



Bioactive compounds from *Malva rotundifolia* L.

Mohamed-Farid Lahloub¹, Mohamed M. Amer¹, Ahmed Zaki^{1,2}, Ahmed Ashour^{1,3}, Yhiya Amen^{1,3}, Reham M. Samra^{1,*}

¹Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

²National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.

³Division of Systematic Forest and Forest Products Sciences, Department of Agro-Environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

Received: 12-05-2017 / Revised Accepted: 31-07-2017 / Published: 01-08-2017

ABSTRACT

Eight compounds were isolated for the first time from the methanolic extract of *Malva rotundifolia* L. Based on spectroscopic data and mass techniques, the structures of these compounds were identified as palmitic acid (**1**), β -sitosterol (**2**), $5\alpha,8\alpha$ -epidioxyergosta-6,22-dien-3 β -ol (**3**), di-(2-ethylhexyl)phthalate (**4**), β -sitosterol 3-O- β -D-glucoside (**5**), methyl linoleate (**6**), 3-oxo- α -ionol β -D-glucopyranoside (**7**) and megastigman-7-ene-3,5,6,9-tetrol (**8**). All compounds except **2** and **5** were examined for their anti-allergic and melanin synthesis activities on RBL-2H3 and B16 melanoma cells, respectively. The most active compound was **3** which significantly suppressed the release of β -hexosaminidase and therefore it can be used as anti-allergic substance. The same compound also showed melanin synthesis inhibition but other compounds (**1**, **4**, **6**, **7** and **8**) showed melanin synthesis stimulatory effects at the same concentration.

Keywords: *Malva rotundifolia* L., Palmitic acid, β -Hexosaminidase, Melanin

Address for Correspondence: Reham M. Samra, Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt. E-mail:rehamsamraa@yahoo.com

How to Cite this Article: Mohamed-Farid Lahloub, Mohamed M. Amer, Ahmed Zaki, Ahmed Ashour, Yhiya Amen, Reham M. Samra. Bioactive compounds from *Malva rotundifolia* L. World J Pharm Sci 2017; 5(8): 171-177.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License, which allows adapt, share and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.



INTRODUCTION

Family Malvaceae includes over 75 genera and 1000 species of tropical and temperate distribution, especially in America [1, 2]. This family is represented by several genera, such as, *Althaea* (hollyhock, marshmallow); *Sida*; *Abutilon* (Indian mallow); *Gossypium* (cotton); *Hibiscus* (rose mallow) [2]. Other well-known plants are the mallows or cheeseweeds (*Malva*). The name cheeseweeds is due to the separation of the fruit compartment into sections resembling pieces of circular cheeses of old fashioned grocery stores [3]. Genus *Malva* is reported to have flavonoids [4, 5], anthocyanins [6], steroids [7, 8], phenolics [9], aliphatic compounds [10, 11], coumarins [9] and others. *Malva* genus has demonstrated different biological effects such as antioxidant [12, 13], anti-inflammatory [14, 15], antimicrobial [16, 17], hypoglycemic [18], hepatoprotective [19], and other biological activities include counter-irritant activity [20]. Furthermore, it is also reported to reduce the occurrence of the cardiovascular diseases and prevent thrombus formation [21], and treatment of hand eczema [22].

There is no report concerning the chemical composition of *M. rotundifolia* L. However, little biological studies were published [23]. Therefore, the purpose of this study was to investigate the chemical constituents of *M. rotundifolia* L. and evaluate their effects on melanin synthesis and their anti-allergic activities.

On the other hand, we measured the anti-allergic activities of the isolated compounds by using rat basophilic leukemia mast cells (RBL-2H3) which used as a pattern of human mast cells. Mast cells have vital roles in allergic diseases. Activation of mast cells by an allergen causes exocytosis. Upon exocytosis, the allergic mediator β -hexosaminidase released linearly with histamine from mast cells [24]. Any substance that suppresses the release of these inflammatory mediators is considered to be a potential anti-allergic substance [25].

