

Isolation of a megastigman glycoside and an indol derivative from *Malva nicaeensis* All.

Gülin RENDA ^{1*} , Michal ŠORAL ^{2,3} , Didem ŞÖHRETOĞLU ⁴ 

¹ Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61100 Trabzon, Turkey.

² Analytical Department, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava, Slovak Republic.

³ Central Laboratories, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, SK-812 37 Bratislava, Slovak Republic.

⁴ Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Sıhhiye 06100 Ankara, Turkey.

* Corresponding Author. E-mail: gulingurhan@yahoo.com (G.R.); Tel. +90-532-333 11 33.

Received: 16 April 2021 / Revised: 26 July 2021 / Accepted: 30 July 2021

ABSTRACT: *Malva* species are used for the treatment of cough, digestive problems, asthma, urticaria. *Malva nicaeensis* is a medicinal food plant like the other *Malva* species. To the best of our knowledge, no phytochemical studies on *Malva nicaeensis* have been reported up till now. In the present study, we aimed to assess the antioxidant effect of different extracts of *M. nicaeensis*, and to isolate the major metabolites of *M. nicaeensis*. DPPH radical scavenging effect of crude methanol extract, chloroform, ethyl acetate subextracts as well as remaining water extract prepared from the aerial parts of *M. nicaeensis* were tested. Phytochemical studies on the water and ethyl acetate subextract led to the isolation of a megastigman glycoside; roseoside and indole-3-carboxylic acid. The structures of the isolated compounds were identified on the basis of 1D- and 2D-NMR experiments. Biological activity test results support the ethnobotanical data of the plant.

KEYWORDS: *Malva nicaeensis*; roseoside; megastigman glycoside; indole-3-carboxylic acid; Malvaceae; DPPH.

1. INTRODUCTION

The genus *Malva* (Malvaceae) is represented by 9 species in Turkish Flora [1]. *Malva* species are known as 'mallow' and aerial parts are eaten and also used as medicine since ancient times. *Malva nicaeensis* is an herbaceous perennial plant; it has been used for the treatment of cough, fever, poisoning, gastrointestinal complaints, cancer and gynecological diseases and also externally as painkiller, wound healer, against rheumatism and burns [2-5]. Reportedly, *M. nicaeensis* exhibits antileishmanial effect against *Leishmania major*, and it inhibits pancreatic lipase [6,7]. Up to date, terpenes, phenolic compounds, flavonoids and anthocyanin compounds were isolated from *Malva* species [8-11]. Kaempferol 3-*O*- β -glucopyranoside, 3-*O*-(6''-*O*-trans-*p*-coumaroyl)- β -D-glucopyranoside, 7-*O*- β -D-glucopyranoside, 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside and 3,7-*O*-diglucoside as well as quercetin 3-*O*- β -D-glucopyranoside, 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside and apigenin 7-*O*- β -D-glucopyranoside were isolated from *Malva crispa* L. [8]. Six steroidal lactones (sylvestrosterol A-C, sylvestrogenin A-C), a homomonoterpenic glucoside (malvanoyl glucoside), malvone A, β -sitosterol-3- β -D-glucopyranoside along with phenolic acids (4-hydroxybenzoic acid, 4-methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 2-hydroxybenzoic acid, 4-hydroxy-2-methoxybenzoic acid, 4-hydroxycinnamic acid, 4-hydroxydihydrocinnamic acid, 4-hydroxy-3-methoxydihydrocinnamic acid, ferulic acid), 4-hydroxybenzyl alcohol, tyrosol, linalool, linalool-1-*o*ic acid were isolated from *Malva sylvestris* Linn. [9,10]. Also (6*R*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-one, blumenol A, (+)-dehydrovomifoliol, (3*R*,7*E*)-3-hydroxy-5,7-megastigmadien-9-one, (3*S*,5*R*,6*S*,7*E*,9*R*)-5,6-epoxy-3,9-dihydroxy-7-megastigmene and (3*S*,5*R*,6*R*,7*E*,9*R*)-3,5,6,9-tetrahydroxy-7-megastigmene were isolated from *M. sylvestris* [10]. However, no data from phytochemical studies on *Malva nicaeensis* have been available up till now.

