developing the chromatograms. Spray reagents used were Aluminum chloride spray reagent for flavonoids[10], Ferric chloride spray reagent for phenolic compounds[11] and Folin-Ciocalteu used for determination of total polyphenolic content obtained from Loba-Chemie (Mumbai,india).

Equipment and Apparatus

Chromatographic jars and glass columns of different dimensions were used for TLC and CC. UV lamp (λ_{max} = 254 and 330 nm, Shimadzu), a product of Hanovia lamps, for location of fluorescent spots. Bruker NMR-spectrophotometer: ^{1}H NMR, 400 MHz,^{13} C-NMR, 100 MHz, Japan. Chemical shift values were recorded in δ ppm.

Material for Biological Evaluation

Drugs and chemicals

Indomethacin was obtained from (Eipico, Egyptian Int. Pharmaceutical Industries Co under license of Merck & Co. N.J.U.S.A) as anti-inflammatory drug, Carrageenan from Sigma Company for induction of oedema, Paracetamol (acetaminophen) provided by Egypt International Pharmaceutical Industries Company (Cairo Egypt) used for liver damage, Silymarin provided by Amoun Pharmaceutical Industries Company (Cairo, Egypt) as hepatoprotective, Diclofenac (Eipico, Egyptian Int. Pharmaceutical Industries Co under license of Merck & Co. N.J.U.S.A) as analgesic and Transaminase kits (Bio-Merieux Co, France) were utilized in assessing serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes.

Experimental animals were obtained from animal house colony at the National Research Centre (Dokki, Giza, Egypt). Adult male albino rats of Sprauge Dawely Strain weighing 120-140 gram body weight used to anti-inflammatory, analgesic and hepatoprotective activities. The animals were kept under the same hygienic conditions and on a standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein-95% pure (10.5%) and starch (54.3%). Water was supplied *ad libitum*. The study was performed according to the international rules and guidelines of the Ethical Committee of the National Research Centre for experimental animal use.

Isolation and identification of the main constituents of the ethyl acetate fraction

Five grams of the ethyl acetate fractionated extract of the flowering aerial parts were applied on a vacuum liquid chromatography column (VLC), (4x12 cm), packed with 150 g silica gel H. Gradient elution was performed using methylene chloride, methylene chloride/ethyl acetate, ethyl acetate, ethyl acetate/methanol and methanol. The polarity was gradually increased by 5% stepwise. Fractions (100 ml each) were collected and monitored by

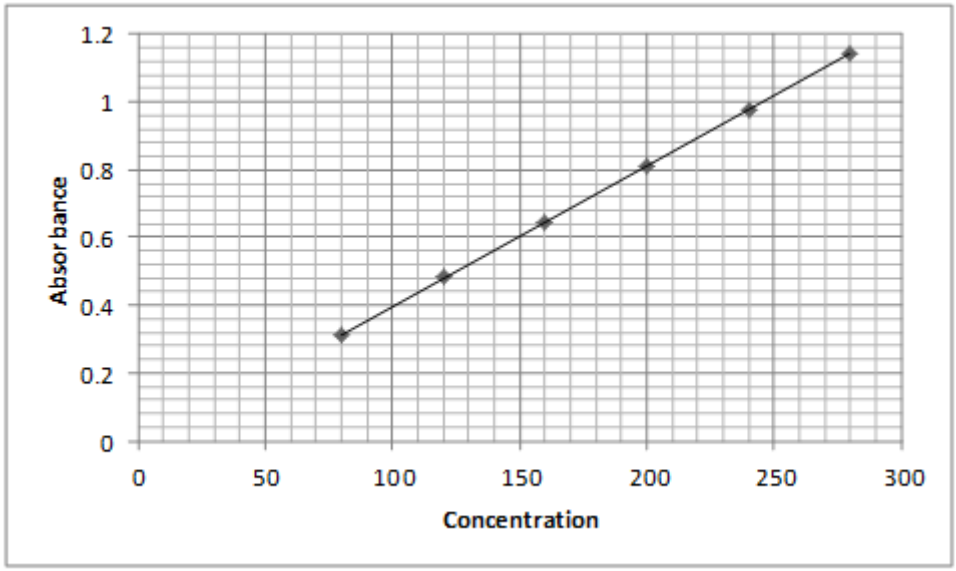


Figure 1: Calibration curve of standard gallic acid.

Table 1: Absorbance of the different concentrations of standard gallic acid.

Concentration (µg/ml)	Absorbance*
80	0.315
120	0.482
160	0.642
200	0.809
240	0.978
280	1.141

*Average of three determinations

Table 2: Absorbances of the different concentrations of standard quercetin.