Besides, we evaluated the effect of the isolated compounds on melanin synthesis. Production of melanin and skin pigmentation are the function of melanocytes. These melanocytes synthesize melanin upon irradiation by ultraviolet and then melanin transports to keratinocytes to absorb energy and protect against sunburn. Nevertheless, abnormal melanogenesis results in pigmentary disorders, such as hypopigmentation or hyperpigmentation, which are of clinical and cosmetic concerns. Many lightening compounds such as arbutin, are used to modulate melanogenesis [26]. For elimination of the

influence of the cytotoxicity of the compounds, the MTT assay was done in parallel for both experiments.

MATERIAL AND METHODS

General: 1D and 2D spectra of compounds were recorded on Bruker AVANCE III 400 MHz equipped with a 5-mm PABBO probe head. Samples were dissolved in CDCl_3 or CD_3OD and the chemical shifts are expressed in ppm relative to the TMS acting as an internal standard. TLC was performed on silica gel F₂₅₄ aluminum plate (20 × 20 cm, Fluka) (Merck, Germany). The detection was done at UV₂₅₄ nm using ultra violet lamp, (Desaga, Germany) working at 254 and 366 nm. Using 0.5% vanillin (Sigma) in conc. H_2SO_4 -EtOH (1:19) as spraying reagent followed by heating, spots were visualized. Isolation and purification procedures were performed using solvents of reagent grade, purified by distillation (Fischer chemicals). Column chromatography (CC) was performed on silica gel G 60-230 mesh (Merck, Germany).

Plant material: The plant material used in this study consists of all aerial parts (with flowers and seeds) and roots of *M. rotundifolia* L. This plant was collected from Saint Catherine's Monastery, Egypt in the South Sinai Governorate on March, 2013. The plant identity was kindly confirmed by Dr. Ibrahim Mashaly, Professor of Ecology, Faculty of Science, Mansoura University, Egypt. A voucher specimen (No. 2013/MR) was kept at the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University.

Extraction and isolation procedures: The powdered dried plant (400 g) was extracted by percolation with MeOH (5 x 1 L). The combined extracts of methanol were concentrated under reduced pressure at 40 °C until a syrupy consistency is obtained. Drying was done in a desiccator over anhydrous CaCl_2 till a constant weight (66 g). The dried extract was suspended in water-methanol and subjected to successive extraction with petroleum ether (5 x 200 mL), methylene chloride (4 x 200 mL), ethyl acetate (4 x 200mL) and *n*-butanol (5 x 200 mL). Under reduced pressure, the collected extracts were evaporated to dryness to afford 7 g (10.6%) petroleum ether extract (Fraction A), 1.4 g (2.1%) methylene chloride extract (Fraction B), 1 g (1.5%) ethyl acetate extract (Fraction C) and finally 5 g (7.5%) *n*- butanol extract (Fraction D).

1. Isolation of compounds: Fraction A was loaded on silica gel column (350 g, 50 x 3.5 cm) and subjected to gradient elution with petroleum ether –

EtOAc starting with 100% petroleum ether. Similar fractions were pooled together on the basis of similarity in R_f values. Collected fractions were subjected to purification through chromatography and repeated crystallization to give compounds **1** (10 mg), **2** (30 mg), **3** (7.1 mg), **4** (10.2 mg) and **5** (10 mg).

Fraction B was loaded on silica gel column (100 g, 40 x 2.5 cm) and subjected to gradient elution with petroleum ether – EtOAc starting with 5% EtOAc. The latter elution afforded fractions which were collected on the basis of similarity in R_f values. Collected fractions were subjected to purification through chromatography and repeated crystallization to give compound **6** (8 mg).

Fraction D was loaded on silica gel column (150 g, 45 x 2.5 cm) and subjected to gradient elution using ethyl acetate : methanol : water (100 : 0 : 0, 100 : 5 : 4, 100 : 8 : 6, 100 : 10 : 7 and 100 : 13 : 11). Similar fractions were pooled together on the basis of similarity in R_f values. Collected fractions were subjected to purification through chromatography and repeated crystallization to afford compounds **7** (2.5 mg) and **8** (3.8 mg).