Oxidative stress is defined as a serious imbalance between oxidation and antioxidants, "a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage." The reactive

How to cite this article: Renda G, Šoral M, Şöretoğlu D. Isolation of a megastigman glycoside and an indol derivative from *Malva nicaeensis* All. J Res Pharm. 2021; 25(5): 564-568.

oxygen species generate oxidative stress, a deleterious process that can damage all cell structures. Reactive oxygen species play an important role in etiology of various diseases including cancer, neurodegenerative, infectious and inflammatory illnesses. Epidemiological studies show that plant-derived foods can protect against many degenerative diseases. Aromatic and medicinal plants are a good source of natural antioxidants which may serve as valuable ingredients for different applications, including pharmaceuticals, functional foods and food supplements [12-16].

In the present study, to evaluate the scientific basis of the above mentioned ethnopharmacological use of *M. nicaeensis*, we aimed to test the DPPH radical scavenging effect of the crude extract of *M. nicaeensis*, and to isolate and identify metabolites from the titled plant.

2. RESULTS AND DISCUSSION

2.1. Antioxidant effect

The crude methanol extract was dispersed in water and partitioned with chloroform and ethyl acetate, respectively to obtain subextracts. Radical scavenging activity of the methanol extract and all subextracts were examined using DPPH test. The DPPH radical scavenging properties of extracts are given in Table 1. Among the tested subextracts, water subextract showed the radical scavenging activity (60% inhibition) at the dose of 198 µg/ml.

Table 1. DPPH radical scavenging effects of crude methanol and subextracts of *M. nicaeensis*.

Extract	DPPH ± S.D. (SC ₅₀ , µg/ml)
Methanol	476.43 ± 0.05
Chloroform	1846.30 ± 0.12
Ethyl acetate	736.50 ± 0.08
Water	161.52 ± 0.10
Gallic acid	0.07 ± 0.01

Previously, the IC₅₀ values of MeOH and water extracts of *M. parviflora* leaves were determined as 89.03 ± 2.65 and 76.67 ± 0.29 µg/ml [17]. The water extract of leaves, flowers, immature fruits and leafy flowered stems of *M. sylvestris* scavenged DPPH with IC₅₀ values of 18.49, 35.30, 10.07, 19.41 mg/ml, respectively [18]. In a study conducted in Jordan, the ABTS radical scavenging effect of MeOH and water extracts of 51 plant species, including *M. nicaeensis*, were investigated -the antioxidant activity of the water and methanol extract of *M. nicaeensis* was found as 21.8 and 24.4 µmol TE/g, respectively [19].

2.2. Structure elucidation of isolates

A megastigmane glycoside **1** (Figure 1) was isolated from the water subextract whereas an indol derivative was isolated from the ethylacetate subextract. The structure of these compounds were identified as roseoside (**1**) and indole-3-carboxylic acid (**2**) by comparison of their spectroscopic data with those reported in the literature [20-23].

The occurrence of a megastigmane glycoside in the genus *Malva* has not been reported before. This study will be first phytochemical study on *M. nicaeensis*. Roseoside has been isolated from two Malvaceae plants, *Urena lobata* and *Abutilon theophrasti* so far. However, occurrence of roseoside or any megastigmane glycoside has never been reported from genus *Malva* [24,25].

3. CONCLUSION

In conclusion, almost all extracts of *M. nicaeensis* exhibit DPPH radical scavenging effects. Notably, roseoside and indole-3-carboxylic acid were isolated from the titled plant and it is the first report occurrence of indole-3-carboxylic acid as well as a megastigmane glycoside in genus *Malva*.

