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# HYPOGLYCEMIC EFFECT OF PHENOLIC CONSTITUENTS FROM *MYRTUS COMMUNIS* L.

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## ABSTRACT

In this study, the aqueous extract from 90% methanol extract of *Myrtus communis* L. leaves was analyzed for their phenolic acids content qualitatively and quantitatively using HPLC. Eleven free phenolic acids representing 31.7 % were identified from the aqueous extract of *Myrtus communis* L. leaves. Elagic, cinnamic and sinapic acids were the most abundant free phenolic acids (19.5 %, 18.6 % and 13.2 %, respectively). Extensive isolation of the aqueous and ethyl acetate extracts resulted in four known compounds gallic acid (1), Ellagic acid (2), 3,4,3'-tri-O-methyl ellagic acid (3) and 3'-O-methylellagic acid 4-O-β-D-glucopyranoside (4). Hypoglycemic activity was confirmed using streptozotocin induced diabetic rats with 400 mg purified aqueous extract of leaves and 100 mg of isolated ellagic acid. These doses were nearly normalized the hyperglycemia within three hours after oral gastric intubations. This study confirmed the Folk medicinal use of *Myrtus communis* L. leaves as hypoglycemic agent.

## INTRODUCTION

Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the Myrtaceae family, grows spontaneously throughout the Mediterranean area and has been used for medicinal, food and spice purposes since ancient times. The leaves and fruit are traditionally used as antiseptic, disinfectant and as hypoglycemic agents for treatment of diabetes mellitus [1-3]. In Folk medicine, the fruits of the plant are used in the treatment of various infectious diseases, including diarrhea and dysentery, whereas the leaves are used as antiseptic and until now several studies have indicated that myrtle herbs could be used as a source of antioxidant and anti-mutagenic agents [4]. Generally, these studies were mainly focused towards the phenolic compounds in myrtle extracts [5]. The previous phytochemical constituents revealed the presence of volatile oils, flavonoids and phenolic compounds. The plant tissues are generally used in preparation of extracts with important pharmacological and antimicrobial activity that is usually ascribed to essential oils, polyphenols and hydrolysable tannins. The aim of the present study was the investigation of the phenolic acids contents of *Myrtus communis* L. leaves. It is worth noting that phenolic acids have interesting and multidirectional pharmacological properties such as anti-bacterial, anti-viral and hypoglycemic effects [6]. Thus identification and quantification of phenolic acids in leaves of *Myrtus comrnunis* L. appears interesting from both a biological and eco-physiological point of view.

## Experimental

### Plant material

Leaves of *Myrtus communis* L. was collected from the Eastern area of Libya and identified by Prof. Hussein Bossela (Faculty of science, Al-azhar University, Cairo, Egypt). A voucher specimen is deposited in Pharmacognosy Department, Faculty of Pharmacy, Al-azhar University, Cairo, Egypt and Department of Science and Food Technology, Faculty of Agriculture, Omar Al-Mokhtar University, Al-Baidha, Libya.

### Extraction

One Kg of dried leaves of *Myrtus communis* L. were exhaustively extracted with 90% methanol. The extract was concentrated in vacuum at 40 °C resulting in a greenish brown residue (82 g). The residue was suspended in $H_2O$ (500 ml), filtered and successively partitioned with petroleum ether 40-60 °C (0.5 L x 3) (3.5 g), chloroform (0.5 L x 3) (2.2 g), and ethyl acetate (0.5 L x 3) (13 g). The remainder aqueous extract was concentrated in vacuum (31.3 g) and kept in a refrigerator for chemical and biological study.

### Chromatographic Methods

**1- Paper chromatography**

Whatman No. 1, mobile phase: S1= acetic acid: water (15:85)

**2- Thin layer chromatography.**

Silica gel plates 60-$F_{254}$ (10 x 20 cm, Merck), mobile phase; S2 = benzene: ethyl acetate: formic acid (80:20:10), S3 = chloroform: acetone: formic acid (80:20:10), S4 = benzene, methanol: acetic acid (90:16:4). Cellulose plates (10 x 20 cm, Merck), mobile phase; S5 = benzene: acetic acid: water (6:7:3).

The purified aqueous extract was subjected to qualitative analysis by PC and TLC against standard phenolic acids. The chromatograms were visualized under UV light ($\lambda$ = 254 nm), before and after exposure to ammonia vapors. All chromatograms were also analyzed after spraying with 2% $FeCl_3$ in 0.5 N HCl solutions in day light. $R_f$ of each phenolic acids and their color reactions were recorded in Table 1.