2. Identification of the isolated compounds:

Eight compounds were isolated for the first time from *Malva rotundifolia* L. methanolic extract. Compounds **1-8** (Figure 1) were identified by comparing their data with published data in literature [27-35]. These compounds are palmitic acid **1**, β -sitosterol **2**, $5\alpha,8\alpha$ -epidioxyergosta-6,22-dien-3 β -ol **3**, di-(2-ethylhexyl)phthalate **4**, β -sitosterol 3-O- β -D-glucoside **5**, methyl linoleate **6**, 3-oxo- α -ionol β -D-glucopyranoside **7** and megastigman-7-ene-3,5,6,9-tetrol **8**.

Cell line and culture conditions: Rat basophilic leukemia (RBL-2H3) and B16 melanoma cells were obtained from Riken Bioresource Center, Japan and preserved in 10% FBS brought from Thermo Fisher Science, Tokyo, Japan in Eagle's minimal essential medium (EMEM) purchased from Nissui Seiyaku, Tokyo, Japan. Optimum pH and temperature were maintained by providing 5% CO₂ and keeping cells at a constant temperature 37 °C. p-nitrophenyl-N-acetyl- β -glucosaminide and calcium ionophore A23187 were obtained from Sigma-Aldrich, St. Louis, MO, USA. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Tokyo Chemical Industry, Tokyo, Japan.

Anti-allergic assay: The anti-allergic effects of the compounds were investigated by the β -hexosaminidase inhibition assay. A modified RBL-2H3 was used as a cell-based model of allergic

response [36]. Using a 96-well plate, the cells were placed at a density of 1×10^6 cells/well. After incubation in EMEM for 48 h, the medium was replaced with 100 μ L of Tyroid buffer (5 mM KCl, 130 mM NaCl, 1 mM MgCl₂·6H₂O, 1.4 mM CaCl₂, 1 mg BSA, 10 mM HEPES, 5.6 mM glucose, pH 7.2) and 1 μ L of each compound (n = 3) was added. Simultaneously, 1 μ L 100% DMSO (negative control) and quercetin 50 mg/mL in DMSO (positive control) were tested and the plate was incubated again for 30 min in CO₂ incubator at 37 °C. Thereafter, the sample was removed and to each well, 100 μ L of the Tyroid buffer and 2 μ L calcium ionophore A23187 were added and incubated for 30 min. After incubation, 50 μ L of the supernatant were gathered from each well and in a new 96-well plate mixed to 50 μ L of 1 mM substrate solution (p-nitrophenyl-N-acetyl- β -glucosaminide). On a shaker, the mixture was incubated for 1 h at room temperature. 100 μ L of stop solution (100 mM sodium carbonate, pH=10) were added. Finally, the absorbance was measured at 405 nm. In another well plate MTT experiment was performed in parallel for measurement of the cytotoxicity of compounds. To each well of the other well plate, 50 μ L of MTT reagent in phosphate buffered saline (PBS, 250 μ g/mL) were added to cells (1×10^6 cells/well) after the cells were treated with each compound for 1 h. In a humidified atmosphere of 5% of CO₂ at 37 °C, the plates were incubated for 4 h. After removal of the medium, 1.0 mL isopropanol (containing 0.04N HCl) was added, and after overnight keeping in dark, the absorbance was measured at 570 nm using a microplate reader (Shimadzu, Japan).

B16 melanoma cell line assay: This assay was carried out as previously described [37, 38] with little modifications [39]. Using two 24-well plates, a mouse B16 melanoma cells were placed at a density of 1×10^5 cells/well in media free from samples for 24 h. After 24 h incubation, 2 μ L of the test sample (n = 3) and 998 μ L of fresh media replaced the old media. Simultaneously, 2 μ L 100% DMSO (negative control) and arbutin 50 mg/mL in DMSO (positive control) were tested. After 48 h incubation, the medium was replaced with fresh medium containing each sample. The remaining adherent cells were assayed after 24 h then, the medium was removed and the cells were washed with PBS from one plate. The melanin content was determined by dissolving the cell pellet in 1.0 mL of 1N NaOH. The crude cell extracts were kept overnight in dark and then were assayed for melanin content by using a microplate reader (Shimadzu, Japan) at 405 nm. To each well of the other well plate for determination of cell viability, we added 50 μ L of MTT reagent in PBS (250 μ g/mL). In a humidified atmosphere of 5% of CO₂

at 37 °C, the plates were incubated for 4 h. After removal of the medium, 1.0 mL isopropanol (containing 0.04N HCl) was added, and after overnight keeping in dark, the absorbance was measured at 570 nm.

