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(Research Article)

Phytochemical and Biological Study of Melissa officinalis (Family Lamiaceae) Cultivated in Egypt

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Abstract: Phytochemical investigation of 70% aqueous methanol extract of leaves of lemon balm (*Melissa officinalis*L., *Lamiaceae*) was subjected to different chromatographic separation techniques. The structures of isolated compounds were determined based on spectral data (UV, ^{1}H-NMR, ^{13}CNMR, and mass spectrometry). Also, assessment of the antioxidant, anticonvulsant, anti-inflammatory as well as antimicrobial activities was determined using different techniques. **Result:** three isoflavones;4'- methoxy genistein [Biochanin A] **(1)**,6, 8 dimethoxy- biochanin A -7 - *O*- α - L- rhamnopyranosyl (1'''-6'') - *O* - β - D- glucopyranoside **(2)**, isoflavone 6, 8dimethoxy 7-*O*- β- D rutinoside, 4'-*O*- α- L-rhamnopyranoside (**3)**.Besides six known flavonoids; hyperin **(4)**, luteolin 7-*O*- rhamnoside**(5)**, scutellarein – 4',7 dimethyl ether**(6)**, nepetin - 7- methyl ether**(7),**luteolin**(8),**and apigenin **(9),**in addition to caffeic acid**(10)**, chlorogenic acid **(11)** isolated for the first time from *Melissa officinalis***.** The examined extract revealed significant anticonvulsant activity, effective in inhibition of both acute & chronic inflammation. Scutellarein – 4',7-dimethyl ether **(6)** showed remarkable inhibition against fungi and exhibited the highest antibacterial effect against both Gram +ve and Gram -ve bacteria. The above evidence suggests that *Melissa officinalis* leaves are a good supply of natural isoflavones that can be utilized to avoid convulsion, and inflammation, as well as an antioxidant and antimicrobial supplement.

Keywords: *Melissa officinalis*; isoflavones; antioxidant; anticonvulsant; anti-inflammatory; antimicrobial.

1. INTRODUCTION

The Lamiaceae (Labiatae) is a flowering plant family frequently identified as the mint family. The enlarged Lamiaceae contains about 236 genera and 7200 species a botanical family, most of them are herbaceous and annuals or perennials growing all over the world, and few tropical species are trees[1,2]. Genus *Melissa* belongs to the family Lamiaceae, which is known as lemon balm, is a perennial or annual herb, mostly square in cross-section, with entire leaves.

Melissa comprises about six species distributed throughout the world. *Melissa officinalis* L. is an aromatic herb native to southern Europe, W. Asia, and The Mediterranean Sea region, this plant is now widely cultivated. Lemon balm has been used in medicinal folkloric remedies[3]. It has been used to treat a variety of health diseases, including the neurological, antimicrobial, central nervous system, anti-malignant, cardiovascular, respiratory, and antidepressant problems, as well as to improve memory in dementia patients. An early recommendation claimed that it might be used for any problems that were thought to come from a nervous system issue[4].

Many additional medical kinds of literature have mentioned *Melissa officinalis* (Mo). Because of its fresh scent, Mo is also utilized in the culinary sector and aromatherapy[5]. Phytochemical studies carried out on *Melissa* species led to the isolation of several essential oils, phenolic acids, some

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flavonoids, and saponins[6]. A wide range of biological activities was studied for *Melissa* species such as antioxidant, anticancer[7, 8], and anti-diabetic effects[9]. Recently, *Melissa officinalis* sharpened memory and repels insects[10].

2. METHODS

2.1. Plant material

Melissa officinalis aerial pieces were collected during the flowering stage in July 2010 from plants cultivated in Agriculture Research Centre, Faculty of Agriculture, and Cairo University, Egypt. The plant identification was established by Dr. Moafi El- Ghadban, Professor of Aromatic and Medicinal Plants, Agriculture Faculty, Cairo University, Egypt. Voucher specimens (Mo-3) were stored in the Pharmacognosy Department, Faculty of Pharmacy, and AL-Azhar University, Egypt.

2.2. Chemicals

The majority of the solvents used in the extraction and isolation were sourced from Al - Gomhoria Company for medicines and medical supplies, Cairo, Egypt. Chemicals that are used to test antioxidant action, DPPH (2,2-Diphenyl-1-picrylhydrazyl), deoxyribose (DOR), tertiary butyl hydroquinone (TBHQ), and Ascorbic acid were purchased from Sigma -Aldrich, Chemical Co, St Louis, Mo, USA and ICN Pharmaceutical incorporation, USA. Authentic reference flavonoid and phenolic compounds were supplied by Agriculture Research Centre, Food Technology Research Institute, Giza, Egypt.

2.3. Experimental animals and microorganisms

Male and female Wistar albino rats were kept under the same hygienic conditions and well balanced diet and water in animal house of Faculty of Pharmacy, AL-Azhar University, Egypt. Animal treatments are followed the ethical and institutional guidelines of the care and use of laboratory animals. The animal care was conducted in accordance with the ethical procedures and policies approved by the animal care and use committee at the Faculty of Pharmacy, Al-Azhar University, Egypt (No 348). Gram positive bacteria, Gram negative bacteria and fungi were obtained from Regional Center for Mycology and Biotechnology.