4. MATERIALS AND METHODS

4.1. General

Silica gel 60 F254 precoated TLC plates (Merck) were used for monitoring fractions during chromatographic separations. Sephadex LH-20 (Sigma), Silica gel 60 (Merck), Reverse phase silica gel (Rp-C18,

Merck) was used for column chromatography. Chloroform was purchased from Merck and all the other chemicals were obtained from Sigma. The compounds were detected by UV fluorescence and 1% vanillin/H₂SO₄ spray, followed by heating at 100 °C for 2 min. NMR spectra were recorded in CD₃OD on two Varian/Agilent VNMRs 600 NMR spectrometers (Palo Alto, CA) (frequencies of 600 and 151 MHz for ¹H and ¹³C nuclei, respectively) at room temperature. ¹H and ¹³C NMR data are presented in table 2.

Table 2. ¹H and ¹³C NMR data for compound 1 (¹H: 600 MHz, ¹³C: 151 MHz, CD₃OD).

Position	¹ H δ / ppm	Signal multiplicity	J values / Hz	¹³ C δ / ppm
1	-	-	-	42.2
2	2.15	d	17.0	50.4
3	2.52	d	17.0	
4	-	-	-	201.0
5	5.87	m	*	127.0
6	-	-	-	167.1
7	-	-	-	79.8
8	5.86	m	*	131.4
9	5.86	m	*	135.1
10	4.42	m	*	77.0
11	1.29	d	6.2	20.9
12	1.03	s	-	24.4
13	1.04	s	-	23.1
1'	1.92	d	1.3	19.3
2'	4.34	d	7.8	102.5
3'	3.17	dd	9.1, 7.8	75.0
4'	3.34	m	*	77.9
5'	3.25	m	*	71.4
6'	3.23	m	*	77.8
	3.63	dd	11.7, 5.6	
	3.85	dd	11.7, 2.1	62.6

* Not determined due to overlapping

4.2. Plant material

The aerial parts of *M. nicaeensis* were collected from Trabzon, Erdoğdu district in May 2012. The plant material was identified by Assoc. Prof. Dr. Gülin Renda. A voucher specimen (HUEF12018) has been deposited at the Herbarium of Hacettepe University, Faculty of Pharmacy.

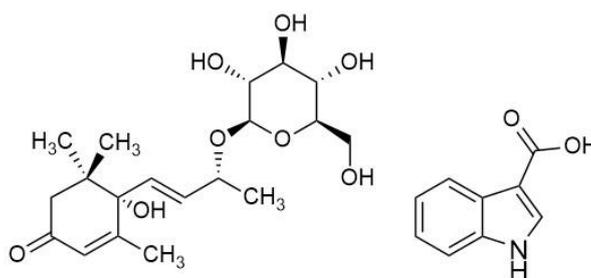


Figure 1. Compounds isolated from *M. nicaeensis*

4.3. DPPH scavenging assay

This assay was conducted according to the method of Yayli et al. Briefly, sample at various concentrations (138, 277, 415, 555, 690 µg/ml) and reference antioxidant, L-ascorbic acid, were added to a solution of 1.5x10⁻⁵ M DPPH radical in MeOH (1 ml), and the reaction mixture was shaken vigorously. After incubating at 37 °C for 30 minutes, the remaining DPPH was determined by spectrophotometry at 517 nm. The radical scavenging activity of each sample was expressed using the ratio of the absorption of DPPH (%) relative to the control DPPH solution (100%) in the absence of the sample by the equation 1. All tests were performed three times.

$$\% \text{ DPPH Radical Scavenging} = [\text{CA} - \text{EA}] / \text{CA} \times 100 \quad (\text{Eq.1})$$