RESULTS AND DISCUSSION

Anti-allergic assay: In the present study, the inhibitory activities of the isolated compounds on β -hexosaminidase release were shown at different concentrations (Table 1). The most active compound was **3** which significantly suppressed the release of β -hexosaminidase without cytotoxicity at final concentrations of 50, 100 and 200 μ M. Compounds **1**, **4**, **7** and **8** showed moderate activities in inhibition of β -hexosaminidase release at a concentration of 50 μ M. The least active compound was **6** that caused a minor suppressive effect on the release of β -hexosaminidase (~4% inhibition) at 100 μ M.

B16 melanoma cell line assay: In this study, the effects of the isolated compounds on melanin

synthesis in B16 melanoma cells were shown at different concentrations (Table 2). The most active compound was **3** that showed melanin synthesis inhibition (~49%) but caused little melanocytotoxicity (~13%) at a final concentration of 10 μ M. Other compounds (**1**, **4**, **6**, **7** and **8**) showed melanin synthesis stimulatory effects at the same concentration.

CONCLUSION

This study resulted in isolation of eight compounds for the first time from the methanolic extract of *Malva rotundifolia* L. 5 α ,8 α -epidioxyergosta-6,22-dien-3 β -ol is a promising compound that can be used as a safe anti-allergic substance. Also, the same compound is a promising compound as a new skin whitening agent for treating hyperpigmentation through inhibition of melanin synthesis. Moreover, the study resulted in isolation of some compounds with melanin synthesis stimulatory effect.

Table (1): Cell viability and effects of the isolated compounds on β -hexosaminidase release from RBL-2H3 basophilic leukemia cells.

	50 μ M		100 μ M		200 μ M	
	β -hexosaminidase release	CV	β -hexosaminidase release	CV	β -hexosaminidase release	CV
1	72.340 \pm 3.250	97.560 \pm 2.812	76.595 \pm 9.594	98.943 \pm 4.263	78.723 \pm 10.054	99.837 \pm 3.088
3	63.551 \pm 5.665	113.771 \pm 8.632	53.271 \pm 6.321	112.280 \pm 7.265	60.280 \pm 9.121	93.421 \pm 5.359
4	71.495 \pm 7.193	110.877 \pm 2.528	92.990 \pm 13.025	116.184 \pm 6.315	103.271 \pm 2.428	108.771 \pm 5.896
6	111.214 \pm 7.971	103.728 \pm 1.331	96.261 \pm 8.411	109.210 \pm 4.091	126.168 \pm 4.282	105.789 \pm 7.033
7	81.775 \pm 12.720	106.096 \pm 1.449	74.299 \pm 27.231	108.859 \pm 3.352	86.915 \pm 23.886	103.903 \pm 2.187
8	71.028 \pm 7.009	112.412 \pm 2.729	91.588 \pm 5.307	117.500 \pm 3.034	123.364 \pm 16.958	118.464 \pm 2.210

Data presented as means \pm SD (n=3), CV, cell viability (%). Quercetin was used as a positive control at 5 μ M, CV=101.27 \pm 4.634, β -hexosaminidase release =50.00 \pm 12.141.

Table (2): Cell viability and effects of the isolated compounds on melanin synthesis using B16 melanoma cells.