2.4. Investigation of proteins and amino acids

The proteins and amino acids of the samples were evaluated using Csomos and Simon-Sarkadi technique[12].

2.5. Isolation of phytoconstituents

45Air-dried powdered *Melissa officinalis* aerial parts (1kg) were exhaustively macerated with 70% methanol. (4 x 3L, 1.5h each) After stripping off the solvent under reduced pressure and low temperature ($\simeq$ 50 ºC), affording 250 g of total dry extract. The brownish sticky residue obtained was successively defatted with petroleum ether (3 x 1L), followed by methylene chloride (3 x 1L), followed by suspended in the least amount of distilled water and treated with an excess of methanol (ten folds for desalting) and filtered to give 6, 45, 45 and 120 g of each solvent respectively. The methanol fraction (125g.) was subjected to the column of polyamide S6 chromatography (130 × 3.5 cm). Water was used to begin elution, then a mixture of water /methanol of stepwise decreasing polarities up to pure methanol, which yielded 40 fractions of 500 mL each. By using PC, UV light, spray reagents, and using silica gel plates. Seven collective fractions (A-G) were created by combining comparable fractions which were exposed to consecutive column chromatography on Sephadex LH-20, silica gel, and/or microcrystalline cellulose with different solvent systems to afford eleven pure compounds. All separation processes were followed by 2D-PC and CoPC using Whatman No. 1 paper with BAW for the first run (S_1) and AA for the second run(S_2), [BAW: *n*-BuOH/ AcOH/H_2O (4:1:5, top layer); AA: 15% aqueous AcOH].

2.6. Experimental data of isolated compounds

Compound 1

Yellowish amorphous powder (25 mg), R_f value 0.30 (BAW); purple fluorescence below UV- light, changed to light green by $FeCl_3$ and unclear change with ammonia vapours and Naturstoff spray reagent. **UV-** spectral data λ_{max} (nm) (MeOH) : 261, 330 (sh), (+ NaOMe) : 249, 273, 327, (+ $AlCl_3$) : 273, 310, 375 , (+$AlCl_3$ / HCl) : 273, 310 (sh), 373, (+ NaOAc) : 272 , 327, (+NaOAc / H_3BO_3) : 262, 330(sh). ^{1}H and ^{13}C NMR data are presented in Tables 1 and 2.

Compound 2:

Yellowish white powder (20 mg), R_f value 0.064 (AA); the same chromatographic properties on PC as the previous compound (1). **UV-** spectral data λ_{max} (nm) (MeOH): 248, 273, 340; (+NaOMe): 259, 285, 400; (+NaOAc): 275, 342; (+NaOAc / H_3BO_3): 276, 340; (+$AlCl_3$): 275, 353; (+$AlCl_3$ / HCl): 275, 353. Negative ESI/MS: *m/ź* 651 $[M-H]^-$. ^{1}H NMR data are presented in Table 1.

Compound 3:

Yellowish white amorphous powder (10 mg), R_f value0.12 (AA); the same chromatographic properties on PC as compounds (1, 2). **UV-** spectral data λ_{max} (nm) (MeOH): 248(sh), 273, 340, (+ NaOMe): 259(sh), 285, 400, (+NaOAc): 275, 342, (+NaOAc / H_3BO_3): 276, 340, (+$AlCl_3$): 275, 353, (+$AlCl_3$ / HCl): 275, 353. Negative ESI /MS: *m⁄ź* 783 $[M-H]^-$. ^1H NMR data are presented in Table 1.

Compound 4:

Yellow powder (50 mg), R_f value 0.24 (AA); deep purple fluorescence beneath UV- light, yellow fluorescence by ammonia and turned to orange by Naturstoff spray reagent. The **UV**- spectral data λ_{max} (nm) (MeOH): 259, 266(sh), 299(sh), 359, (+NaOMe): 272, 327, 410,(+NaOAc): 271, 325, 393, (+NaOAc / H_3BO_3): 262, 298, 397, (+$AlCl_3$): 275, 303(sh), 433, (+$AlCl_3$ / HCl): 271, 300, 364(sh), 402. Negative ESI / MS showed that $[M-H]^-$ at 464 (467.73) together with diagnostic fragment at *m⁄ź* 300 (303) which were assigned to $[M\text{-hexose}]^-$ = $[Quercetin\text{-}2H]^-$. ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

Compound 5:

Pale yellow amorphous powder (20 mg), R_f value0.77 (AA); purple fluorescence under UV-light, change to yellow on exposure to NH_3. **UV** spectral data λ_{max} (nm) (MeOH): 264.83, 286, 303, 336.66, (+NaOMe): 259, 324(sh), 379, (+NaOAc): 276, 308, 335, (+NaOAc / H_3BO_3): 312, 352, (+$AlCl_3$): 269, 321, 360.96, (+$AlCl_3$ / HCl): 269, 294, 313,335. ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

Compound 6:

Yellow needle crystals (30 mg), R_f value0.82 (S_1); dark purple fluorescence under UV- light, turned to green with $FeCl_3$ and dark purple fluorescence with ammonia vapours. Negative **ESI / MS**: *m⁄ź* 314 $[M-H]^-$ and major fragment at *m⁄z* 286 which are a characteristic fragment of methoxylated flavonoids. ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