CA = Control absorbance EA= Extract absorbance

4.4. Extraction and isolation

The shade-dried and powdered aerial parts of *M. nicaeensis* (480 g) were extracted with 1.5 l MeOH at room temperature for 6 hours. The same process was repeated 5 times, and the extracts were combined (53.74 g). The concentrated combined MeOH extract was suspended in 300 ml H₂O:MeOH (9:1) and then partitioned stepwise with CHCl₃ (300 ml, 2 times) and EtOAc (300 ml, 2 times). The organic solvents were evaporated to dryness to give CHCl₃ (10.0 g), EtOAc (1.68 g) subextracts, while the H₂O phase was lyophilized to yield remaining water extract (rH₂O, 40.0 g). Isolation studies were conducted on remaining water extract. The extract was subjected to C18-Vacuum Liquid Chromatography (LiChroprep C18-VLC) eluting with H₂O:MeOH gradient (100:0→0:100); the separation provided 20 fractions. Fr. 5 (586.0 mg) was applied to Sephadex LH-20 CC and then the fractions were collected and monitored with TLC. Those showing the same chromatographic pattern were pooled and applied to silicagel column, gradient elution was performed with CHCl₃:MeOH:H₂O (90:10:1→61:32:7) to afford compound **1** (12.3 mg). Ethyl acetate extract (1.68 g) was subjected to Sephadex LH-20 CC. 19 fractions were obtained after elution with MeOH. Fr.5-19 (1.20 g) were combined and separated by silicagel column chromatography using the gradient mixture of CHCl₃:MeOH:H₂O (90:10:1→70:30:3). 76 fractions were collected. Fr.5-7 (147 mg) were combined and purified with Sephadex LH-20 CC to yield compound **2** (7.1 mg).

Acknowledgements: This research was supported by the Scientific Research Foundation of the Karadeniz Technical University, Turkey (Project number THD-1202).

Author contributions: Concept – G.R.; Design – G.R, M.S, D.S; Supervision – G.R.; Resources – G.R., M.S; Materials – G.R.; Data Collection and/or Processing – G.R., M.S; Analysis and/or Interpretation – G.R., M.S, D.S ; Literature Search – G.R., M.S, D.S; Writing – G.R., D.S.; Critical Reviews – G.R., M.S, D.S.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- [1] Cullen J. *Malva* L. In: Davis PH (editor). Flora of Turkey and the East Aegean Islands. Vol 2, Edinburg University Press, Edinburgh. 1967; pp. 404-408.
- [2] Tuzlacı E, Eryaşar Aymaz, P. Turkish folk medicinal plants, Part IV: Gönen (Balıkesir). *Fitoterapia*. 2001; 72(4): 323-343. [CrossRef]
- [3] Tuzlacı E. Şifa Niyetine, Türkiye'nin Bitkisel Halk İlaçları. Alfa Basım Yayım Dağıtım Ltd. Şti., İstanbul, Turkey. 2006.
- [4] Uzun E, Sarıyar G, Adsersen A, Karakoç B, Ötük G, Oktayoglu E, Pirildar S. Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. *J Ethnopharmacol*. 2004; 95: 287-296. [CrossRef]
- [5] Yeşilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, Takeda Y, Takaishi Y. Traditional medicine in Turkey V. Folk medicine in the inner Taurus Mountains. *J Ethnopharmacol*. 1995; 46: 133-152. [CrossRef]
- [6] Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. A review of natural products with antileishmanial activity. *Phytochemistry*. 2005; 12(6-7): 514-35. [CrossRef]
- [7] Bustanji Y, Mohammad M, Hudaib M, Tawaha K, Al-Masri IM, AlKhatib HS, Issa A, Alali FQ. Screening of some medicinal plants for their pancreatic lipase inhibitory potential. *Jordan J Pharm Sci*. 2011; 4(2): 81-88.
- [8] Matlawska I, Sikorska M. Flavonoids from flowers of *Malva crispa* L. (Malvaceae). *Acta Pol Pharm*. 2004; 61(1):65-68.
- [9] Mustafa A, Ali M. New steroidal lactones and homomonoterpenic glucoside from fruits Of *Malva sylvestris* L. *Acta Pol Pharm*. 2011; 68(3): 393-401.
- [10] Cutillo F, D'Abrosca B, Dellagrecia M, Fiorentino A, Zarrelli A. Terpenoids and phenol derivatives from *Malva sylvestris*. *Phytochemistry*. 2006; 67(5): 481-485. [CrossRef]
- [11] Veshkurova O, Golubenko Z, Pshenichnov E, Arzanova I, Uzbekov V, Sultanova E, Salikhov S, Williams HJ, Reibenspies JH, Puckhaber LS, Stipanovic RD. Malvone A, a phytoalexin found in *Malva sylvestris* (family Malvaceae). *Phytochemistry*. 2006; 67(21): 2376-2379. [CrossRef]
- [12] Halliwell B, Gutteridge MC. The antioxidants of human extracellular fluids. *Arch Biochem Biophys*. 1990; 280(1): 1-8. [CrossRef]
- [13] Hollman PCH, Geelen A, Kromhout D. Dietary flavonol intake may lower stroke risk in men and women. *J Nutr*. 2010; 140(3): 600-604. [CrossRef]