Concentration (µg/ml)	Absorbance*
5	0.04
10	0.111
20	0.248
40	0.385
80	0.771
100	0.963

*Average of three determinations

TLC. Similar fractions were pooled together to yield two collective fractions. Fractions I and II were employed for isolation and purification of individual compounds.

Fraction I:

*(*0.5g; TLC: one major spot, R_f value 0.6 in S_3 in addition to other minor spots. The fraction was chromatographed on sephadex LH-20 column (27x2 cm) using methanol: water (80:20) for elution. Fractions of 10 ml each were collected and similar fractions were pooled together on the basis of its TLC spotting. The collected similar fractions were evaporated till dryness and then further purified on RP-18 column using methanol:water (50:50) for elution, to give 14 mg of white needle crystals (compound 1).

Fraction II

(0.5 g; TLC: one major spot, R_f value 0.8 in S_3. It was further purified on several (Sephadex LH-20) columns using methanol: water (80:20) for elution, to yield 16 mg of yellow powder (compound 2).

Identification of the isolated compounds was performed through co-chromatography, as well as physicochemical and spectral analysis. Structure elucidation was based on interpretation of spectral data including those of UV and NMR (^{1}H NMR and ^{13}C NMR).

In-vivo biological evaluation

The methanolic extract (70%) of flowering aerial parts at different concentrations (125, 250 & 500 mg/Kg b.wt.) was subjected to evaluation of its anti-inflammatory, analgesic and hepatoprotective activities. Statistical analysis [12] was carried out by using one way analysis of variance (ANOVA) for anti-inflammatory and hepatoprotective activities. Two-way ANOVA followed by Tukey multiple comparison tests were used for statistical analysis of analgesic activity.

Assessment of anti-inflammatory activity

The anti-inflammatory activity was evaluated by using Carrageenan-induced rat paw oedema method[13]. Paw oedema was induced by a sub-plantar injection of 100 µl of 1% of sterile carrageenan suspension in saline into the right hind paw. The tested concentrations and indomethacin (standard anti-inflammatory, positive control, 10 mg/Kg b.wt.) were orally administered to experimental animals (n=8). The oedema component of inflammation was quantified by measuring hind footpad immediately before carrageenan injection and 1-3h after carrageenan injection. Oedema was expressed as a percentage of change from control (pre-drug) values using a Plethysmometer (7141: UGO Basile, Comerio, Italy[14].

Assessment of analgesic activity

The analgesic activity was carried out using hot plate method[15]. Rats were divided into 5 groups (n=6, each). The first group was used as control. The second group received diclofenac (a standard analgesic, positive

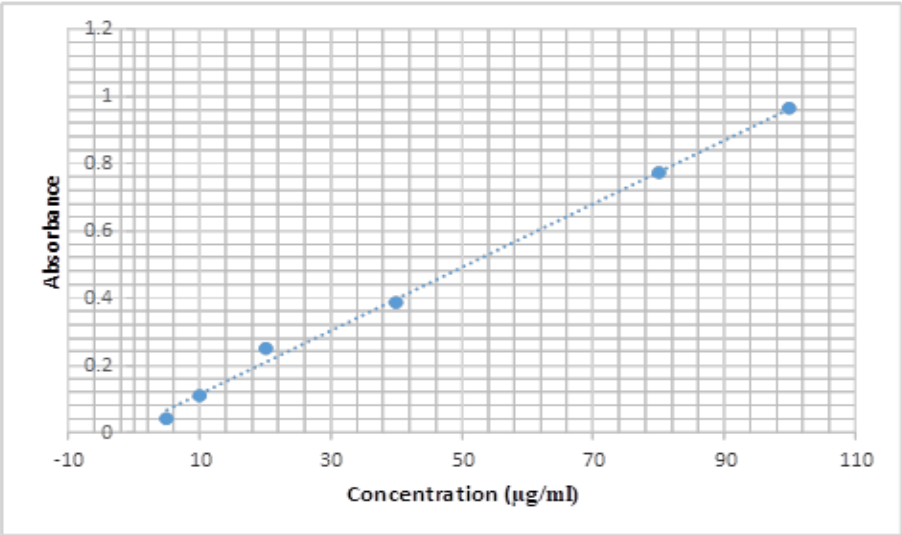
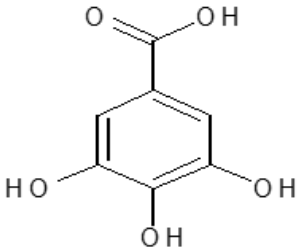
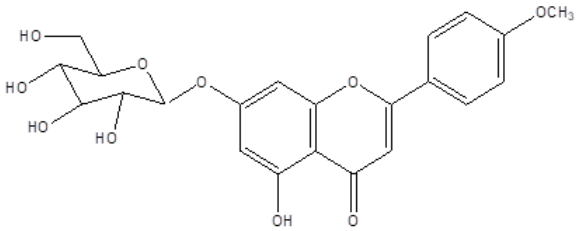


Figure 2: Calibration curve of standard quercetin.