Compound 7:

Yellowish brown crystals (30 mg), R_f value0.80 (S_1); dark purple fluorescence under UV- light, dark purple fluorescence on exposure to NH_3 and give green with $FeCl_3$. ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

2.7. Antioxidant assay

Firstly, we make characterization of antioxidant activity via non-enzymatic lipid peroxidation test[11]by determining the inhibitory activity of different concentrations of methanol extract of (Mo) as well as probucol (as antioxidant reference) on lipid peroxidation which is stimulated by iron/ascorbate system. In the second part, the mechanisms of action of tested extract as an antioxidant *in vitro* were investigated through different tests including diphenyl picryl hydrazine (DPPH) assay[14], deoxyribose (DOR) test[15], and superoxide radical scavenging test[16]. Finally, the pro-oxidant activity of the tested extract was investigated *via* bleomycin-dependent DNA damage[17] using different concentrations of methanol extract of (Mo) as well as TBHQ as a reference. A percent inhibition versus concentration was drawn using linear regression fitting. The IC50 value was calculated using the concentration that caused 50% inhibition.

2.8. Anticonvulsant activity

The anticonvulsant effect was determined according to Vernadakis and Woodbury[18].

2.9. Anti-inflammatory activity

Acute and chronic anti-inflammatory properties of methanol extract of *Melissa officinalis* were tested according to methods described by winter *et al*[19]and Ramprasath *et al.*[20] respectively**.**

2.10. Antimicrobial activity

Compounds **4, 6**, methanol, and water extracts of *Melissa officinalis* were subjected to antimicrobial testing. Using the agar well diffusion method[21], several microorganisms were employed to investigate antibiotic activity by pouring the nutrient agar medium into sterilized plates (12 cm diameter). For each bacterium culture, the test was repeated three times: Penicillin G and Streptomycin were utilized as standard antibacterial medicines. Separately the antifungal activity of examined samples was screened in vitro by agar well diffusion method[22]. For each fungus, the test was repeated three times. Antifungal standard medications included Clotrimazole and Itraconazole. Agar plate method[23] was used to determine the minimum inhibitory concentration (MIC) of the investigated substances.

3. RESULTS

3. 1. Quantitative determination of total amino acids

Different herbal formulae in the market used for the treatment of protein malnutrition contain *Melissa officinalis*[24] therefore; it will be interesting to investigate its protein contents. Quantitative determination of total amino acids revealed that total protein = 8.9 g /100 g extract and 15 amino acids were identified in both free and total amino acids; alpha-amino adipic acid is of the highest percent of the detected amino acids while phenylalanine is of the lowest percent.

Table 1: ^{1}H NMR data of compounds **1-7** (500 MHz, DMSO-d_6**)**

Position	1	2	3	4	5	6	7
2	**8.18 (s)**	**8.46 (s)**	**8.47 (s)**				
3					**6.98 (s)**	**6.78 (s)**	**6.90 (s)**
5				-	-	-	-
6	**6.39 (d, 1.8 Hz)**			**6.17 (d, 1.8 Hz)**	**6.71 (d, 1.8 Hz)**	**6.3 (d, 2.5 Hz)**	**6.19 (d, 2.5 Hz)**
7				-	-	-	-
8	**6.8 (d, 1.8 Hz)**			**6.38 (d, 1.8 Hz)**	**6.73 (d, 1.8 Hz)**	**7.44 (s)**	**6.80 (s)**
2′	**7.66 (d, 10.7 Hz)**	**7.46 (d, 8.4 Hz)**	**7.65 (d, 10 Hz)**	**7.54 (brs)**	**7.45 (dd, 2.5, 8)**	**7.92 (d, 9.2 Hz)**	**7.5 (d, 2.3 Hz)**
3′	**7.32 (d, 8.2 Hz)**	**7.05 (d, 8.4 Hz)**	**7.04 (d, 10 Hz)**	-	-	**7.1 (d, 8.6 Hz)**	-
5′	**7.32 (d, 8.2 Hz)**	**7.05 (d, 8.4 Hz)**	**7.04 (d, 10 Hz)**	**6.81 (d, 9.2 Hz)**	**6.86 (d, 8 Hz)**	**7.1 (d, 8.6 Hz)**	**6.94 (d, 10 Hz)**
6′	**7.66 (d, 10.7 Hz)**	**7.46 (d, 8.4 Hz)**	**7.65 (d, 10 Hz)**	**7.54 (brs)**	**7.45 (dd, 2.5, 8)**	**7.92 (d, 9.2 Hz)**	**7.6 (dd, 2.37 Hz)**
1′′		**5.43 (d, 7.5 Hz)**	**5.39 (d, 7.5 Hz)**	**5.42(brd, 6 Hz)**	**5.3 (brs)**		
1‴		**4.11 (brs)**	**4.25 (brs)**				
1⁗			**4.93 (brs)**				
CH_3- 6''		**1.1 (d, 6.85 Hz)**		-	**1.46 (d, 6.8 Hz)**		
CH_3- 6'''			**1.2 (d, 6.8 Hz)**				
CH_3- 6⁗			**1.04 (d, 6.8 Hz)**				
6-OCH_3		**3.85 (s)**	**3.86 (s)**	-		-	**3.7 (s)**
7-OCH_3				-	-	**3.86 (s)**	**3.90 (s)**
8-OCH_3		**3.77 (s)**	**3.76 (s)**				
4'-OCH_3	**3.87 (s)**	**3.69 (s)**		-	-	**3.70 (s)**	-

