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# Phytochemical and biological study of *Malva parviflora* L. grown in Egypt

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**ABSTRACT**

Column chromatography of the light petroleum fraction of *Malva parviflora* L. yielded $\beta$-amyrin, α-amyrine, mixture of $\beta$-sitosterol and stigmasterol, cholesterol, campasterol, ergosterol and $\beta$-sitosterol-*O*-glucoside. GLC of the methylated fatty acids revealed the presence of 14 fatty acids. Linolenic, palmitic and linoleic acids were the more prominent and accounting 28.25, 26.5 and 24.73%, respectively. Ethyl vanillin and chlorophyll A and B were isolated from chloroform fraction. The ethyl acetate fraction afforded kampeferol-3-(6''-*p*-coumaroyl-*O*-$\beta$-D-glucoside (tribuloside). The structure of these compounds was determined by spectral data (IR, UV, EIMS, $^{1}$H and $^{13}$CNMR). The crude ethanol extract as well as its fractions were examined for anti-inflammatory, antimicrobial and cytotoxic activities to indicate promising activity.

**Keywords**: *Malva parviflora;* sterols; flavonoids; fatty acids; anti-inflammatory, antimicrobial; cytotoxic activity.

## INTRODUCTION

*Malva* is a widespread tropical and temperate genus of the family Malvaceae (Zohary, 1987; Boulos, 2000). Many species of this genus are efficient in cough, ulcers in the bladders, intestinal infections, colitis, tonsillitis, gastroenteritis, cholesterol and lipid-lowering, anti-hypertensive, antioxidant, analgesics, emollient, pectoral girdle and arteriosclerosis treatment. In addition, the plants are also used externally as antidandruff, demulcent, softening of tumors and abscess (Nimri *et al*., 1999; Abad *et al*., 2007; Khare, 2007; Afolaya *et al*., 2008; Islam *et al*., 2007-2010; Ishtiaq *et al*., 2012; Tesevic *et al*., 2012).

*Malva parviflora* (known as cheese weed) is growing in waste ground, roadsides and desert plains in Egypt (Zohary, 1987). The plant does not have an especially strong or exciting taste, but does make a pleasant addition to salads and can be cooked as a green. Both the leaves and the immature fruit are edible.

Current literature indicated no reports about the chemistry or biological activity of the Egyptian *M. parviflora*. In this study, we report the isolation and structure determination of the chemical constituents of this plant and their evident anti-inflammatory, antimicrobial and cytotoxic effects.

## MATERIALS and METHODS

### Plant material

*Malva parviflora* L., (syn. *M. flexuosa* Hornem.) family Malvaceae was collected in the flowering stage in June 2008 from the roadsides in the vicinity of Banha, province Kalubya, Egypt. The identification was kindly verified by Dr. H. Abdel-Baset, Professor of Plant Taxonomy, Faculty of Science, Zagazig University, Egypt. A voucher specimen is deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

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## Methods and apparatus

All solvents were analytical grade. Melting points were determined on Digital, electro-thermal LTD (England) and were uncorrected. IR was determined on a Pye Unicam SP 3000 and IR spectrophotometer, Jasko, FT/IR-460 plus. Shimadzu U.V. 1700 spectrophotometer (Japan) was used for U.V. spectral analysis. Mass spectra were recorded on Shimadzu GC-MS-QP 1000 EX mass spectrometer at 70 eV. NMR spectra were obtained using Varian MAT 300 MHz. GLC analysis of methyl ester of the total fatty acids was carried out on HP-6890 GC mode gas chromatograph equipped with FID. The column (30m, i.d. 320 μm, film thickness 0.25μm) was packed with 5% polyethylene. Temperature program 70 to 220 $^{o}$C (15 $^{o}$C/min).

## Authentics

Authentic sterols, sugars and flavonoids were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University. Reference samples of fatty acids methyl esters were obtained from National Research Center Cairo, Egypt. Diclofenac sodium, ampicillin and nystatin were obtained from Arab Drug Company, Egypt.