Table 1: $R_f$ values of phenolic acids in different solvent systems.

| No. | Phenolic acid | $R_f$ values | | | | | Spot color | |
|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | S5 | Vis. | UV |
| 1 | Cinnamic | 0.62 | 0.82 | 0.96 | 0.68 | 0.61 | -- | pb |
| 2 | *O*-coumaric | 0.53 | 0.71 | 0.92 | 0.59 | 0.50 | o | v |
| 3 | *P*-coumaric | 0.53 | 0.71 | 0.92 | 0.56 | 0.47 | cr | nb |
| 4 | Caffeic | 0.45 | 0.68 | 0.80 | 0.38 | 0.81 | bg | Br |
| 5 | Ferulic | 0.36 | 0.42 | 0.68 | 0.24 | 0.8 | y | b |
| 6 | Ellagic | 0.75 | 0.62 | 0.85 | 0.43 | 0.55 | v | b |
| 7 | Isochlorogenic | 0.70 | 0.10 | 0.16 | 0.2 | 0.4 | B | bg |
| 8 | 3-OH-benzoic | 0.45 | 0.7 | 0.88 | 0.75 | 0.6 | v | b |
| 9 | 4-OH-benzoic | 0.4 | 0.75 | 0.83 | 0.7 | 0.65 | v | b |
| 10 | Sinapic | 0.64 | 0.78 | 0.15 | 0.50 | 0.71 | cr | nb |

pb , pale blue; o, orange; v, violet; cr, crimson; nb, navy blue; bg, brownish green; br, brown; b, blue; y, yellow

**3- HPLC Analysis (qualitative and quantitative).**

HPLC analysis was performed using HPLC-DAD Merck-Hitachi (LaChrom, Tokyo, Japan) equipment consisting of LC-1110 pump and a LC-1210 UV (GBC detector), and 7125 injection valve with a 20 µl sample loop. The separation was carried on $C_{18}$-RP column (250 mm x 4.6 mm hypersil). The flow rate was 1 ML / min. and the run time was 60 min. The experiment was carried out at room temperature and detection with UV at 230-280 nm. Mobile phases (A) 0.8 % formic acid in water (B) acetonitrile. A gradient solvent system was used [7] as shown in Table 2.

Gradient elution was developed with standard phenolic acids, sinapic, *o*, *p*, *m*-coumaric, ellagic, cinnamic, ferulic, caffeic, isochlorogenic and 4-OH benzoic acids were obtained from Fluka Chemie GmbH, (Switzerland). Calibration curve was obtained by plotting peak area against concentration of standard phenolic acids as shown in Table 2.

**Table 2:** Gradient mobile phase system of HPLC technique.

| Time | 0.8 % formic acid in water | Acetonitrile |
|---|---|---|
| 0 | 95 | 5 |
| 20 | 75 | 25 |
| 40 | 50 | 50 |
| 45 -60 | 25 | 75 |

**4- Isolation of the secondary metabolites from aqueous and ethyl acetate fractions.**

TLC experiments of the aqueous and ethyl acetate soluble fractions showed close similarities of the $R_f$ values and gave the same color reactions with vanillin/$H_2SO_4$ spraying reagent, therefore, aqueous and ethyl acetate soluble fractions were chosen for further isolation and identification for their major secondary metabolites separately.

A portion of aqueous fraction (5g) was chromatographed over silica gel column chromatography using mixtures of $CHCl_3$-MeOH (8:-2→ 3:7) to give four fractions AA (950 mg), AB (780 mg), AC (325 mg), and AD (250 mg). Fractions AB and AC were separately re-chromatographed over silica gel column chromatography using mixtures of $CHCl_3$-MeOH (8:-2→ 3:7) to afford sub-fractions (AB-1 and AC-1), each was further purified by repeated silica gel chromatography (8:2 → 5:5) and Sephadex LH-20 column chromatography using (MeOH) as eluent to afford compounds **1** (31mg) and **2** (84 mg).

A portion of the ethyl acetate soluble fraction (5 g) was chromatographed over silica gel column chromatography using mixtures of $CHCl_3$-MeOH (8:-2→ 3:7) to give five fractions EA (840 mg), EB (510 mg), EC (312 mg), ED (214 mg) and EE (280 mg). Fractions B and C were separately re-chromatographed over silica gel column chromatography using mixtures of $CHCl_3$-MeOH (8:-2→ 3:7) to afford sub-fractions (EB-1 and EC-1), each was further purified by repeated silica gel chromatography (8:2→5:5) and Sephadex LH-20 column chromatography using (MeOH) as eluent to afford compounds **3** (24 mg) and **4** (18 mg).