3. 2. Identification of separated phytoconstituents

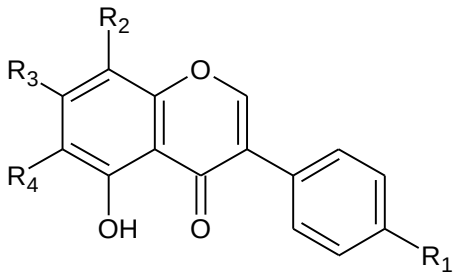
Methanol extract of *Melissa officinalis* aerial parts was fractionated using different chromatographic techniques that resulted in the isolation of eleven compounds. Spectral approaches such as ^{1}H NMR, ^{13}C NMR, and ESI-MS were used to identify them. Following complete acid hydrolysis, CoPC also tested standard genuine sugars and aglycones. Compound **1** exhibited UV spectral information and chromatographic characteristics of 5, 7, and 4' - oxygenated isoflavone aglycone[25]. The isoflavone identity of compound **1** was also supported by the characteristic location of H-2 resonance at about δ8.48 ppm. This ^{1}H-NMR data was compared with that reported before[26]. Hence, compound **1** was confirmed as 4'- methoxy genistein (Biochanin A).

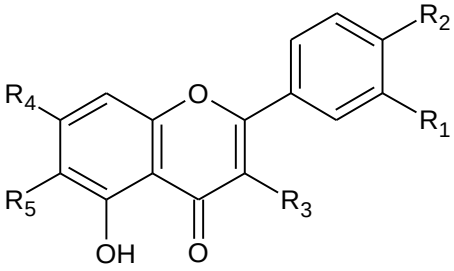
Compound **2** exhibited UV spectral information and chromatographic characteristics of isoflavone - 7 - *O* -glycoside[27]. In the aqueous phase of full acid hydrolysis, glucose and rhamnose were detected (CoPC, aniline hydrogen phthalate reagent). The ^{1}H-NMR spectrum showed the relative downfield position of **X**-type doublet (H-3′/5′) at δ_H 7.05 (Δ + ~ 0.2 ppm) was characteristic for 4'- *O* - methoxyl, which was given as singlet at 3.69. Also, the characteristic location of H-2 resonance at about δ_H 8.14 ppm proved the nature of isoflavone. The absence of both H-8 and H-6 together with the presence of another two singlets at δ_H 3.85 and 3.77 each represent three protons indicative of methoxylation at C-6 and C-8. Also, the existence of β. anomeric proton signal at δ_H 5.4 (H-1") just in the case of 7 - O - glycoside structures is this regarded as a typical position. [28, 29] and reading with NaOAc indicate glycosidation on OH-7.

The presence of terminal rhamnopyranosyl on C-6" of glucoside was established from the distinguishing δ value of anomeric proton at δ_H 4.11 (brs) plus doublet of its CH_3- group at δ_H 1.1.

Table 2: ^{13}C NMR data of compounds **1** and **4-7** (125MHz, DMSO-d_6).

Position	1	4	5	6	7
2	154.5	156.84	164.28	164.51	164.51
3	122.7	133.78	103.42	103.54	103.86
4	180.8	177.93	181.79	182.79	182.79
5	162.4	161.72	161.32	153.17	153.17
6	99.5	99.17	99.77	132.38	132.38
7	164.9	164.65	163.09	159.13	159.14
8	94.2	94.04	94.28	92.08	92.08
9	158.0	156.66	156.43	152.58	152.58
10	104.9	104.44	106.17	103.86	105.60
1′	121.4	121.66	121.90	120.98	121.49
2′	130.6	115.70	114.12	129.05	110.70
3′	115.5	145.27	145.65	113.58	148.58
4′	155.9	148.90	150.31	164.39	147.34
5′	115.5	116.64	116.42	113.58	116.5
6′	130.6	122.14	119.30	129.05	120.98
1″		101.29	100.09		
2″		74.54	70.38		
3″		76.88	72.24		
4″		70.34	72.31		
5″		78.04	69.64		
6″		61.39	60.99		
CH_3- 6‴			17.71		
6-OCH_3	-	-	-		60.57
7-*O* CH_3	-	-	-	56.52	56.98
4'-*O*CH_3	57.21	-	-	56.32	-





1. R_1= OCH_3; R_2=R_4=H; R_3=OH

2. R_1=R_4= R_2=OCH_3; R_3=*O*-rutinose

3. R_1=*O*-rh; R_2= R_4= OCH_3; R_3= *O*-rutinose

4. R_1=R_4=OH; R_2= OH; R_5=H; R_3=*O*-gal

5. R_1=R_2=OH; R_3=H; R_4=*O*-rh; R_5= H

6. R_1= H; R_2=R_4=OCH_3; R_5=OH

7. R_1=OH; R_3=H; R_4=R_5= OCH_3; R_2= OH

Figure 1. Structures of isolated compounds (1-7) from *Melissa officinalis* aerial parts

These data were compared with those of 6, 8 demethoxylated derivatives which were reported before from *Pongamia pinnata*[30]

Hence, compound **2** was first isolated from the plant and requires ^{13}C-NMR & 2D-NMR to confirm a correlation between carbons and protons of glycosidation and methoxylation. :6, 8 dimethoxy-Biochanin A -7 - *O*- α - L- $^{1}C_{4}$- rhamnopyranosyl (1'''-6") - *O* - β - D- $^{4}C_{1}$- glucopyranoside (6, 8 dimethoxy- Biochanin A -7 - *O*- β- D- rutinoside).