## Extraction and fractionation

The air-dried powdered plant material of *M. parviflora* L. (5.4 kg) were exhaustively extracted with 85 % ethanol (20 L.). The concentrated ethanolic extract (570 g) was suspended in distilled water and fractionated by extraction with light petroleum (6 x 500 ml), chloroform (5 x 500 ml), and ethyl acetate (7 x 500 ml). The collected fractions were separately concentrated to yield 81.3, 29.7 and 11g, respectively.

## Chromatography of the light petroleum fraction

The light petroleum fraction (30 gm) was chromatographed on a silica gel column (150 × 4 cm, 450 g) packed in light petroleum (b.p. 60-80 $^{o}$C) and the polarity increased gradually using dichloromethane and methanol. Fractions of 250 ml each were collected, concentrated under vacuum and monitored by thin layer chromatography (TLC) using chloroform: methanol (9: 1 v/v) as developing system. TLC was visualized with 10% $H_2SO_4$ spray reagent. Fractions 17-21 were crystallized in methanol to gave 60 mg of compound **1** (colorless needles $R_f$ 0.73). Column fractions 24-31 were collected, concentrated and subjected to preparative TLC to afford 70 mg of compound **2** (white needles $R_f$ 0.71). Fractions 35-39 gave 300 mg of compound 3 (white needles, $R_f$ 0.43) while, fractions 45-51 were crystallized from methanol to gave 80 mg of compound **4** (white needles $R_f$ 0.40). Column fractions 56-65 gave 150 mg of compound **5** (white needles $R_f$ 0.37). Crystallization of column fractions 66-75 gave 120 mg of compound **6** (colorless needle $R_f$ 0.34) and the collected fractions 135-155 gave 500 mg of compound **7** (white powder $R_f$ 0.13).

## Analysis of the fatty acid contents

About 0.2 g of the resulted fatty acids after **saponification** of the petroleum ether fraction was esterified according to the method reported by Christie (1993), and the obtained methylesters were subjected to GLC analysis. Results are reported in Table 1.

## Chromatography of the chloroform fraction

The chloroform fraction (20 gm ) was chromatographed on silica gel column (4 ×

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150 cm, 300 g) packed in light petroleum and the polarity was increased gradually using dichloromethane then methanol. The eluted fractions were screened by TLC using $CHCl_3$:MeOH (8:2 v/v) as developing system. Repeated crystallization of fractions 21-30 gave 30 mg of compound **8** (white fine powder, $R_f$ 0.8). The collected fractions 31-45 gave 10 mg of material **9** (dark blue crystals, two spots with $R_f$ 0.71 and 0.65) and trials to isolate them in progress.

**Chromatography of the ethyl acetate fraction**

The ethyl acetate fraction (11g) was subjected to column chromatography on silica gel column (3 × 100 cm, 150 g) packed in dichloromethane and the polarity was increased gradually using methanol. The eluted fractions were monitored with TLC using butanol : acetic acid : water (4: 1: 5; upper layer). Fractions 51-70 eluted with ($CHCl_3$ -MeOH; 3:1 v/v) afforded 500 mg of brown residue. This residue was purified on Sephadex LH-20 column (2 x 50 cm, 20 g) packed in methanol to give 50 mg of compound **10** (yellow crystals, $R_f$ 0.42) after crystallization from methanol.

