Thermal analysis on the effect of quercetin-3-rutinoside-7-rhamnoside and flavonoids on model phospholipid membranes

Christos Ganos[1,2], Nikolaos Naziris[1], Maria Chountoulesi[1], Konstantia Graikou[2], Costas Demetzos[1,*]

[1]Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimioupolis Zografou 15771, Athens, Greece

[2]Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis Zografou 15771, Athens, Greece

*Prof. Costas Demetzos, demetzos@pharm.uoa.gr

Key words: Flavonoids; Bilayers; Thermotropic effect; Biological effect

- **Introduction**

The phytochemical analysis of the methanolic extract of the greek endemic plant *Rindera graeca* resulted to the isolation of the flavonoid 3-rutinoside-7-rhamnoside-quercetin (RGMS1), which was identified through LC/MS and NMR (**Figure 1**, **Table 1**). Structurally related flavonoids are in the market as medicinal products for the protection of blood capillaries. Therefore, this study was conducted in order to assess and compare the ability of RGMS1 and a selected group of flavonoids to interact with DPPC (dipalmitoylphosphatidylcholine) model membranes and alter their thermodynamic properties, as an effort to simulate interactions of these biomolecules with biological membranes.

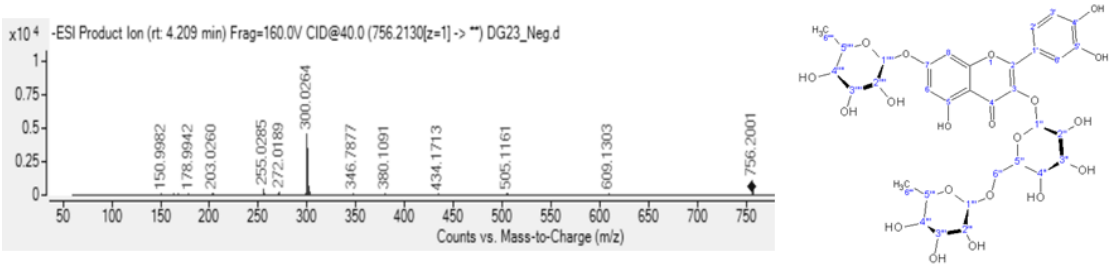

Figure 1: MS spectra and formula of RGMS1.

Table 1: Phenolic metabolites, identified through LC/MS analysis of *Rindera graeca* methanolic extract

No	Compound	Molecular Formula	$[M-H]^-$ m/z	m/z	Reference
1	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0852	191	Civra et al. 2017
2	Caffeic acid	$C_9H_70_4$	179.0326	135,134,89	Chen et al. 2011
3	Rutin	$C_{27}H_{30}O_{16}$	609.1445	300,271, 301	Civra et al. 2017
4	Quercetin 3-rutinoside-7-rhamnoside	$C_{33}H_{40}O_{20}$	755,2013	300, 609, 179	Lata & Mittal 2017

5	Bisodium rabdosiin salt	$C_{36}H_{30}0_{16}Na_2$	741.1369	717, 396, 360, 161, 133	Tufa et al. 2018
6	Rabdosiin	$C_{36}H_{30}0_{16}$	717.1391	396, 360, 161, 133	Tufa et al. 2018
7	Salvianolic acid A isomer	$C_{26}H_{22}O_{10}$	493.0985	265, 185	Chen et al. 2011
8	Rosmarinic acid	$C_{18}H_{16}O_8$	359.0797	197, 179, 161, 133	Standard
9	Lithospermic acid B	$C_{36}H_{30}0_{16}$	717.1497	537, 519, 475, 339, 197	Liu et al. 2011

- **Material and Methods**

The flavonoid RGMS1 was isolated from the Greek endemic plant *Rindera graeca* and structurally determined through LC-MS and ^{1}H-NMR. Rutin (Merck 500 017), quercetin (Extrasynthese 0066) and RGMS1 [1] were mixed with DPPC (Avanti Polar Lipids, Inc.) at 9:0.1/0.5 molar ratios, fully hydrated with HPLC-grade H_2O and subjected to differential scanning calorimetry (DSC). A DSC822^e Mettler-Toledo (Schwerzenbach, Switzerland) was utilized for this purpose. All thermotropic parameters, including the lipids' main transition temperature T_m and transition enthalpy ΔH_m, were calculated with the Mettler-Toledo STAR^e software.