Complete acid hydrolysis of compound**3**: glucose and rhamnose (aqueous phase). The ^{1}H-NMR spectrum of this compound was more or less the same as of compound **2** with two differences directly proving the glycosidation on C-4' instead of methoxylation. The first difference was the appearance of another α anomeric proton at 4.93 of rhamnose moiety and doublet of its CH_3 group at 1.04 which was confirmed by the downfield shift of **X-** type, doublet of (H-3'/5') at δ7.04. Based on the information presented above and a comparison to previously published data of isoflavone isolated from *Salvia triloba*[31], compound **3** was expected to be isoflavone 6, 8 dimethoxy 7-*O*- β- D rutinoside, 4'-*O*- α- L- rhamnopyranoside. Compound **3** was first isolated from the plant and further confirmation by ^{13}C, Cosy, HMBC or HMQC should be carried out for complete elucidation.

In addition to the previous compounds, there is also one flavonol glycoside, Quercetin -3- *O*- *β* - galactopyranoside or Hyperin [32](**4**), one flavone glycoside; luteolin 7-*O*- rhamnoside[33](**5**), four flavones aglycones: scutellarein – 4',7 dimethyl ether[34](**6**), nepetin - 7- methyl ether[35](**7**), luteolin[36](**8**), apigenin[37] (**9**) together with two phenolic acids; Caffeic acid[38] (**10**) and Chlorogenic acid[39] (**11**). They were identified by CoPC against authentic samples. Compounds (**1-7**) were isolated for the first time from *M. officinalis*.

3.3. Antioxidant activity

3.3.1. Characterization of antioxidant activity:

Characterization of antioxidant activity is done *via* a non-enzymatic lipid peroxidation test: The antioxidant effect of different concentrations of tested extract of *Melissa officinalis* as well as probucol (as antioxidant reference) was calculated as percent inhibition of TBARS formation. It was observed that all tested concentrations had significant antioxidant effects in a dose-dependent manner; the gradual increase of concentration of *Melissa officinalis* extract was followed by the gradual increase in the percentage of inhibition of non-enzymatic lipid peroxidation but, to less extent than the effect of standard antioxidant substance as probucol.

3.3.2. DPPH assay (free radical scavenging activity):

The ability of *Melissa officinalis* extract, BHT, and TBHQ to act as a donor for electron or hydrogen atoms was measured. The antioxidant effect of *Melissa officinalis* extract was calculated as the percent change of absorbance compared to control. A percent inhibition versus concentration was drawn using linear regression fitting, and the IC50 value was determined. *Melissa officinalis* extract had no inhibitory effect up to 100 mM in comparison to BHT and TBHQ.

3.3.3. Deoxyribose test (DOR test) (Hydroxyl radical scavenging):

The antioxidant properties of *Melissa officinalis* extract were calculated as percent change of absorbance compared to control. A percent inhibition versus concentration was drawn using line regression fitting. The fraction of non-enzymatic lipid peroxidation inhibition of *Melissa officinalis* extract was more than that of standard antioxidant substance **[**tertiary butyl hydroquinone (TBHQ)**]**. On the other hand, there is a similarity between them in that each gradual increase in concentration is followed by a gradual increase in the percentage of inhibition of non-enzymatic lipid peroxidation.

3.3.4. Superoxide radical scavenging test:

The ability of plant extract to scavenge $O_2^{\cdot}$or inhibit the xanthine oxidase enzyme activity was investigated using different concentrations of methanol extract of *Melissa officinalis* and TBHQ (as a reference). *Melissa officinalis* extract had no inhibitory effect in comparison to TBHQ.

3.3.5. Prooxidant activity test (bleomycin-dependant DNA damage):

Finally, the pro-oxidant activity of the tested extract was investigated via bleomycin-dependent DNA damage using different concentrations of methanol extract of *Melissa officinalis* as well as TBHQ as a reference. *Melissa officinalis* extract seemed to have no pro-oxidant activity in the concentration up to 100 µM.

3.4. Anticonvulsant effect

Melissa officinalis methanol extract (tested doses 200, 250, and 300 mg /Kg b.wt.) proved to have potent anticonvulsant activity in comparison to diphenylhydantoin (the widespread antiepileptic drug). It showed a potency of nearly 1.1, 1.2, and 1.3 times the reference drug respectively. The gradual increase in tested doses, followed by a gradual increase in electric convulsive threshold proved the increase in % of response (Figure 2).