**Biological studies**

**Anti-inflammatory activity**

The anti-inflammatory activity of the crude ethanolic extract of the aerial parts of *M. parviflora* was studied using the hind paw edema method as reported by Winter *et al.* (1962). Diclofenac sodium was used as reference standard. Twenty one male albino rats were divided into three groups of seven rats each. The first group served as control and was given gum acacia mucilage (7%). The second group received diclofenac sodium at the dose of 4 mg/kg body weight (BW) and the third group was given the total alcoholic extract (120 mg/kg, BW) suspended in 7% gum acacia. All treatments were administered orally. Thirty min. later, paw edema was induced by subcutaneous injection of 0.1 mL carrageenan (1% suspension in saline) into the subplantar surface of the right hind paw of each animal (including control), while 0.1 mL normal saline was injected into the left hind paw. The hind paw diameter was measured, using a Vernier Caliber, just before the injection of carrageenan and 1, 2, 3, 4, 5, and 24 h after the injection, and the mean thickness of the edema was calculated and compared with that of the controlled inflamed rats. Since the time course of the effect was plotted against percentage increase in paw edema, the area under the curve (*AUC*) was considered as the cumulative anti-inflammatory effect during the entire observation period. Because the *AUC* represents the integrated anti-inflammatory effect (variation of paw diameter) during the observation period, it includes both the maximal response and the duration of action. The *AUC* relating variation of edema to time was obtained using the trapezoidal rule [13]. Total inhibition (*TI*, %) was calculated for each group and at each record, using the following equation:

$TI\ (\%) = (AUC\ \text{control} - AUC\ \text{treated}) \cdot 100 / AUC\ \text{control}$. The result was recorded in Table 2.

**Antimicrobial activity**

The crude ethanolic extract and its fractions (light petroleum, chloroform, ethyl acetate and aqueous) of *M. parviflora* were dissolved in DMSO (10 mg/ 100 µL, w/v) and assayed by the agar well diffusion method (Perez *et al.*,1990). The tested Gram positive bacteria was *Staphylococcus aureus* (ATCC 6538), while the Gram-negative was *Escherichia coli* (ATCC 10536). *Candida albicans* (ATCC 10231) was the used yeast. Medium was inoculated with microorganisms, and wells were filled with 0.1 mL of each extract. Ampicillin and

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nystatin were used as standard antibacterial and antifungal, respectively. The plates were incubated at 37 °C in case of bacteria and 30 °C in case of the fungi for 48 h. The diameter of the zone of inhibition (in mm) of each well was measured and recorded in Table 3.

**Cytotoxic activity**

Potential cytotoxicity of the crude ethanolic extract of *M. parviflora* was investigated using the sulfo-rhodamine-B assay reported by Skehan *et al.* [15]. The tested cell lines were: colon carcinoma (HCT-116), breast carcinoma (MCF-7), liver carcinoma (Hep G2), cervical carcinoma (Hela) and normal fibroblast (BHK-21). Cells were placed on a 96-multiwell plate (104 cells/well) for 24 h before treatment with the ethanolic extract of *M. parviflora* to allow attachment of cells to the walls of the plate. Different concentrations of the samples (1, 2.5, 5, and 10 $\mu$g/mL) were added to the cell monolayer, and triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the samples for 48 h at 37 °C in an atmosphere of 5% $CO_2$, and then cells were fixed, washed, and stained with sulfo-rhodamine-B stain. Excess stain was removed with acetic acid and attached stain was recovered with Tris-EDTA buffer. The optical density (O.D.) was measured in an ELISA reader at 564 nm. Control cells were treated with vehicle alone. The fraction of cell survival was calculated as follows: survival fraction = O.D. (treated cells)/O.D. (control cells). The relation between the surviving fractions against the samples concentration was plotted to obtain the survival curve of each tumor cell line. With the same manner, the effect of the chloroform fraction, ethyl acetate fraction, chlorophyll and tributoside was investigated against liver carcinoma cell line. Doxorubicin was used as standard cytotoxic drug. The results were recorded in Table 4.

**Statistical analysis**

All experiments were repeated at least three times. The results were performed using the *t*-test and a probabilistic value; $p < 0.05$ was considered significant for biological assays.

## RESULTS and DISCUSSION

**Phytochemical results**

Altogether 7 compounds (**1-7**) were isolated and identified from the light petroleum fraction of the ethanolic extract of *M. parviflora* (Figure 1). Two compounds (**8, 9**), were isolated and identified from the chloroformic fraction, however one flavonoid glycoside (**10**) was isolated and identified from the ethyl acetate fraction. The chemical structures of the isolated compounds were identified by interpretation of their spectral data including IR, UV, MS, NMR and comparison with literature data as well as authentic substances from our laboratory.