	10 μ M		20 μ M		40 μ M	
	MC	CV	MC	CV	MC	CV
1	130.270 \pm 6.895	105.3 \pm 2.540	128.468 \pm 3.689	91.571 \pm 6.734	115.135 \pm 1.178	94.147 \pm 11.652
3	51.699 \pm 2.568	87.414 \pm 3.638	50.323 \pm 2.021	81.555 \pm 1.382	39.077 \pm 1.699	74.972 \pm 3.202
4	153.398 \pm 13.507	106.437 \pm 3.910	100.809 \pm 2.684	104.303 \pm 1.783	84.061 \pm 1.560	88.571 \pm 2.216
6	143.408 \pm 2.283	102.496 \pm 2.409	179.378 \pm 4.348	102.389 \pm 3.561	80.131 \pm 5.249	90.477 \pm 1.884
7	178.154 \pm 11.406	94.008 \pm 6.424	161.770 \pm 8.717	101.248 \pm 3.0115	175.800 \pm 4.846	92.617 \pm 3.590
8	127.053 \pm 16.090	100.610 \pm 1.956	136.714 \pm 0.911	97.254 \pm 1.685	88.888 \pm 8.375	94.317 \pm 2.861

Data presented as means \pm SD (n=3), MC, melanin content (%); CV, cell viability (%). Arbutin was used as a positive control at 50 mg/mL, CV=99.227 \pm 1.507, MC=47.477 \pm 2.707.