**Biological methods**

**I) Hypoglycemic effect of ethyl acetate soluble fraction of *Myrtus communis* L. on streptozotocin induced diabetic rats [8-11].**

Twenty male western albino rats (100-150 g) were used and allowed for free access to tap water and laboratory chow. Rats were divided into four groups A, B, C and D. Group A was used as control non diabetic, group B was used as control diabetic, group C was used as diabetic rats treated with the aqueous extract (200 mg) and group D was used as diabetic rats treated with the aqueous extract (400 mg). Diabetes was induced by intraperitonial injection of streptozotocin at a dose 65 mg/Kg body weight (freshly dissolved in 0.01 mol/L citrate buffer, pH 4.5) as a single dose. This dose is to induce type I diabetes. Animals were allowed for free access to tap water and laboratory chow then subjected after 2 days for non fasting blood glucose determination using blood glucose meter (Accu-chek, Germany). Blood samples were collected by

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cutting tail with sharp razor. Normal rats and that showed marked elevation in blood glucose level were allowed for oral administration of aqueous extract with 200 and 400 mg/ Kg body weight/ day in divided doses (every 12 h). Oral administration of the drug was carried out using gastric intubation. After 2 days of drug administration, normal and diabetic rats (treated and non treated) were subjected for non fasting blood glucose level determination then the drug was stopped for 2 days then diabetic rats were subjected for blood glucose level determination again. Each reading was the average of three measurements as shown in Table 3.

Table 3: Hypoglycemic effect of aqueous extract of *Myrtus communis* on streptozotocin diabetic rats.

| No. | Non fasting B. G. before treatment/SE | Non fasting B. G. after 2 days of treatment/ SE | Non fasting B. G. after 2 days of stopping treatment/ SE |
|---|---|---|---|
| Group A (normal rats) | | | |
| 1 | 91 ± 5.5 | 90 ± 4.5 | |
| 2 | 93 ± 7.1 | 89 ± 6.4 | |
| 3 | 87 ± 6.3 | 79 ± 3.7 | |
| 4 | 94 ± 4.2 | 84 ± 7.4 | |
| 5 | 86 ± 3.8 | 80 ± 9.5 | |
| Group B (control diabetic without any treatment) | | | |
| 1 | 412 ± 8.2 | 384 ± 11 | 399 ± 8.7 |
| 2 | 379 ± 9.4 | 406 ± 7.1 | 384 ± 9.1 |
| 3 | 401 ± 10.3 | 391 ± 6.4 | 411 ± 10.6 |
| 4 | 349 ± 9.9 | 381 ± 5.8 | 434 ± 12.1 |
| 5 | 371 ± 11.3 | 394 ± 7.6 | 358 ±10.2 |
| Group C (diabetic rats + aqueous extract 200 mg/Kg) | | | |
| 1 | 333 ± 7.3 | 290 ± 3.2 | 392 ± 10.3 |
| 2 | 391 ± 9.6 | 340 ± 4.6 | 401 ± 4.5 |
| 3 | 400 ± 8.9 | 341 ± 8.7 | 421 ± 5.4 |
| 4 | 405 ± 12.4 | 388 ± 6.1 | 482 ± 7.5 |
| 5 | 410 ± 10.5 | 380 ± 4.5 | 444 ± 6.5 |
| Group D (Diabetic rats + aqueous extract 400 mg/Kg ) | | | |
| 1 | 423 ± 9.4 | 149 ± 4.1 | 349 ± 6.9 |
| 2 | 357 ± 8.1 | 131 ± 3.9 | 412 ± 11.1 |
| 3 | 369 ± 11.9 | 134 ± 5.6 | 376 ± 8.4 |
| 4 | 413 ±12.6 | 153 ± 6.3 | 391 ± 6.7 |
| 5 | 388 ±10.4 | 121 ± 4.7 | 422 ± 9.8 |

**II) Time course of 400 mg of aqueous extract of *Myrtus communis*.**

Fifteen male western albino rats (100-150 g) were used and allowed for free access to tap water and laboratory chow. Rats were divided into four groups A, B and C. Group A was used as control non diabetic and group B was used as control diabetic rats while group C was used as diabetic rats that treated with the aqueous extract from *Myrtus communis*. After confirmation of hyperglycemia as in first experiment, diabetic rats were administrated 400 mg/Kg body weight. Diabetic rats were subjected for blood glucose determination after 2h, 3h and 4h from oral drug administration as shown in Table 4. Each reading was the average of three measurements.