The isolated compounds include $\beta$-amyrin (**1**), α-amyrin (**2**), a mixture of $\beta$ -sitosterol and stigmasterol (**3**), cholesterol (**4**), campasterol (**5**), ergosterol (**6**), and $\beta$-sitosterol-*O*-$\beta$-D-glucoside (**7**) (Goad and Akishisa, 1997; Hamdan *et al.*, 2011), ethyl vanillin (**8**) (de Jager *et al.*, 2007), chlorophyll A and B (Harborne, 1984) , tribuloside (**10**) (Hafez *et al.*, 2003). The isolated compounds are reported here for the first time as constituents of *M. Parviflora*.

Fourteen fatty acids were detected in the aerial parts of *M. Parviflora* L. (Table 1); constituting about 99.9 % of its fatty acids content. The saturated fatty acids comprise about 36.23% of the total amount of the analyzed fatty acids of the plant. Linoleic acid (di-unsaturated), linolenic acid (tri-unsaturated) and palmitic acid (saturated)

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represented by 24.73, 28.25 and 26.5 % of the total fatty acid contents.

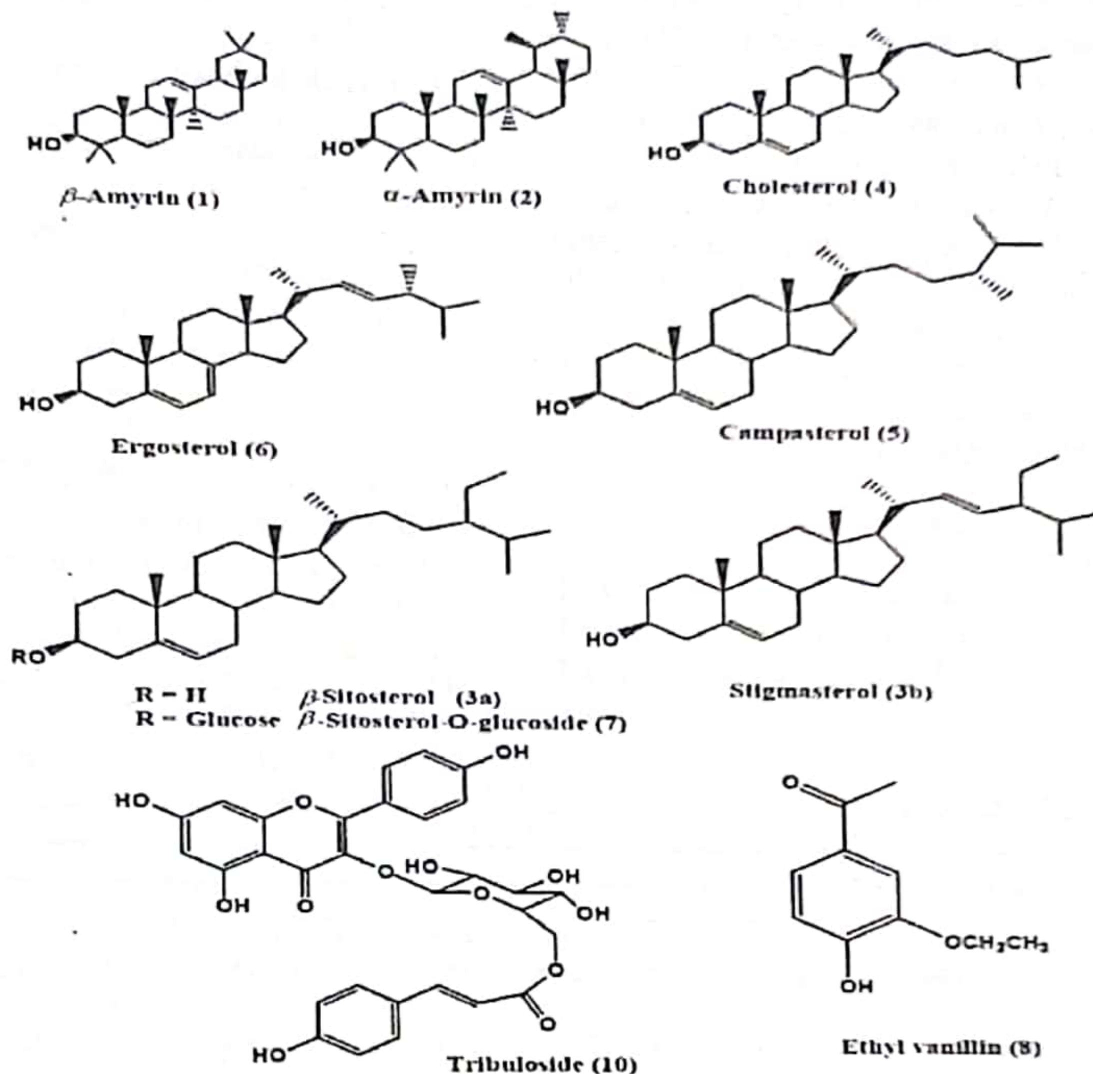


**Figure 1. Isolated compounds from *Malva parviflora***

Table (1): Results of GLC analysis of fatty acid methyl esters from the lipid fraction of *Malva Parviflora* L.

| RT | Area % | Systematic Name | Trivial Name |
|---|---|---|---|
| 9.474 | 0.78 | Decanoic | Capric |
| 12.953 | 0.59 | Dodecanoic | Lauric |
| 16.445 | 1.31 | Tetradecanoic | Myristic |
| 20.048 | 26.5 | Hexadecanoic | Palmitic |
| 20.504 | 1.29 | *cis*-9-Hexadecanoic | Palmitoleic |
| 23.320 | 3.83 | Octadecanoic | Stearic |
| 23.870 | 7.15 | *cis*-9-Octadecanoic | Oleic |
| 24.990 | 24.73 | *cis*-9,12-Octadecadienoic | Linoleic |
| 26.254 | 28.25 | *cis*-9,12,15-Octadecatrienoic | Linolenic |
| 27.22 | 0.8 | Eicosanoic | Arachidic |