- [14] Hollman PCH, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem Tox.* 1999; 37: 937-942. [CrossRef]
- [15] Bhatt ID, Rawat S, Ranbeer S, Rawal RS. Antioxidants in medicinal plants. In: Chandra S, Lata H, Varma A. (editors). *Biotechnology for Medicinal Plants*. Springer-Verlag, Berlin. 2013, pp. 295-326.
- [16] Alfa HH, Arroo RRJ. Over 3 decades of research on dietary flavonoid antioxidants and cancer prevention: What have we achieved? *Phytochem Rev.* (2019); 18: 989-1004. [CrossRef]
- [17] Bouriche H, Meziti H, Senator A. In vivo anti-inflammatory and antioxidant effects of *Malva parviflora* leaf extracts. *Acta Hort.* 2010; 854: 23-30. [CrossRef]
- [18] Barros L, Carvalho AM, Ferreira ICFR. Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: a comparative study of the nutraceutical potential and composition. *Food Chem Toxicol.* 2010; 48(6): 1466-1472. [CrossRef]
- [19] Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem.* 2007; 104: 1372-1378. [CrossRef]
- [20] Yamano Y, Ito M. Synthesis of optically active vomifoliol and roseoside stereoisomers. *Chem Pharm Bull.* 2005; 53(5): 541-546. [CrossRef]
- [21] Wang Y-S, Liao Z, Li Y, Huang R, Zhan H-B, Yang J-H. A New Megastigmane diglycoside from *Litsea glutinosa* (Lour.) C. B. Rob. *J Braz Chem Soc.* 2011; 22 (11): 2234-2238. [CrossRef]
- [22] Burton G, Ghini AA, Gros EG. ¹³C NMR spectra of substituted indoles. *Magn Reson Chem.* 1986; 9: 829-831. [CrossRef]
- [23] Quyen VT, Nam VV, Hieu TV, Vinh LC, Huong DTM, Minh LTH, Murphy B, Minh CV, Cuong PV. Secondary metabolites from marine bacterium *Streptomyces* sp. G039. *Vietnam J Chem.* 2015; 53(2e) 142-145.
- [24] Su C, Qi B, Wang J, Ding N, Wu Y, Shi X-P, Zhu Z-X, Liu X, W X-H, Zheng J, Tu P-F, Shi S-P. Megastigmane glycosides from *Urena lobata*. *Fitoterapia.* 2018; 127: 123-128. [CrossRef]
- [25] Mamadaliyeva NZ, Sharopov F, Giraultc J-P, Wink M, Lafont R. Phytochemical analysis and bioactivity of the aerial parts of *Abutilon theophrasti* (Malvaceae), a medicinal weed. *Nat Prod Rep.* 2014; 28(20): 1777-1779. [CrossRef]

This is an open access article which is publicly available on our journal's website under Institutional Repository at <http://dspace.marmara.edu.tr>.