**III) Hypoglycemic effect of ellagic acid from *Myrtus communis* L. on streptozotocin diabetic rats.**

Fifteen male western albino rats (100-150 g) were used and allowed for free access to tap water and laboratory chow. Rats were divided into three groups A, B and C. Group A was used as control non diabetic and group B was used as control diabetic rats while group C was used as treated diabetic rats with ellagic acid isolated from *Myrtus communis* L.. After confirmation of hyperglycemia as in first experiment, diabetic rats were administrated 100 mg/ Kg/ day body weight as a single dose. Normal and control diabetic rats were allowed for saline administration as placebo. Diabetic rats (control and treated) were subjected for blood glucose determination after 3h from oral administration of the saline and drug respectively as shown in Table 5. Then, rats were allowed for drug withdrawal and subjected for blood glucose level determination after 2 days of stopping drug administration. Each reading was the average of three measurements.

**Table 4:** Time course of 400 mg of aqueous extract of *Myrtus communis*.

| No. | Non fasting B. G. Before treatment | Non fasting B. G. after treatment for 2h | Non fasting B. G. after treatment for 3h | Non fasting B. G. after treatment for 4h |
|---|---|---|---|---|
| Group A (control non diabetic) | | | | |
| 1 | 89 ± 5.2 | 94 ± 6.4 | | |
| 2 | 92 ± 6.1 | 81 ± 8.5 | | |
| 3 | 95 ± 6.4 | 80 ± 6.3 | | |
| 4 | 90 ± 4.3 | 78 ± 9.2 | | |
| 5 | 88 ± 3.2 | 101 ± 8.7 | | |
| Group B (control diabetic) | | | | |
| 1 | 401 ± 7.4 | 410 ±10.2 | | |
| 2 | 391 ± 5.1 | 431 ± 11.3 | | |
| 3 | 379 ± 6.9 | 411 ± 9.9 | | |
| 4 | 415 ± 9.6 | 427 ± 9.7 | | |
| 5 | 410 ± 10.1 | 454 ±12.3 | | |
| Group C (diabetic rats treated with 400 mg of ethyl acetate soluble fraction). | | | | |
| 1 | 413 ± 7.6 | 199 ± 3.4 | 143 ± 6.2 | 165 ± 8.1 |
| 2 | 395 ± 5.4 | 174 ± 4.6 | 119 ± 4.3 | 130 ± 4.9 |
| 3 | 409 ± 6.4 | 181 ± 5.3 | 139 ± 4.1 | 148 ± 7.6 |
| 4 | 396 ± 7.9 | 166 ± 3.5 | 129 ± 3.8 | 141 ± 6.1 |
| 5 | 422 ± 9.8 | 211 ± 4.1 | 154 ± 5.4 | 172 ± 5.5 |

**Table 5:** Hypoglycemic effect of ellagic acid from *Myrtus communis* leaves on streptozotocin diabetic rats.

| No. | Non fasting B. G. before treatment/SE | Non fasting B. G. after 100 mg/ kg / day of ellagic acid | Non fasting B. G. after 2 days of stopping treatment/ SE |
|---|---|---|---|
| Group A (normal rats) | | | |
| 1 | 91 ± 5.5 | 90 ± 4.5 | |
| 2 | 93 ± 7.1 | 89 ± 6.4 | |
| 3 | 87 ± 6.3 | 79 ± 3.7 | |
| 4 | 94 ± 4.2 | 84 ± 7.4 | |
| 5 | 86 ± 3.8 | 80 ± 9.5 | |
| Group B (control diabetic without any treatment) | | | |
| 1 | 450 ± 8.2 | 419 ± 11 | 431 ± 8.7 |
| 2 | 392 ± 9.4 | 416 ± 7.1 | 401 ± 9.1 |
| 3 | 411 ± 10.3 | 399 ± 6.4 | 401 ± 10.6 |
| 4 | 394 ± 9.9 | 398 ± 5.8 | 414 ± 12.1 |
| 5 | 381 ± 11.3 | 404 ± 7.6 | 398 ±10.2 |
| Group C (diabetic rats + 100 mg/ kg / day of ellagic acid) | | | |
| 1 | 433 ± 9.4 | 194 ± 4.1 | 423 ± 6.9 |
| 2 | 375 ± 8.1 | 141 ± 3.9 | 397 ± 11.1 |
| 3 | 399 ± 11.9 | 154 ± 5.6 | 333 ± 8.4 |
| 4 | 431 ±12.6 | 153 ± 6.3 | 441 ± 6.7 |
| 5 | 418 ±10.4 | 131 ± 4.7 | 402 ± 9.8 |

## Results and Discussion

PC, TLC, and HPLC analysis of the remained aqueous extract obtained from *Myrtus communis* leaves, revealed the presence of several free phenolic acids that was shown in Table 6.