Compound (1) Gallic acid



Compound (2) Acacetin-7-O- glucoside

Figure 3: Compounds isolated from the flowering aerial part of *Lavandula pubescens*.

Table 3: Acute Anti-inflammatory effect of different concentrations of the methanolic extract of *Lavandula pubescens* flowering aerial parts.

Groups	Dose in mg/Kg b.wt.	Oedema volume (% change from baseline)		
		1 h	2 h	3 h
Control	-	69.71±2.63	96.17±3.80	100.60±4.81
Indomethacin	10	44.33±2.06*	33.11±1.21*	24.17±1.78*
Methanolic extract	125	44.67*±2.10	55.51*±3.57	62.96*±1.12
	250	49.79*±1.63	45.71*±2.42	53.93*±3.65
	500	40.84*±3.26	45.78*±1.83	38.58*±3.55

The data represent the mean± standard error of the mean (n=8)
*Statistically significant from zero time: $P< 0.05$.

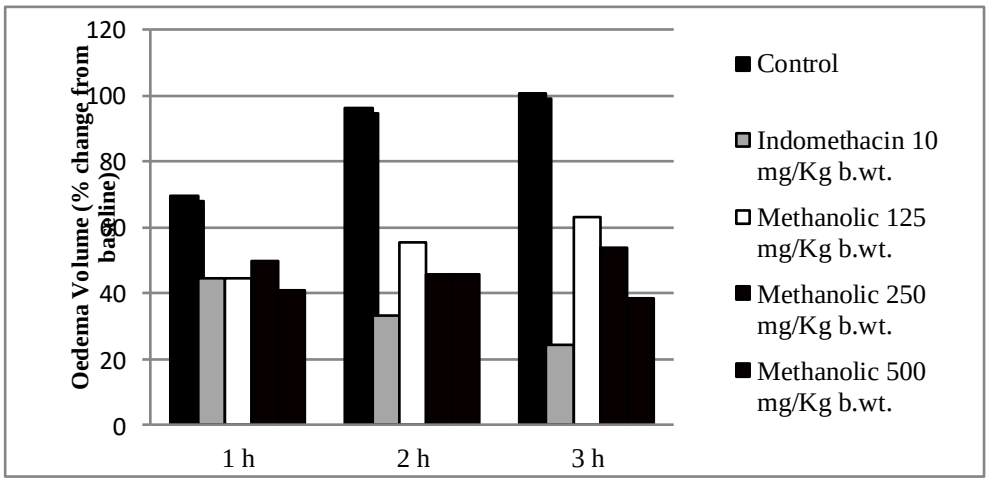


Figure 4: The acute anti-inflammatory activity of different concentrations of the methanolic extract of *Lavandula pubescens* flowering aerial parts.

control, 10 mg/Kg b.wt.) orally. The other three groups received the tested extract at doses of 125, 250 and 500 mg/Kg b.wt., orally. Each rat was placed on a hot plate maintained at the temperature of 55±1 °C and the latency period was recorded with a stop watch which represents the time taken for the rat to react to the pain stimulus. The response to pain stimulus included; jumping, raising and licking of hind foot was noticed.

Assessment of hepatoprotective activity

The experimental animals were divided into 6 groups (n=6, each). The first group (negative control group) received 1% tween 80, orally. In the second group (paracetamol group), rats were fasted for 18 hours and paracetamol was orally administered in a single dose of 600 mg/kg. The third group received the reference hepatoprotective drug silymarin (50 mg/kg.b.wt) in addition to paracetamol (positive control). The other three groups were pre-treated with the different concentrations of the methanolic extract orally (125, 250 & 500 mg/Kg b.wt.). This procedure was carried out for 2 weeks before induction of hepatic damage by paracetamol. At the end of the experiment (24h after the dose of paracetamol), rats were anesthetized with ether, blood samples were collected via the retro-orbital plexusinto plain centrifuge tubes and subjected to centrifugation (2500 rpm, at 4°C, for 15 min). Sera were then isolated and saved for biochemical analysis[16] of AST and ALT. Animals were afterwards sacrificed by decapitation and liver tissue immediately isolated, washed with ice cold saline (0.9% NaCl) and saved in neutral buffered 10% formalin solution for further histopathological examination.

Histopathological examination of the isolated fresh liver tissues

Autopsy samples were taken from the liver of rats of the different groups and fixed in 10% formalin for 24 hours. Washing was carried out with tap water then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 ˚C in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for examination through the light electric microscope[17].