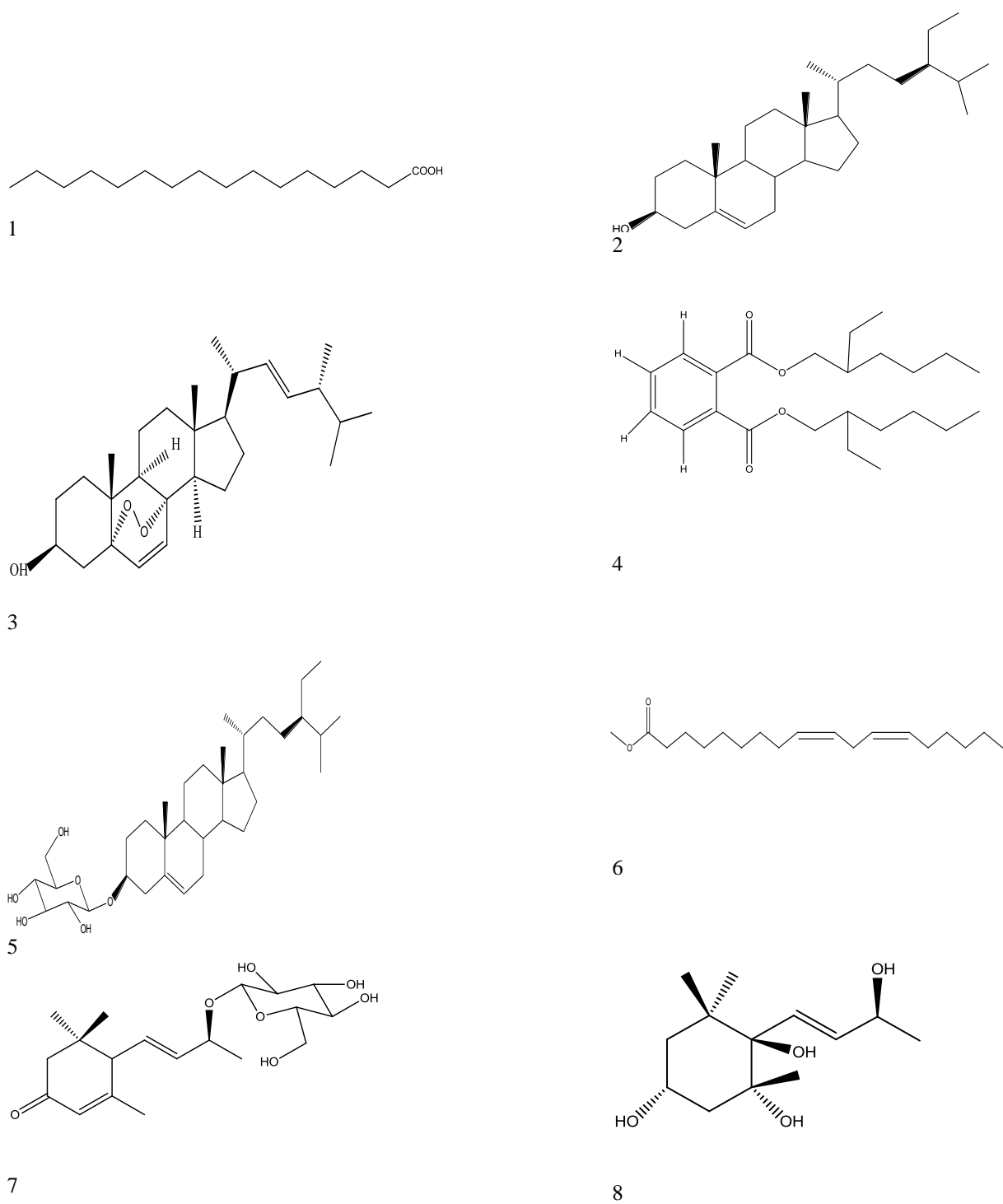


Figure (1) Structures of the isolated compounds (1-8)