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Table 6: HPLC analysis of phenolic acids obtained from leaves of *Myrtus communis*.

| No. | Phenolic acids | $R_t$ (min.) | % | $R_f$ standard |
|---|---|---|---|---|
| 1 | 4-OH-benzoic | 9.7 | 1.7 | 9.9 |
| 2 | Isochlorogenic | 10.8 | 0.4 | 10.5 |
| 3 | 3-OH-benzoic | 12.1 | 0.5 | 12.2 |
| 4 | Caffeic | 14.8 | 1.8 | 14.5 |
| 5 | *p*-coumaric | 19.5 | 2.2 | 19.6 |
| 6 | *m*-coumaric | 22.4 | 3.8 | 22.1 |
| 7 | Ferulic | 23.4 | 2.3 | 23.3 |
| 8 | Sinapic | 25.4 | 4.2 | 25.1 |
| 9 | *O*-coumaric | 27.9 | 2.8 | 27.5 |
| 10 | Ellagic | 29.1 | 6.2 | 28.9 |
| 11 | Cinnamic | 33.2 | 5.9 | 31.9 |

The total percentage of free phenolic acids was 31.7 %. Eleven free phenolic acids were identified with different percentages. They were 4-OH-benzoic, Isochlorogenic, 3-OH-benzoic, Caffeic, *P*-Coumaric, *m*-Coumaric, Ferulic, Sinapic, *O*-Coumaric, Ellagic and Cinnamic acids. Ellagic acid and Cinnamic acid were the most pronouncing in the test sample (6.2 % and 5.9 %, respectively).

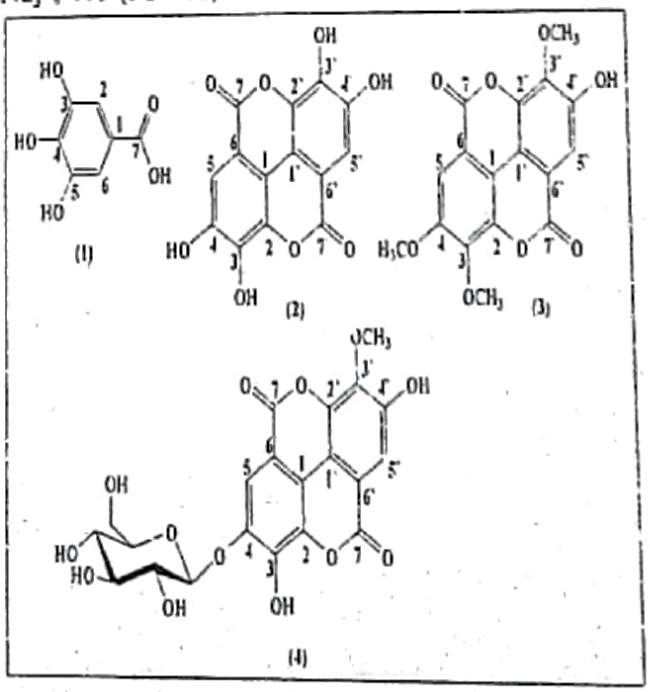
**Identification of Isolated Compounds**

**Compound 1 (Gallic acid):** White powder, UV $\lambda_{max}$ 272, 364 nm (methanol); IR (KBr) $Cm^{-1}$: 3445 (br. OH), 2925 (Carboxyl), 1590 (aromatic ring). $^1$H-NMR ($CD_3OD$): $\delta_H$ 7.11 (2H, s. H-2, 6). $^{13}$C-NMR ($CD_3OD$) $\delta_C$: 122.10 (C-1), 112.26 (C-2, C-6), 146.90 (C-3, C-5), 138.86 (C-4), and 169.16 (C-7).ESI-MS (positive mode) *m/z* 171 $[M + H]^+$, 153 $(M + H- H_2O)$ and 125 $(M - COOH)^+$.

**Compound 2 (Ellagic acid):** Amorphous powder, UV $\lambda_{max}$ 215, 250sh, 258, 272 and 351 nm. IR spectrum (KBr) $Cm^{-1}$: 3518, 3396 and 3262 (free and hydrogen bonded OH groups), 1737 and 1726 $cm^{-1}$ (ester carbonyl groups), and 1620, 1587 and 1494 (aromatic rings). $^1$H-NMR ($CD_3OD$): $\delta_H$ 7.72 (1H, s, H-5), 7.86 (1H, s, H-5`), $^{13}$C- NMR ($CD_3OD$) $\delta_C$: 112.32 (C-1), 136.10 (C-2), 143.65 (C-3), 149.87 (C-4), 111.12 (C-5), 108.35 (C-6), 161.20 (C-7), 112.65 (C-1`), 136.10 (C-2`), 143.50 (C-3`), 149.98 (C-4`), 110.95 (C-5`), 108.35 (C-6`), 161.55 (C-7`). ESI-MS (positive mode) *m/z* 303 $[M + H]^+$ and 325 $[M + Na]^+$.