RESULTS AND DISCUSSION

The results obtained in this study showed a significant level of phenolic compounds in the ethanolic extract (90%) of flowering aerial parts. The results are recorded in (Table 1) and represented in (Fig.1)

The phenolic content was expressed as gallic acid equivalent and was deduced from the pre-established calibration curve (Fig.1) and calculated using the following equation[8].

$$Y= 0.0041x-0.016,\ R^2=1$$

Where, Y= absorbance, x= corresponding concentration (µg/ml) and R^2= correlation coefficient. The absorbance of the ethanolic extract of flowering aerial parts of *Lavandula pubescens* was 0.944 nm corresponding to the phenolic content of extract of the flowering aerial parts which was 234.17±0.37 µg GAE/ mg of sample.Many human's disorders such as atherosclerosis, arthritis, Alzheimer disease, cancer etc., are suggested to the increase in the concentrations of the free radicals[18]. Phenolic compounds are ubiquitous secondary metabolites in plants, commonly known to have antioxidant effect[19]. The use of natural phenolic antioxidants from plants does not induce serious side effects, while synthetic antioxidants were found to have genotoxic effect[20].

In the present study, the total flavonoids content of ethanolic extract was also determined using aluminium chloride colorimetric method. The flavonoid content was calculated as quercitin and was deduced from the pre-established standard calibration curve (Fig. 2) using the following equation[9]:

$$Y= 0.009x+0.018,\ R^2=0.996$$

Where, Y= absorbance, x= corresponding concentration (µg/ml) and R^2= correlation coefficient.

The absorbance of the ethanolic extract of the flowering aerial parts was 1.227 nm corresponding to flavonoid content 134.36±0.067 µg quercetin/ mg.

Flavonoids are secondary plant metabolites known as vitamin P and play an important role in coloring the plant. They are readily ingested by humans and seem to display important anti-inflammatory, anti-allergic and anti-cancer activities[21].

A previous study showed a significant linear correlation between the free radical scavenging activity determined and the total flavonoid compounds[22]. Oxidative stress in human body contributes to the pathogenesis of many human diseases. The elimination of free radicals by the intake of antioxidative agents (Phenolic and flavonoids) is important to reduce the oxidative stress and hence for the prevention of chronic disease[23].

Phytochemical investigation of the Ethyl Acetate fraction

TLC screening of the ethyl acetate fraction (S_3) revealed the presence of a series of polyphenols. Attempts to isolate the components that may be responsible of the extract activity were performed through normal and/ reversed column chromatography. Chromatographic fractionation of the ethyl acetate fraction afforded two known phenolic compounds, which as far as the available literature is concerned are for the first time reported from the plant. Structure elucidation was based on physicochemical and spectral analysis as well as by comparison with published data. Structures of isolated compounds are represented in (Fig. 3).

Characterization of the isolated compounds

Compound 1: White needle crystals, 14 mg, m.p. 255-257 °C, R_f 0.6 in S_3 , UV (MeOH), λ_{max} 272 nm ^{1}H-NMR: δ (400 MHz, MeOH), 7.07 (2H, singlet, H-2,6); ^{13}C-NMR: δ (100 MHz,MeOH) 169.4 (C-1), 144.95 (C-4 and C-6), 137.98 (C-5), 121.17 (C-2), 108.89 (C-3 and C-7). Based on the obtained spectral data, co-chromatography, m.p. and by comparison with published data[24], compound 1 was identified as gallic acid.

Compound 2: Yellow powder, 16 mg, m.p. 260-262 °C, R_f: 0.8 in S_3; UV λ_{max} nm: MeOH, 268,324; NaOMe, 285,359; $AlCl_3$, 277,300,345; $AlCl_3$/HCl, 278,300,338;

Table 4: Analgesic effect of different concentrations of the methanolic extract of *Lavandula pubescens* flowering aerial parts.

Group	Dose in mg/Kg b.wt.	Time		
		1hr	2hr	3hr
Control	-	7.62 ± 1.02	7.62 ± 1.02	7.62 ± 1.02
Diclofenac	10	13.52* ± 0.84	14.40* ± 0.61	14.8* ± 0.34
Methanolic extract	125	11.09* ± 0.68	8.56 ± 0.63	10.96* ± 0.49
	250	13.82* ± 0.99	10.26* ± 0.77	12.58* ± 1.06
	500	8.34@ ± 0.61	10.80* ± 0.47	6.52@ ± 0.53

Each value represents a mean of 5-6 animals ± SE.
*Significantly different from the control group at p<0.05.
@Significantly different from the Diclofenac at p<0.05.