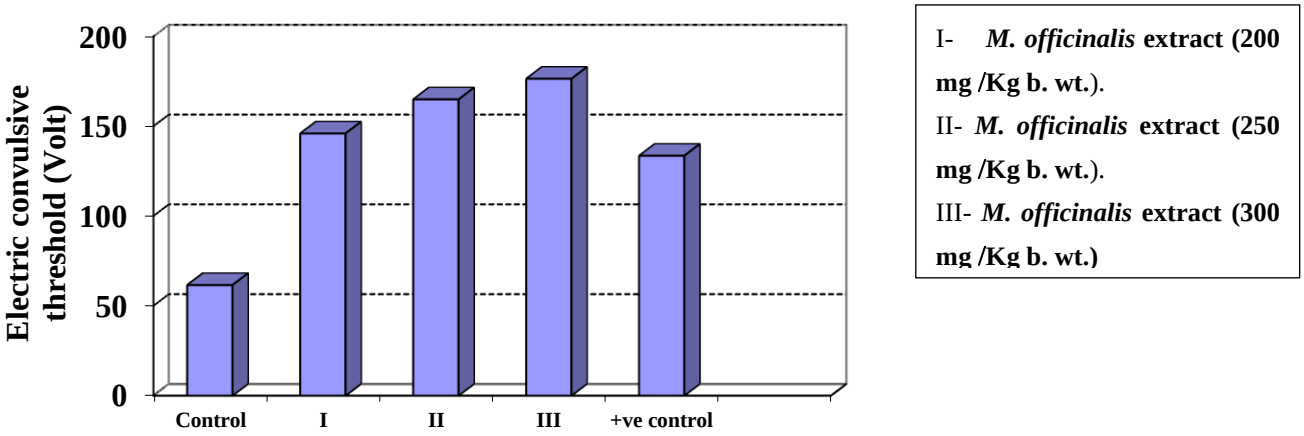


Figure 2. The electric convulsive threshold in adult male rats 1 hour after administration of different doses of *Melissa officinalis* extract.

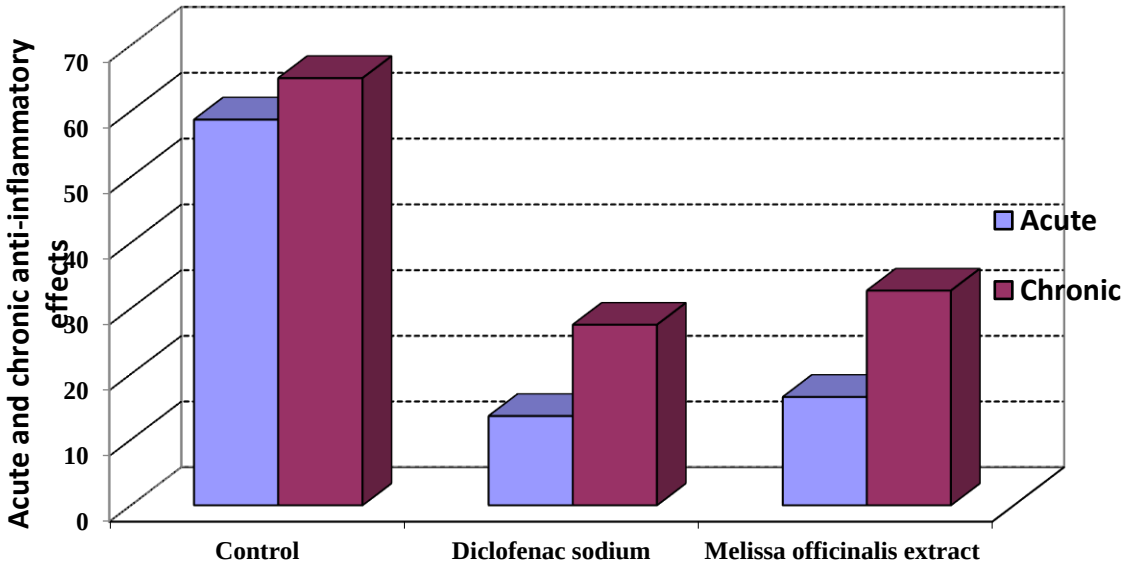


Figure 3. Acute and chronic anti-inflammatory effects of *Melissa officinalis* extract in adult rats.

3.5. Anti-inflammatory activity

The methanol extract of *Melissa officinalis* was significantly effective in reducing the mean cotton pellet weight, which means that they inhibit the granuloma tissue formation due to inhibition of inflammation, in addition to the potency of the extract was nearly matching the effect of diclofenac sodium (reference drug) (Figure 3).

3.6. Antimicrobial activity

All results have complied in Table 3. The growth of *Syncephalastrumracemosum*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* was not affected by any of the tested extracts but the compound **6** (scutellarein – 4',7 dimethyl ether) sample showed remarkable inhibition against other fungi, it also has a strong antibacterial impact against both Gram-positive and Gram-negative microorganisms. Although, water extract did not affect the growth of all fungi it had a notable effect of inhibition on the growth of Gram +ve and Gram-ve bacteria more than that of compound **4** (hyperin). The methanol extract was more effective than both compound 4 and water extract as it showed a significant inhibitory effect on the growth of microorganisms which could be due to the

occurrence of a high percentage of phenolic compound (Figure 4).

Minimum inhibitory concentration (MIC) of tested samples against tested microorganisms was determined. Results showed that compound **6** has the highest activity as antimicrobial since it inhibits the visible growth of microorganisms at least concentration except for its effect on Gram-ve bacteria where methanol extract was more active than it. Also, methanol extract is more effective than both compound **4** and water extract as it had MIC lower than both of them.