**Compound 3 (3,4,3`-tri-O-methyl ellagic acid):** morphous powder, UV $\lambda_{max}$ 372, 356 (sh), 252 nm. IR spectrum (KBr) $Cm^{-1}$: 3425 (OH), 2920, 2850, 1735 (lactone carbonyl), and 1605, 1570, 1455, 1360, (aromatic rings). $^1$H-NMR ($CD_3OD$): $\delta_H$ 7.72 (1H, s, H-5), 7.80 (1H, s, H-5`). $^{13}$C- NMR ($CD_3OD$) $\delta_C$: 111.65 (C-1), 142.10 (C-2), 139.50 (C-3), 153.10 (C-4), 110.30 (C-5), 112.70 (C-6), 156.63 (C-7), 111.50 (C-1`), 142.25 (C-2`), 140.19 (C-3`), 152.85 (C-4`), 109.90 (C-5`), 112.78 (C-6`), 157.40 (C-7`), 56.77 ($OCH_3$-C-2`), 58.10 ($OCH_3$-C-3`), 58.66 ($OCH_3$-C-4`). ESI-MS (positive mode) *m/z* 345 $[M + H]^+$ ($C_{17}H_{12}O_8$).

**Compound 4 ( 3`-O-methylellagic acid 4-O-β-D-glucopyranoside):** White needle crystals, UV $\lambda_{max}$ 263, 355 nm. IR spectrum (KBr) $Cm^{-1}$: 3415 (OH), 1710 (lactone carbonyl), and 1610, 1575, (aromatic rings). $^1$H-NMR ($CD_3OD$): $\delta_H$ 7.79 (1H, s, H-5), 7.65 (1H, s, H-5`), 3.98 (3H, s, $OCH_3$-3`), 4.93 (H-1``), 3.54 (m, H-2``), 3.38 (m, H-3``), 3.22 (m, H-4``), ,3.52 (m, H-5``), 3.62, 3.78 (m, $H_2$-6``). $^{13}$C- NMR ($CD_3OD$) $\delta_C$: 109.35 (C-1), 139.45 (C-2), 142.18 (C-3), 149.55 (C-4), 111.12 (C-5), 115.30 (C-6), 157.82 (C-7), 113.67 (C-1`), 144.10 (C-2`), 141.78 (C-3`), 151.64 (C-4`), 114.55 (C-5`), 113.20 (C-6`), 157.60 (C-7`), 60.10 ($OCH_3$-C-3`); glucose moiety: 103.12 (C-1``), 74.45 (C-2``), 75.66 (C-3``), 69.90 (C-4``), 78.10 (C-5``), 62.28 (C-6``). ESI-MS (positive mode) *m/z* 501 $[M + Na]^+$, 479 $(M + H)^+$




**Structure elucidation of Isolated Compounds**

**Compound 1** was obtained as a white powder. ESI-MS showed the molecular ion peak at m/z 170 $(M)^+$ and 171 $(M+H)^+$ which are compatible with a molecular formula $C_7H_6O_5$. The IR spectrum exhibited strong absorptions at 3445, 1590 and 2925 $cm^{-1}$ indicating the existence of hydroxyl, aromatic and carboxyl functionalities.

The $^1$H-NMR spectrum of **1** clearly showed two equivalent protons of two *meta* coupled aromatic signals at δ 7.11 of a sharp singlet, H-2 and H-6. These signals together with signals due to the presence of three singlet protons observed at δ 4.82 (3H, OH-3, 4 and 5) were also observed. The $^{13}$C-NMR of **1**, was similar to those of gallic acid [12], showing two symmetric methine carbon signals at δ112.26 (C-2 and C-6), signals of tetra-substituted quaternary carbons δ 122.10 (C-1), 146.90 (C-3 and C-5), and δ 138.86 (C-4), and a carboxyl group at δ 169.16. Accordingly, on the basis of the spectral data and a comparison with the data reported previously [12]. The structure of compound **1** was determined to be 3,4,5-trihydroxybenzoic acid (Gallic acid).