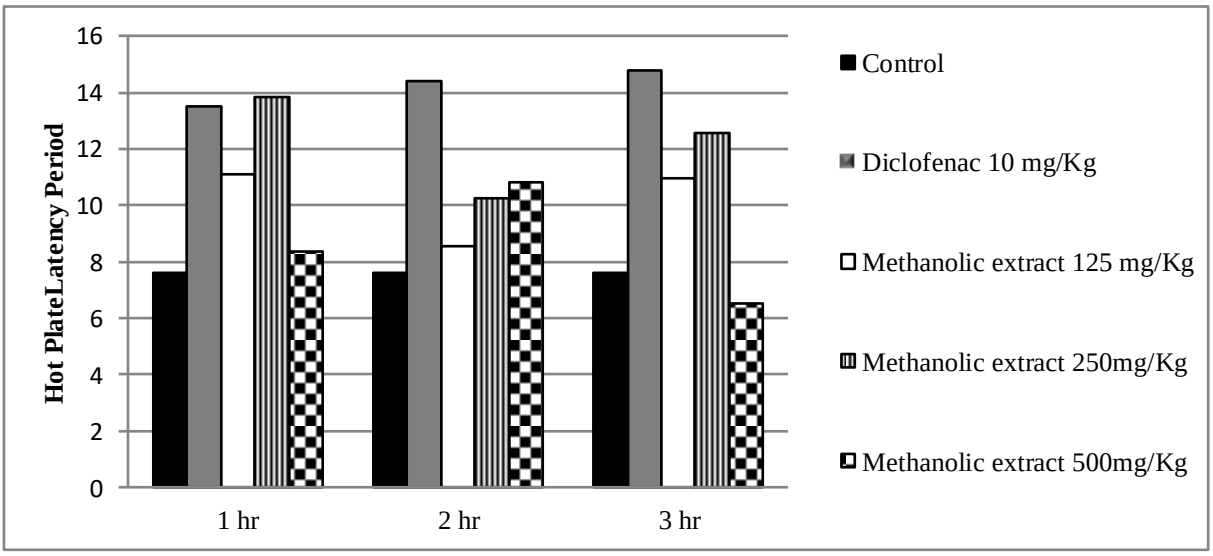


Figure 5: The analgesic activity of different concentrations of the methanolic extract of *Lavandula pubescens* flowering aerial parts.

^{1}H-NMR: δ (400 MHz, DMSO), 12.9 (1H,s,5-OH), 6.94 (1H,s,H-3), 6.395 (1H,d,H-6 J= 2.3), 6.82 (1H,d,H-8 J=2.3), 7.19 (2H,d,3',5' J=8.6), 5.04 (1H,d,glc. H-1"), 3.87 (3H,s,4' OCH_3), 8.08 (2H,d,2',6' J=8.6); ^{13}C-NMR: δ (100 MHz, DMSO), 162 (C-2), 102 (C-3), 183 (C-4), 159 (C-5), 98 (C-6), 164 (C-7), 93 (C-8), 161.92 (C-9), 105 (C-10), 126 (C-1'), 128.75 (C 2',6'), 117.05 (C-3',5'), 56.38 (OCH_3), 152 (C-4'), 94 (glc-C-1"), 73.24 (glc-C-2"), 76.69 (glc-C-3"), 69.82 (glc-C-4"), 76.18 (glc-C-5"), 60.89 (glc-C-6"). From the above findings and through comparison with published data[25], compound 2 was identified as Acacetin-7-O- glucoside.

Another three flavonoids were previously isolated from the dried aerial parts of both *Lavandula coronopifolia* and *L. pubescens* were collected from Gebel El-Shayeb area, Egypt and identified spectroscopically as hypolaetin 8-O-glucuronide, hypolaetin 4-methyl ether 8-O-glucuronide and isoscutellarein 8-O-glucuronide[26].

Anti-inflammatory activity

The anti-inflammatory activity of the methanolic extract of *Lavandula pubescens* flowering aerial parts was evaluated at three different concentrations 125, 250 & 500 mg/Kg b.wt. compared with 10 mg/Kg b.wt. indomethacin. The extract exhibited anti-inflammatory activity at all the tested doses represented by a significant decrease in the weight of oedema (Table 3, Fig. 4). All the tested doses of the extract demonstrated a significant decrease in the weight of oedema. The anti-inflammatory effect was dose-dependant. The potency of the three tested doses (125, 250 & 500 mg/Kg b.wt.) were 49.2 %, 61 % and 81.15 % respectively compared to indomethacin as positive control (potency = 100%).

Surveying the available literature, few reports were found concerning the anti-inflammatory activity of *Lavandula* species[5], whilst nothing was traced regarding anti-inflammatory effect of of *Lavandula pubescens*. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medicine for the treatment of diseases and disorders linked with inflammation, pain and fever[27]. Long-term administration of NSAID may cause severe complications including gastric ulcer, renal damage, bronchospasm and cardiac abnormalities due to their nonselective inhibition of cyclooxygenases (COX) enzymes[27,28,29]. Therefore, new anti-inflammatory, analgesic drugs having lesser effects are being searched all over the world as alternatives to NSAIDs[30]. Medicinal plants derived natural compounds such as flavonoids, lignans and polyphenols are scientifically proved to relieve inflammation, pain and fever[31,32,33].