- **Results and Discussion**

The thermotropic effect of flavonoids on model phospholipid membranes is presented in **Figure 2** and **Table 2**, where various phenomena that concern the biological effect of this particular class of biomolecules through thermodynamics are observed. The polar headgroup mobility, the degree of packing and the final fluidity of the membrane, which is reflected on the observed alterations of its phase transition, are affected by the adhesion, penetration and deep incorporation of molecules. In this way, the chemical structure and 3D conformation of the latter can significantly alter the biophysics of biological membranes, through the disruption, the alteration of their organization, or even the creation of new metastable phases, which may prove to be functional or dysfunctional for the cells and capillary endothelium [2]. Most of all, quercetin-3-rutinoside-7-rhamnoside was found to greatly alter the DPPC thermodynamic parameters in a concentration-dependent manner, tending to increase membrane fluidity. These results are attributed to the flavonoid's 7-rhamnose, which might affect its 3D structure and consequently, its intramembrane conformation.

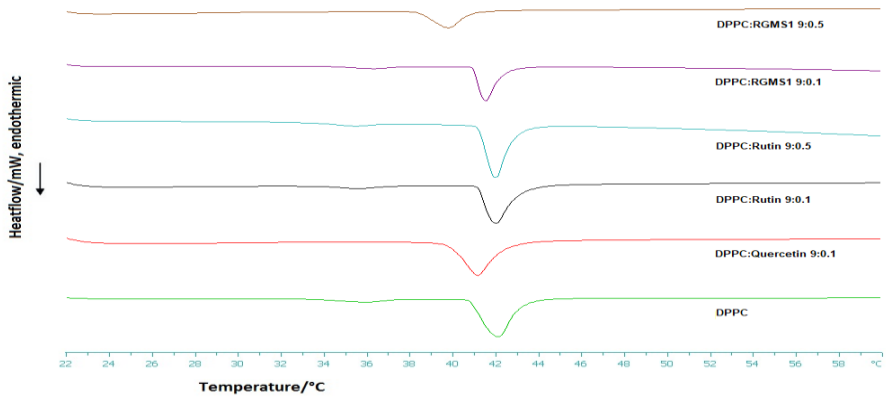

Figure 2: DSC diagrams of DPPC bilayers with incorporated flavonoids.

Table 2: Thermotropic parameters of DSC diagram.

Sample	**Molecular Ratio**	**$T_{onset,m}$ (°C)**	**T_m (°C)**	**$\Delta T_{1/2,m}$ (°C)**	**ΔH_m (Jg^{-1})**	**$T_{onset,s}$ (°C)**	**T_s (°C)**	**$\Delta T_{1/2,s}$ (°C)**	**ΔH_s (Jg^{-1})**
DPPC	-	41.05	41.88	1.45	45.01	34.56	36.54	2.10	5.08
DPPC:Quercetin	9:0.1	39.59	40.79	1.43	48.47	-	-	-	-
DPPC:Rutin	9:0.1	40.91	41.62	1.17	43.15	33.80	35.29	1.87	3.81
DPPC:Rutin	9:0.5	41.00	41.66	0.98	48.13	33.64	35.26	1.78	4.14
DPPC:RGMS1	9:0.1	40.76	41.27	0.84	27.06	34.19	36.21	1.91	3.18
DPPC:RGMS1	9:0.5	38.23	39.59	1.42	21.76	-	-	-	-

- **Conclusions**

By studying the acquired data, it has been concluded that all the examined flavonoids interact with DPPC model membranes. Their thermotropic behavior should be correlated with their pharmacological effect, in terms of blood capillary-related diseases [3]. It is obvious that more studies are needed to prove such a biophysical approach. The results from our study could be used as a starting point to introduce thermodynamics and thermal analysis methods to the development of innovative therapies for severe vascular diseases.

- **References**

[1] Ganos C.G. Phytochemical study on *Rindera graeca* (A.DC) Boiss. & Heldr (Boraginaceae) - Biological activities. Dissertation in Faculty of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Products, NKUA, Athens, 2018.

[2] Matsingou C., Hatziantoniou S., Georgopoulos A., Dimas K., Terzis A., Demetzos C. Labdane-type diterpenes: thermal effects on phospholipid bilayers, incorporation into liposomes and biological activity. *Chem. Phys. Lipids*. 138, 1-11, 2005.

[3] Goniotaki M., Xatziantoniou S., Dimas K., Wagner M., Demetzos C. Encapsulation of naturally occurring flavonoids into liposomes: physicochemical properties and biological activity against human cancer cell lines. *J. Pharm. Pharmacol*. 56, 1217-1223, 2004.