Table3. Antimicrobial activities and minimum inhibitory concentrations (µg/mL)(MIC)of tested samples against tested micro-organisms

Sample / Tested microorganisms	Methanol extract		Water extract		Comp.4		Comp. 6		Reference drug (ST.)	
Fungi	**Mean ± SD**	**MIC (µg/ mL)**	**Mean ± SD**	**MIC (µg/ mL)**	**Mean ± SD**	**MIC (µg/ mL)**	**Mean ± SD**	**MIC (µg/ mL)**	**Clotrimazole**	
									Mean ± SD	**MIC (µg/ mL)**
***Candida albicans* (RCMB 005009**)	**14.6± 0.5**	**125**	**NA**	**NA**	**12.8± 0.12**	**125**	**16.7± 0.12**	**62.5**	**18.9±0.3**	**7.81**
***Syncephalastrum racemosum* (RCMB 035003)**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**19.1±0.02**	**3.9**
***Aspergillus fumigatus* (RCMB 002008)**	**19.3± 0.7**	**7.81**	**NA**	**NA**	**11.3± 0.32**	**250**	**18.2± 0.32**	**7.81**	**22.3±0.7**	**0.97**
***Geotricum candidum* (RCMB 052001).**	**20.3± 0.3**	**3.9**	**NA**	**NA**	**16.2± 0.26**	**62.5**	**21.3± 0.26**	**0.97**	**25.3±0.8**	**0.12**
G + ve bacteria									**Penicillin G**	
***Staphylococcus aureus* (RCMB000106)**	**19.8± 0.3**	**3.9**	**15.2± 0.9**	**125**	**14.3± 0.12**	**125**	**21.3± 0.15**	**0.97**	**24.6±0.5**	**0.12**
***Bacillis subtilis* (RCMB 000101)**	**22.1± 0.8**	**0.97**	**20.4± 0.7**	**3.9**	**16.8± 0.15**	**62.5**	**24.6± 0.24**	**0.24**	**26.4±0.4**	**0.06**
***Streptococcuspneumonia* (RCMB 000101)**	**20.1± 0.5**	**1.95**	**16.2± 0.5**	**62.5**	**10.1± 0.32**	**500**	**21.4± 0.32**	**0.97**	**25.7±0.2**	**0.12**
***Micrococcus luteus* (RCMB000109).**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**20.1±0.5**	**3.9**
G - ve bacteria									**Streptomycin**	
***Pseudomonas aeruginosa* (RCMB000102)**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**20.4±0.3**	**3.9**
***Escherichia coli* (RCMB 000103)**	**21.3± 0.5**	**1.95**	**14.2± 0.6**	**125**	**13.6± 0.32**	**12.5**	**16.3± 0.11**	**62.5**	**26.7±0.9**	**0.06**
Salmonella typhimurium (RCMB 000104)	**25.6± 0.7**	**0.12**	**19.2± 0.1**	**7.81**	**18.2± 0.32**	**7.81**	**22.4± 0.34**	**0.97**	**28.7±0.3**	**0.015**
***Klebsiella pneumonia* (RCMB000111).**	**24.3± 0.1**	**0.24**	**16.1± 0.9**	**62.5**	**21.3± 0.26**	**0.97**	**23.2± 0.37**	**0.12**	**27.3±0.7**	**0.03**

4. DISCUSSION

Since many manufactured medications have harmful and/or toxic effects, focus has switched to naturally occurring pharmaceuticals. For the first time, isoflavone aglycone and isoflavone glycosides were extracted from *M. officinalis* as a result of the phenolic and flavonoid extraction process described in this study. In the course of the current *M. officinalis* investigation, the extract possessed strong hydroxyl radical scavenging activity in a concentration-dependent manner. This inhibitory percentage may be favorably associated with the

plant's flavonoidal content. Flavonoids are secondary metabolites that exist naturally in plants; they are beneficial to human health. Given that free radicals and singlet oxygen species are to blame for many

illnesses, they are very potent scavengers against the majority of oxidizing chemicals[40]. *M. officinalis* extracts exhibited a potent anticonvulsant activity in comparison to diphenyl hydantoin. Phenolic and flavonoids compounds have been reported to possess CNS activities[41]. The extract of *Melissa officinalis* has appreciable anti-inflammatory property like the impact of reference drug.