Analgesic activity

Table 5: Effect of different concentrations of the methanolic extract of *Lavandula pubescens* flowering aerial parts on Serum alanine aminotransferase (ALT), aspertate aminotransferase (AST).

Group	Dose mg/Kg b.wt.	Serum ALT (U/L)	Serum AST (U/L)
Paracetamol	600	121.51*±11.52	612.3*±20.55
Silymarin+Paracetamol	50 +600	60.80@ ± 3.55	413.30* ± 11.96
	125+600	131.0*±6.85	431.0*±32.87
MeOH extract+	250+600	91.75±8.562	400.8±35.87
Paracetamol	500+ 600	90.50*±8.562	308.5±21.61

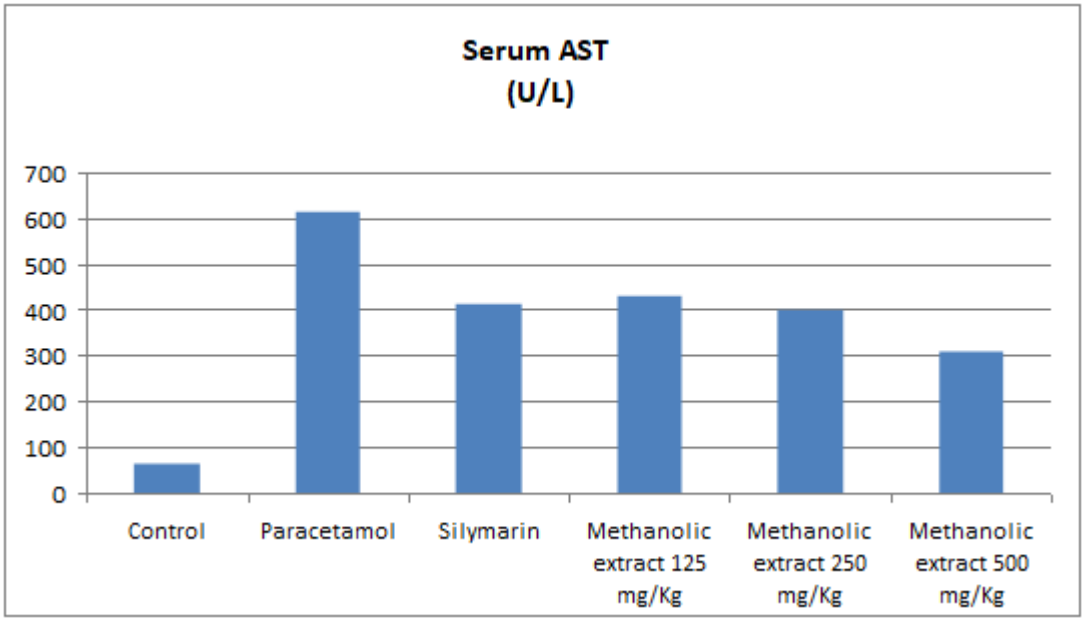


Figure 6: Effect of different concentrations of the methanolic extract of *Lavandula pubecens* flowering aerial parts on the level of AST in Paracetamol-induced hepatic damage in rats.