| 27.51 | 1.45 | all *cis*-5, 8, 11, 14 Eicosanoic | Arachidonic |
| 28.94 | 1.65 | Docosanoic | Behenic |
| 29.354 | 0.83 | *cis*-13-Docosanoic | Erucic |
| 31.603 | 0.77 | Tetracosanoic | Lignoceric |

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## Anti-inflammatory activity

As shown in Table 2, the intradermal injection of 0.1 mL carrageenan (1%) in the rat hind paw significantly increased the paw thickness at all specified time points. On the other hand, oral pretreatment with *M. parviflora* alcoholic extract significantly decreased rats hind paw edema thickness compared to the control group. Our results are in agreement with that reported for the plant growing in Algeria, Nigeria and South Africa (Shale *et al.*, 2005; Bouriche *et al.*, 2009& 2011; Afolayan *et al.*, 2010)

## Antimicrobial activity

The aqueous fraction showed significant activity against all tested microorganisms (Table 3). On the other hand the crude ethanolic extract showed substantial activity against *Candida albicans*. Light petroleum, chloroform and ethyl acetate fractions exhibited a moderate activity against the tested organisms.

## Cytotoxic activity

The crude ethanolic extract of *M. parviflora* inhibit significantly the growth of liver carcinoma cells (Hep G2) *in vitro* (Table 4). The $IC_{50}$ value was 0.87μg/w ($IC_{50}$ for control; doxorubicin 0.6μg/w). Bio-guided fractionation revealed that the chloroform fraction, ethyl acetate fraction, chlorophyll (material 9) and tribuloside (compound 10) show $IC_{50}$ 2.28, 5.23, 0.93 and 3.2μg/w, respectively. Our results suggest a detailed study on the use of *M. parviflora* extract as a potent cytotoxic drug especially against liver carcinoma cells (Hep G2).