**Compound 2** was obtained as white amorphous powder and gave a blue color with ferric chloride reagent, revealing that compound **2** is a phenolic compound. It gave *quasi*-molecular ion peaks at *m/z* 303 $[M + H]^+$ and 325 $[M + Na]^+$, respectively; In the positive ESI-MS measurement and its molecular formula $C_{14}H_6O_8$ indicated its $M^+$ was 302 mass units. The UV spectrum of compound **2** in MeOH solution showed a characteristic absorption curve with maximum absorptions at 215, 250 sh, 258, 272 and 351 nm. The IR spectrum compound **2** showed absorption bands at 3518, 3396 and 3262 cm-1 (free and hydrogen-bonded OH groups), 1737 and 1726 $cm^{-1}$(carbonyl groups), and 1620, 1587 and 1494 $cm^{-1}$ (aromatic rings). $^1$H- and $^{13}$C-NMR spectral data of compound **2** displayed two isolated $sp^2$ methine aromatic proton and carbon signals at $\delta_H$ 7.72 (1H, s, H-5, $\delta_C$111.12) and $\delta_H$ 7.86 (1H, s, H-5', $\delta_C$110.95). By $^{13}$C-NMR two conjugated ester carbonyl at $\delta_C$ 161.20 (C-7) and 161.55 (C-7'), six oxygenated $sp^2$ quaternary at $\delta_C$ [136.10 (C-2), 143.65 (C-3), 149.87 (C-4), 136.10 (C-2'), 143.50 (C-3') and 149.98 (C-4')] and four $sp^2$ quaternary carbon signals at $\delta_C$ [112.32 (C-1), 108.35 (C-6), 112.65 (C-1'), 108.35 (C-6')] were also observed. On the basis of spectral data and a comparison with the data reported previously [13-16]. Compound **2** was identified as ellagic acid.

**Compound 3** gave a dark-blue color under UV light and a yellow color with alkali, and showed absorptions at $\lambda_{max}$ 372, 356 (sh), 252 nm consistent with an ellagic acid derivative. The molecular formula of compound **3**, $C_{16}H_{10}O_8$ was obtained by EIS-MS at 344 $(M)^+$ and 345 $[M + H]^+$. The $^1$H NMR spectrum of compound **3** displayed only two singlet proton signals at $\delta_H$ 7.72 (1H, s, H-5), and 7.80 (1H, s, H-5') for aromatic protons and three methoxyl singlet signals at $\delta_H$ 3.88 (OMe-3), 3.95 (OMe-4) and 4.05 (OMe-3'), indicating the presence of three methoxyl signals in compound **3**. $^{13}$C-NMR spectrum showed signals for two aromatic carbon at $\delta_C$ 110.30 (C-5), and 109.90 (C-5'), twelve quaternary carbons including two carbonyl groups at δ 156.63 (C-7) and 157.40 (C-7'), and six oxygenated carbons at 142.10 (C-2), 139.50 (C-3), 153.10 (C-4), 142.25 (C-2'), 140.19 (C-3') and 152.85 (C-4'), and three methoxyl carbons at δ 56.77 ($OCH_3$-C-2'), 58.10 ($OCH_3$-C-3') and 58.66 ($OCH_3$-C-4').
On the bases of these spectral data and a comparison with the data reported previously [17, 18], the structure of compound **3** was determine as (3, 4, 3'-tri-O-methyl ellagic acid).

**Compound 4** was obtained as white needle crystals and gave on TLC greenish brown and yellowish color with methanolic $FeCl_3$ and vanillin/$H_2SO_4$, respectively. Bands for hydroxyl (3415 $cm^{-1}$) and chelated carbonyl (1710 $cm^{-1}$) functional groups were suggested by IR, and the UV absorptions at $\lambda_{max}$ 254, 355 nm was similar to that of ellagic acid **2** suggesting that **4** has an ellagic acid skeleton. ESI-MS (positive ion mode) of compound **4** gave a molecular ion at m/z 501 $[M + Na]^+$ which together with the $^1$H- and $^{13}$C-NMR spectral data was consistent with the empirical formula $C_{21}H_{18}O_{13}$. The $^1$H-NMR spectrum of **4** revealed two protons as singlets at δ 7.66 and 7.55, assignable to H-5 and H-5', respectively, by comparing with the $^1$H-NMR data of ellagic acid **2**. The $^1$H-NMR spectrum of **4** also showed an aromatic methoxy group at δ 3.82 (3H, *s*). The sugar was identified as β-D-glucose from the coupling constant of the anomeric proton (δ 4.99, $J$ = 7.8 Hz; H-1''). The position of the glycosidic linkage to the aglycone was confirmed on the basis of HMBC experiment.