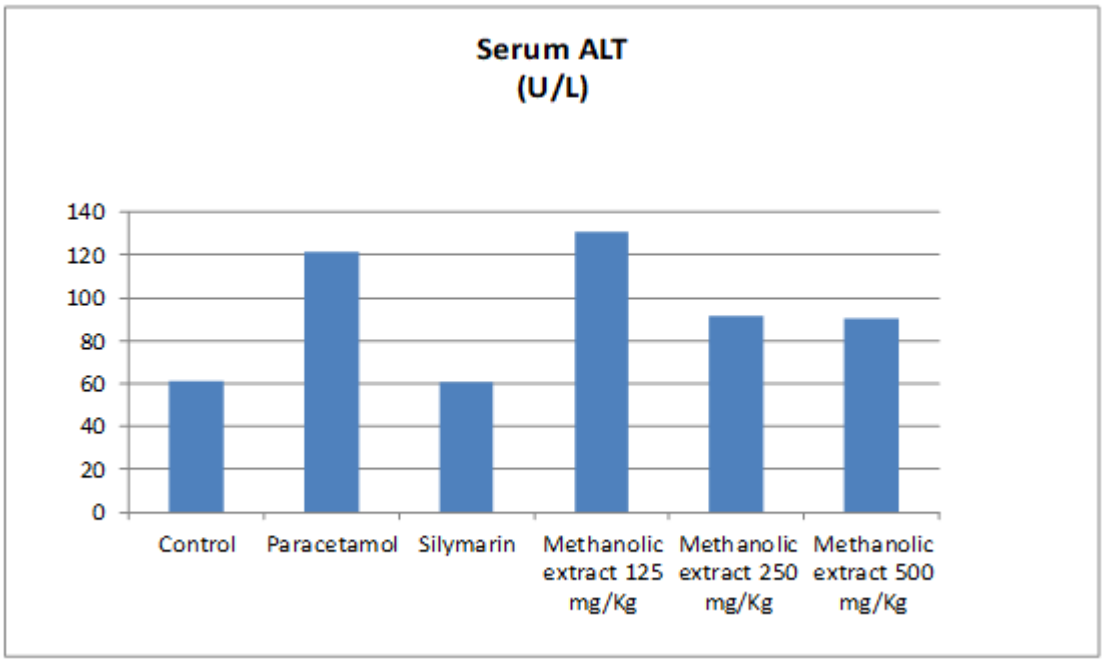


Figure 7: Effect of different concentrations of methanolic extract of *Lavandula pubecens* flowering aerial parts on the level of ALT in Paracetamol-induced hepatic damage in rats.

Tracing the current literature, the leaf extract of *Lavandula angustifolia* demonstrated analgesic activity[5] but nothing was reported concerning the analgesic effect of *Lavandula pubescens*. The analgesic activity of the