REFERENCES

1. Verma B. Introduction to Taxonomy of Angiosperms; PHI Learning Private Limited; New Delhi, India, 2011.
2. Jones S, Luchsinger A. Plant Systematics, 2nd ed.; McGraw-Hill Book Co: New York, 1986.
3. Benson L. Plant Classification; Oxford & IBH publishing Co., Oxford Bldg., N-88 Connaught circus: New Delhi, India, 1970.
4. Matlawska I. Flavonoids from *Malva sylvestris* flowers. Acta Poloniae Pharmaceutica 1994; 51(2): 167-170.
5. Billetter M et al. 8-Hydroxyflavonoid glucuronides from *Malva sylvestris*. Phytochemistry 1991; 30(3): 987-990.
6. Takeda K et al. Malonated anthocyanins in Malvaceae: malonylmalvin from *Malva sylvestris*. Phytochemistry 1989; 28(2): 499-500.
7. Kim J et al. Verticilloside, a new daucosteryl derivative from the seeds of *Malva verticillata*. Natural Product Sciences 2011; 17(4): 350-353.
8. Sharma S, Ali M. A new stigmastane derivative from roots of *Malva parviflora*. Indian Journal of Chemistry Section B 1999; 38(6): 746-748.
9. Della M et al. Antioxidant and radical scavenging properties of *Malva sylvestris*. Natural Product Communications 2009; 4(7): 893-896.
10. Usami A et al. Characterization of aroma-active compounds in dry flower of *Malva sylvestris* L. by GC-MS-O analysis and OAV calculations. Journal of Oleo Science 2013; 62(8): 563-570. 11.
11. Emets T et al. Constituents of *Malva sylvestris* seed oil. Khimiya Prirodnykh Soedinenii 1994; 3: 322-325.
12. Turker M, Dalar A. *In vitro* antioxidant and enzyme inhibitory properties and phenolic composition of *M. neglecta* Wallr. (Malvaceae) fruit: A traditional medicinal fruit from Eastern Anatolia. Industrial Crops and Products 2013; 51: 376-380.
13. Farhan H et al. *In vitro* antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* L. grown in Lebanon. Asian Journal of Pharmaceutical and Clinical Research 2012; 5 (3): 234-238.
14. Bouriche H et al. Anti-inflammatory, free radical-scavenging, and metal-chelating activities of *Malva parviflora*. Pharmaceutical Biology 2011; 49(9): 942-946.
15. Prudente A et al. Pre-clinical anti-inflammatory aspects of a cuisine and medicinal millennial herb: *Malva sylvestris* L. Food and Chemical Toxicology 2013; 58: 324-331.
16. Islam M et al. Antimicrobial and irritant activities of the extracts of *Malva parviflora* L., *Malvastrum coromandelianum* L. and *Amaranthus viridis* L. Pakistan Journal of Pharmaceutical Sciences 2010; 20 (1): 3-6.
17. Cheng C, Wang Z. Bacteriostatic activity of anthocyanin of *Malva sylvestris*. Journal of Forest Research 2005; 17(1): 83-85.
18. Perez G, Rosa M. Evaluation of hypoglycemic activity of the leaves of *Malva parviflora* in streptozotocin-induced diabetic rats. Food & Function 2012; 3(4): 420-427.
19. Hussain L. Hepatoprotective effects of *Malva sylvestris* L. against paracetamol-induced hepatotoxicity. Turkish Journal of Biology 2014; 38: 396-402.
20. Ishtiaq S et al. Identification and evaluation of counter-irritant potential of crude extract of *Malva parviflora* L. by WHO recommended methods. Pakistan Journal of Pharmaceutical Sciences 2012; 25 (3): 589-594.
21. Wang Z. Impact of anthocyanin from *Malva sylvestris* on plasma lipids and free radical. Journal of Forest Research 2005; 16(3): 228-232.
22. Barikbin B et al. *Malva Sylvestris* in the treatment of hand eczema. Iranian Journal of Dermatology 2010; 13(4): 131-134.
23. Drozdova I, Bubenchikov R. Composition and antiinflammatory activity of polysaccharide complexes extracted from sweet violet and low mallow. Pharmaceutical and Chemical Journal 2005; 39(4): 197-200.
24. Weatherly L. A microplate assay to assess chemical effects on RBL-2H3 mast cell degranulation: effects of triclosan without use of an organic solvent. Journal of Visualized Experiments 2013; 81.
25. Zhu Q et al. *In vitro* bioactivities and phytochemical profile of various parts of the strawberry (*Fragaria × ananassa* var. Amaou). Journal of Functional Foods 2015; 13: 38-49.
26. Cheung F et al. Anti-Melanogenic Property of Geoditin A in Murine B16 Melanoma Cells. Marine Drugs 2012; 10: 465-476.
27. Badria F et al. Immunomodulatory triterpenoids from the oleogum resin of *Boswellia carterii* birdwood. Zeitschrift für Naturforschung 2003; 58c: 505-516.
28. Rajput P, Rajput A. Isolation of Stigmasterol and β -sitosterol of leaves of *Corchorus Fasicularis* Lam. Journal of Biological Chemistry 2012; 6: 130-135.
29. Rösecke J, König W. Constituents of the fungi *Daedalea quercina* and *Daedaleopsis confragosa* var. tricolor. Phytochemistry 2000; 54: 757-762.
30. Gauvin A et al. Isolation of bioactive 5 α , 8 α -epidioxy sterols from the marine sponge *Luffariella cf. variabilis*. Canadian Journal of Chemistry 2000; 78: 986-992.
31. Pavia D et al. Introduction to Spectroscopy, 3rd ed.; Thomson learning: UK and USA, 2001.
32. Mustafa A, Ali M. New steroidal lactones and homomonoterpenic glucoside from fruits of *Malva sylvestris* L. Acta poloniae pharmaceutica-Drug Research 2011; 68(3): 393-401.
33. Cho H et al. Phytochemical Constituents of *Salsola komarovii* and Their Effects on NGF Induction. Natural Product Sciences 2014; 20(2): 95-101.
34. Yu Q et al. Staphylionosides A-K: Megastigmane Glucosides from the Leaves of *Staphylea bumalda* DC. Chemical and Pharmaceutical Bulletin 2005; 53(7): 800-807.
35. Cohen H et al. Extraction and Identification of a Plasticizer, Di-(2ethylhexyl)phthalate, from a Plastic Bag Containing Contaminated. Archives of environmental contamination and toxicology 1991; 20: 437-440.
36. Kim S et al. Composition of Liquid Rice Hull Smoke and Anti-Inflammatory Effects in Mice. Journal of Agricultural and Food Chemistry 2011; 59(9): 4570-4581.
37. Dooley T et al. Development of an *in vitro* primary screen for skin depigmentation and antimelanoma agents. skin pharmacology 1994; 7: 188-200.
38. Virador V et al. A standardized protocol for assessing regulators of pigmentation. Analytical Biochemistry 1999; 270: 207-219.
39. Arung E et al. Determination of Antioxidant and Anti-Melanogenesis Activities of Indonesian Lai, *Durio kutejensis* [Bombacaceae (Hassk) Becc] Fruit Extract. Tropical Journal of Pharmaceutical Research 2015; 14: 41-46.