The HMBC spectrum of **4** showed that the anomeric proton of glucose was correlated with C-4 (δ 147.1) of the aglycone moiety, which, in turn, correlated with H-5 (δ 7.66). The position of the methoxyl linkage to ellagic acid was deduced from the HMBC experiment and comparison with model compound **2** [13-16]. The chemical shift of the methyl carbon (δ 60.10) of **4** was similar to that of the 3-O-methyl derivative (δ 60.8, compound **2**), but different from that of the 4-O-methyl derivative (d 56–57), suggesting that the methoxyl group is located at C-3 or C-3' [13-16]. The presence of methoxyl group at C-3' was confirmed by HMBC experiment, in which the H-5' signal (δ 7.55) showed a cross peak with C-3' (δ 140.5), and the C-3' signal, in turn, showed a cross peak with the 3'-methoxyl signal (δ 4.02). These observations and a comparison with the data reported previously [19] indicated unequivocally that compound **4** is 3'-O-methylellagic acid 4-O-β-D-glucopyranoside.

**Biological study**

The aqueous extract obtained from *Myrtus communis* L. leaves exhibited nearly normalization of elevated blood glucose on streptozotocin

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C-3` signal, in turn, showed a cross peak with the 3`-methoxyl signal (δ 4.02). These observations and a comparison with the data reported previously [18] indicated unequivocally that compound 4 is 3`-O-methylellagic acid 4-*O*-β-D-glucopyranoside.

**Biological study**

The aqueous extract obtained from *Myrtus communis* L. leaves exhibited nearly normalization of elevated blood glucose on streptozotocin induced diabetic rats with 400 mg / kg body weight by oral administration route while 200 mg of aqueous extract did not exhibit the same drop of elevated blood glucose of diabetic rats. Normal non diabetic rats did not show a significant decrease in their blood glucose. All diabetic rats that showed drop in blood glucose level retained hyperglycemia after stopping the drug for 2 days. This means that aqueous extract obtained from *Myrtus communis* L. leaves is responsible for the anti-hyperglycemic activity. The maximum decrease in blood glucose was observed after 3 hours of oral administration of the drug. Ellagic acid exhibited a decrease in elevated blood glucose of diabetic rats with 100 mg/ kg body weight by oral administration route. Also all diabetic rats that showed decrease in blood glucose level retained hyerglycemia after stopping the drug for 2 days. Further investigation should be carried to find out whether the phenolic acids constituents are mainly responsible for the anti-hyperglycemic effect or there are others contributing to this activity (synergistically or additively).

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# التأثير الخافض لسكر الدم للمحتوى الفينولي من نبات المرسيل

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يعتبرنبات المرسيل (المرسيم) من الشجيرات التي تنمو على ساحل البحر الأبيض المتوسط وكما ان الطب الشعبى يستخدام نبات المرسيل لعلاج سكر الدم وذلك ما تؤيده هذه الدراسة والتى اثبتت صحة استخدام اوراق نبات المرسيل كعامل مخفض لسكر الدم .

ولقد تم دراسة كمية ونوعية الأحماض الفينولية الحرة باستخدام كروماتوجرافيا السائل ذو الضغط العالى ولقد تم التعرف على احدى عشر حمض فينولى حر بنسبة 31.7% من المستخلص المائى المتبقى من اوراق نبات المرسيل بعد استخلاصه بـ90% كحول ميثيلى ثم تجزئته بالأستخلاص بالبترول الأيثيرى ثم الكلوروفورم ثم خلات الأيثيل بالتوالى. ولقد كانت نسبة حمض الالجيك والسينامك والسينابك من أعلى النسب كالتالى 19.5% و 18.6% و13.2% بالتوالى.

ولقد تم فصل عدد 4 مركبات، اثنان من المستخلص المائى وهما حمض الجاليك وحمض الالجيك بينما تم فصل اثنان من مستخاص خلات الايثيل وهما 3,4,3' ثلاثى ميثوكسى الالجيك و 3' ميثوكسى حمض الاجيك 4- O جلوكو بيرانوسيد

ولقد تم التعرف عليهم باستخدام الاشعة الفوق مغناطيسية و الاشعة تحت الحمراء والرنين النووى المغناطيسى للهيدروجين والكربون 13 احادى وثنائى البعد وكذالك مطياف الكتلة

أما فعالية المستخلص المائى من النبات موضوع البحث كمخفض لسكر الدم فقد تم اثباتها باستخدام الفئران المصابة بالسكر( النوع الأول الذى يعتمد في علاجه على الأنسولين ) بعد تعاطيها 400 مج من المستخلص المائى عن طريق الفم والتى أعادت مستوى السكر تقريبا الى مستواه الطبيعى وذلك خلال ثلاث ساعات من تعاطيها 400 مج من المستخلص المائى .

كما تم التأكد من فعالية مادة حمض الآلجيك المستخلصة من النبات على الفئران المصابة بالسكر( النوع الأول الذى يعتمد في علاجه على الأنسولين ) بعد تعاطيها 100 مج عن طريق الفم والتى خفضت مستوى السكر فى الفئران.