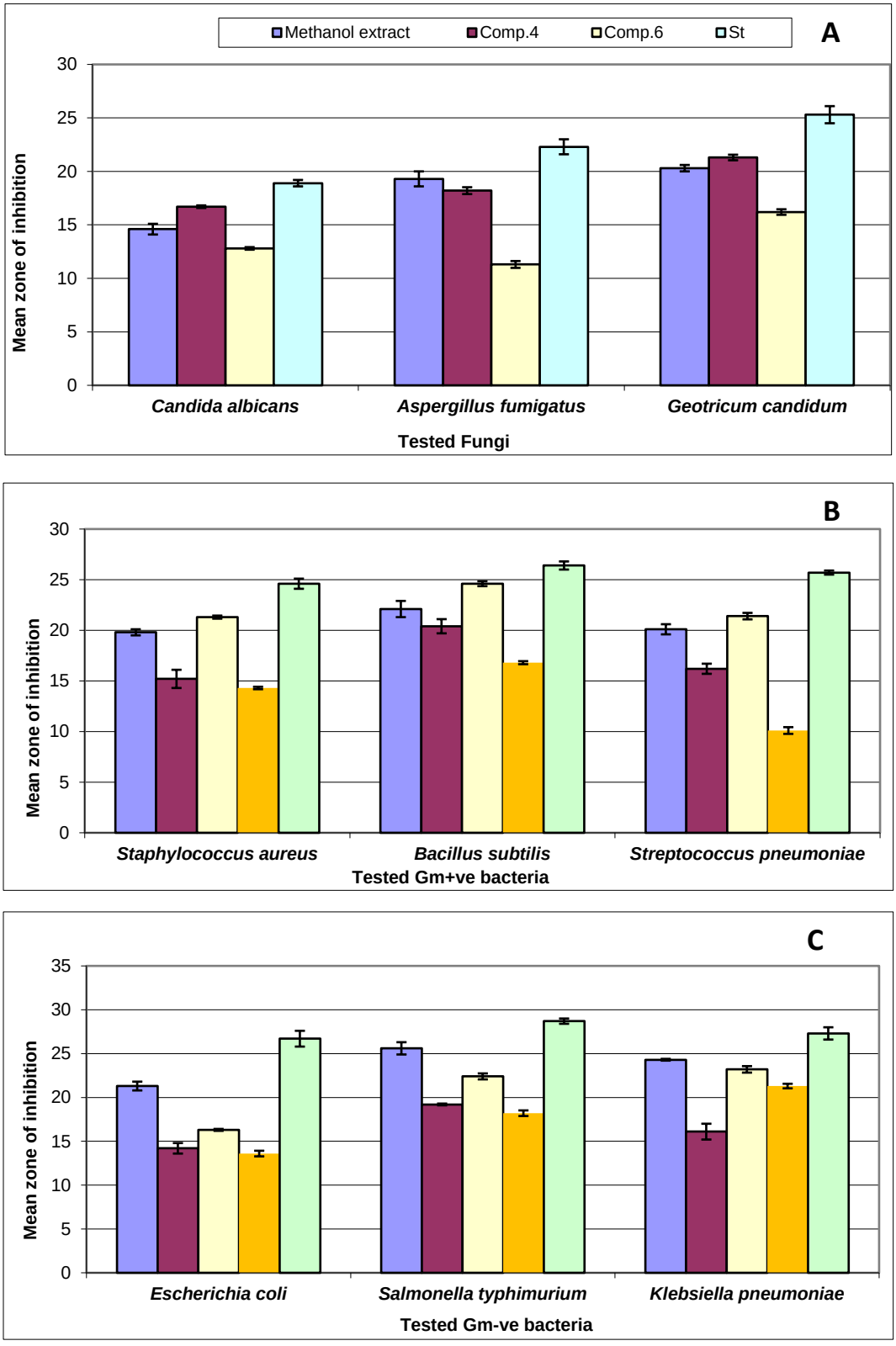


Figure 4. Antimicrobial activities of tested samples against tested micro-organisms

The prior evidence suggests that *Melissa officinalis* has a lot of potential and that it might be used as a natural raw matter for pharmaceutical drugs as it is safe and without side effects, especially since it had another significant hepatoprotective effect. According to Alves et al[42] inhibition zones > 9 mm correspond to active antimicrobial samples. In this work methanol extract and compound 6 exhibited wide spectrum antimicrobial activity against -positive, -negative bacteria and fungi. Compound 6 showed the highest antimicrobial activity against *Staphylococcus aureus*, *Bacillis subtilis*, and *Streptococcus pneumonia* with MIC 0.97, 0.24 and 0.97 respectively. It also showed the highest antifungal effect among the tested samples against *Candida albicans* and *Geotricum candidum* with MIC 62.5 and 0.97 respectively. Methanol extract showed antibacterial activity against *Escherichia coli* and *Salmonella typhimurium* with MIC 0.12 and 0.24 respectively. It exhibited antifungal activity against *Aspergillus fumigates* with MIC 7.81.

5. CONCLUSION

Eleven flavonoids among them three isoflavones for the first time were separated from the dried aerial parts of *Melissa officinalis* extract. This extract possesses potent antioxidant activity and anti-inflammatory and antimicrobial activity. It exhibits a significant anticonvulsant effect supporting the use of the plant as a hypnotic and for the curing of a variety of health problems. Lemon balm's high phenolic contented is chiefly accountable for its therapeutic effects, due to the well-known antioxidant potential of these compounds, so it is important to optimize the extraction conditions to achieve optimal phenolic content and therefore the treatment goals.

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Ethical Statement: The animal care was conducted in accordance with the ethical procedures and policies approved by the animal care and use committee at the Faculty of Pharmacy, Al-Azhar University, Egypt (No 348).

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List of Abbreviations: (Mo): Melissa officinalis; DPPH: diphenyl picryl hydrazine; DOR: deoxyribose; TBHQ: tertiary butyl hydroquinone; Negative ESI /MS: negative electron spray ionization of mass spectroscopy; MIC: minimum inhibitory concentration

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