Table ( 2 ): Results of the anti-inflammatory activity of of *Malva Parviflora* L.

| Treatment | Percentage increase in edema thickness (%) | | | | | |
|---|---|---|---|---|---|---|
| | Time (h) | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 24 |
| Control | 35.36± 2.1 | 57.7 ± 2.8 | 61.2 ±4.4 | 54.48 ± 2.1 | 49.15 ± 3.2 | 17.43± 3.44 |
| Diclofenac sodium | 13.59± 1.03 | 13.44 ± 0.96* | 8.58± 2.36* | 6.61± 2.1* | 5.56 ± 1.77 | 3.26± 0.22* |
| Total extract | 14.76± 0.95* | 33.83±1.38*a | 34.95±0.32* a | 25.52 ± 2* a | 22.52 ± 1.9* | 4.95± 0.52*a |

*significantly different from control, [a] significantly different from diclofenac sodium

Table (3): Antimicrobial activities of *Malva parviflora* L.

| Material | Inhibition zone (mm) | | |
|---|---|---|---|
| | *Staphylococcus aureus* (ATCC6538) | *Escherichia coli* (ATCC10536) | *Candida albicans* (ATCC10231) |
| Ethanol extract | 20 | 18 | 28 |
| Petroleum ether fraction | 20 | 16 | 20 |
| Chloroform fraction | 22 | 18 | 20 |
| Ethyl acetate fraction | 22 | 18 | 18 |
| Aqueous fraction | 30 | 28 | 26 |
| Ampicillin | 30 | 30 | - |
| Nystatin | - | - | 30 |

Total ehanolic extract and its fractions were applied at the concentration of 10 mg/100 μL DMSO, while the used standards (antibiotic and antifungal) were applied at the concentration of 0.1 mg/100 μL.

Table (4): Results of cytotoxic activity of *Malva parviflora* L.

| Tested material | Liver carcinoma (Hep G2) | Cervical carcinoma (Hela) | Breast carcinoma (MCF) | Colon carcinoma (CaCo2) | Normal fibroblast (3T3) |
|---|---|---|---|---|---|
| Total ethanol extract | 0.87μg/W | 5.50 μg/ w | 8.32 μg/w | - Ve | 2.35 μg/w |
| Chloroform fraction | 2.28 μg / w | ND | ND | ND | ND |
| Ethyl acetate fraction | 5.23 μg/w | ND | ND | ND | ND |
| Chlorophyll (9) | 0.93 μg/w | ND | ND | ND | ND |
| Tribuloside "10" | 3.20 μg / w | ND | ND | ND | ND |
| Doxorubicin (standard) | 0.6 μg / w | ND | ND | ND | ND |

-ve, negative result;, ND, not determined

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# دراسة فيتوكيميائية وبيولوجيه لنبات مالفابارفيفلورا الذى ينمو في مصر

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باستخدام عمود السيليكا جيل تم فصل ثمانية مركبات استرولية من خلاصة الاثير البترولى لنبات مالفابارفيفلورا. ومن خلاصة الكلوفورم تم فصل استيل فالنيل وخليط من كلورفيل أ, ب . وتم فصل مركب فلافوتى جيكلوسيد ( تريلوسيد ) من خلاصة خلات الاثتيل. وقد تم اثبات التراكيب البنائية للمركبات المفصولة باستخدام طرق التحليل المختلفة.

كما تم عمل تحليل للاحماض الدهنية باستخدام كروماتوجرافيا الغاز السائلة للإسترالمثيلى لها وأسفر هذا التحليل عن التعرف على 14 حمضا وأن حمض اللينوليك هو الرئيس يليه حمض البلمتيك. وتعتبر هذه هى المرة الاولى لفصل هذه المركبات من نبات (.مالفابارفيفلورا ).

كما تم دراسة تأثير الخلاصات المختلفة للنبات (الكحول ,الايثيرالبيترولى , الكلوروفورم , خلات الإثيل , الماء) كمضاد للالتهابات وضد الميكروبات والتأثير السمى على الخلايا السرطانية وأظهرت التجارب نتائج مشجعه.