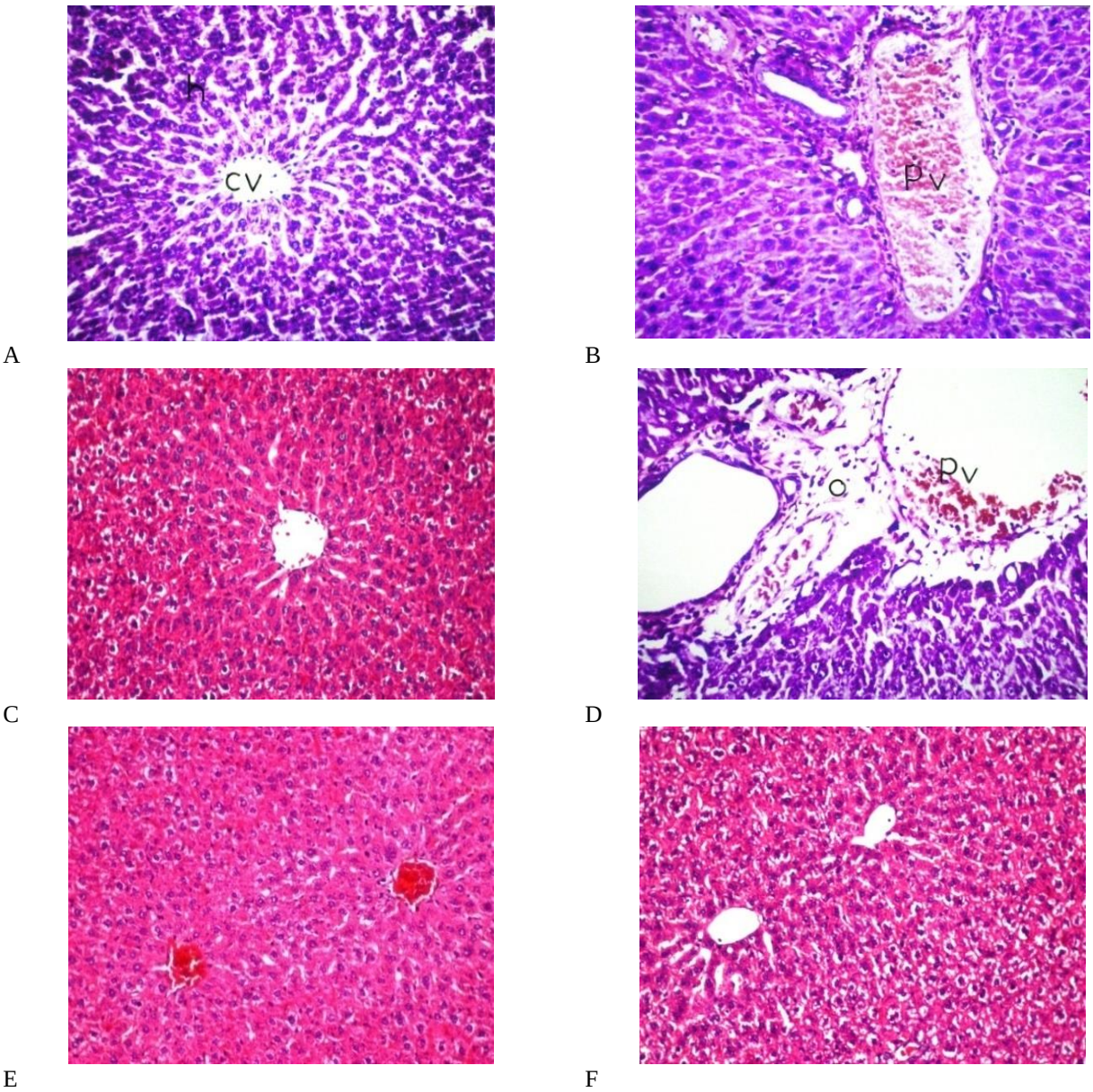


Figure 8: Photomicrographs of liver tissue of: **A**. Rat showing the normal histological structure of the central vein (cv) and hepatocytes (h), **B**. Paracetamol-treated rat (600 mg/kg) showing severe diltation , **C**. silymarin pretreated paracetamol-intoxicated rat (50 mg/kg) showing severe dilatation in portal vein (pv), **D**. extract pretreated paracetamol-intoxicated rat (500 mg/kg) showing no histopahtological alteration. **E**. Extract pretreated paracetamol-intoxicated rat (250 mg/kg) showing no histopahtological alteration. **F**. Mild diltation was observed in central vien (125 mg/kg).

methanolic extract (70%) of *Lavandula pubescens* flowering aerial parts was evaluated at different concentrations 125, 250 & 500 mg/Kg b.wt. compared with 10 mg/Kg b.wt. diclofenac. The extract exhibited analgesic activity at all tested doses represented by a significant increase in the latency period (Table 4, Fig. 5).

Hepatoprotective activity

No information could be traced in the available current literature concerning the study of the hepatoprotective activity of *Lavandula pubescens*. Since some species of *Lavandula* were reported to have antioxidant activity[34-37]therefore, it was deemed of interest to study the hepatoprotective activity of the plant under investigation. Pretreatment with different concentrations of the methanolic extract (70 %) of *Lavandula pubescens* (125, 250 & 500 mg/kg.b.wt), significantly prevented paracetamol-elevated serum levels of AST by (29.6%, 34.54% & 49.6% respectively) and decrease of ALT level at 250 mg/kg.b.wt was 24.49% and at 500 mg/kg.b.wt was 25.52% in rats being compared to the reference hepatoprotective drug silymarin, (32.5% for AST and 49.96 for ALT). Results of biochemical hepatoprotective analysis are presented in (Table 5) and (Figs. 6,7). The results were supported by histopathological examination of the isolated fresh liver tissues (Fig. 8).

Paracetamol is commonly used as antipyretic and analgesic agent. In therapeutic dose of less than 3 g/day, it is usually safe and well tolerated. Paracetamol is

metabolized by liver cytochrome (P450) enzyme (CYP2E1) into *N*-acetyl P-benzoquinoneimine which is a highly reactive metabolic product[38]. The hepatic damage of paracetamol is dependent on the enzymatic activity of CYP2E1 and the availability of glutathione as a hepatoprotector. The metabolism of paracetamol leads to covalently binding to proteins, lipids or nucleic acids and produces oxidative stress by generating free radicals, depletion of reduced glutathione and inducing lipid peroxidation[38]. The resulting oxidative stress affects mitochondrial function and inhibits movement of calcium from cytosol leading to death of hepatocytes[39,40]. High concentrations of serum hepatobiliary enzymes, ALT and AST, are normally present in the liver. Upon necrosis of liver tissues or hepatic damage, these enzymes will be leaked into the circulation resulting in a rise of their serum concentrations[41]. Elevated levels of ALT and AST indicate cellular leakage and loss of functional integrity of cell membrane of hepatocytes[28,41]. A previous study on *Lavandula coronopifolia* demonstrated hepatoprotective properties on the ethanol-induced oxidative stress-mediated cell death in human hepatocellular carcinoma cells and offered new alternatives to the limited therapeutic options to treat liver disease[42]. However in the current study, the methanolic extract (70%) of *Lavandula pubescens* in doses of 250 mg/Kg and 500 mg/Kg prevented paracetamol intoxification and restored the liver tissues to normal (Fig. 8 D & E).

CONCLUSION

The present study showed a reasonable value of the total phenolic and flavonoid content. The methanol extract (70%) of the aerial parts of *Lavandula pubescens* Decne. exerted significant anti-inflammatory, analgesic and hepatoprotective effects through biochemical evaluation. Repeated column chromatography of the ethyl acetate fraction afforded two isolates, which were not previously reported in the genus *Lavandula*, including gallic acid and acacetin-7-O-glucoside. Assessment of the different pharmacological potentialities of *Lavandula pubescens* extract could reflect the traditional medicinal usage of the plant and encouraged its incorporation in herbal formulations after necessary clinical trials.

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