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# Effects of propolis extracts on model membranes


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## Abstract

**The most complex product that can be obtained from beekeeping, growing honey bees (Apis mellifera), is propolis. Propolis (Figure 1) is a product of bees, being produced by mixing resins collected from plants, especially trees, with wax and various substances secreted by them (e.g., substances secreted by their salivary glands). Its chemical composition depends on the geographical area, the local flora and the climatic zone. In this paper we evaluated the effects produced by hydroalcoholic extracts of propolis, obtained from samples collected from different geographical regions of the country, using fluorescence spectroscopy, on two types of model lipid membranes, which mimic the membrane of mammalian and bacterial cells. The effects on membranes were correlated with the amounts of the main phenolic components in the propolis samples (flavones and flavonols, flavanones and dihydroflavonols and the total phenol content), determined using UV-VIS spectroscopy.**


**Figure 1. Raw propolis sample**

## Experimental

**Calibration curves were constructed using a series of five working standard solutions with concentrations in the respective concentration ranges. Three independent determinations were performed at each concentration, and absorbance was plotted against concentration (see Figure 2). In this study, two samples of raw propolis was used to obtain the hidro-alcoholic extracts, first one (P1) from Andrășești, Ialomița county and the second one (P2) from Lipovăț, Vaslui county. The extracts were obtained using a standard procedure and analised for determination of main phenolics constituents (*Bankova V. et al., 2019*). Each sample was analised in triplicate, the results beign presented in Table 1.**

**The lipids used were: DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DPPG (1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt) and cholesterol. All lipids were purchased from Sigma-Aldrich. Unilamellar vesicles that mimic the membrane of mammalian cells were prepared from a mixture of DPPC and cholesterol in a ratio of 85:15 (mol:mol). Similarly, unilamellar vesicles that mimic the bacterial membrane were prepared from a mixture of DPPC and DPPC in a ratio of 85:15 (mol:mol). Unilamellar vesicles, average diameter 200 nm, were obtained by extrusion, using a standard extruder (Avanti Polar Lipids). Final lipid concentration was 50 µM.**

**Concentrations of propolis stock solutions were 8.193 mg/ml – P1 and 5.946 – P2. Stock solutions (extracts) of propolis were prepared in 70% ethanol. In experiments in which the effects of propolis on lipid membranes were followed, its concentration was varied between 0 and 100 µg/ml.**

**The effects of propolis extracts on lipid membranes were studied using fluorescence spectroscopy of Laurdan, a fluorophore sensitive to the number of water molecules it interacts with when it is inserted into the lipid bilayer. For each combination (propolis extract - membrane type) the parameter GP (generalized polarization) was calculated (Figure 3). The concentration of Laurdan in the suspension of LUVs was 100 nM.**

## Results

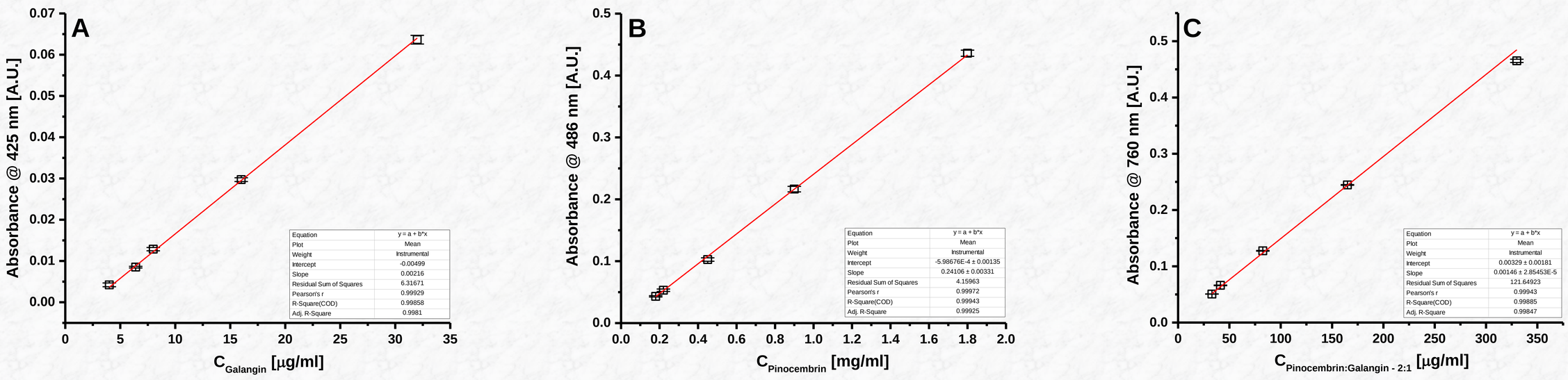


**Figure 2. Calibration curves used for determination of main phenolics constituents of propolis: (A) calibration curve for determination of flavone and flavonol content of propolis extract using galangin as reference compound; (B) calibration curve for determination of flavanone and dihydroflavonol content of propolis extract using pinocembrin as reference compound; (B) calibration curve for determination of total phenolic substances content of propolis extract using a mixture of pinocembrin:galangin (2:1) as reference compound.**

**Table 1. Main phenolics constituents from hidro-alcoholic extracts of propolis**

| Sample | Flavone and flavonol[a] [%] | Flavanone and dihydroflavonol[a] [%] | Total phenolic[a] [%] |
|---|---|---|---|
| P1 - Andrășești, Ialomița county | 7.56 ± 0.14 | 16.61 ± 0.36 | 54.22 ± 1.01 |
| P2 - Lipovăț, Vaslui county | 11.97 ± 0.95 | 21.46 ± 0.20 | 59.30 ± 0.76 |

[a] Mean of three different measurements ± SD

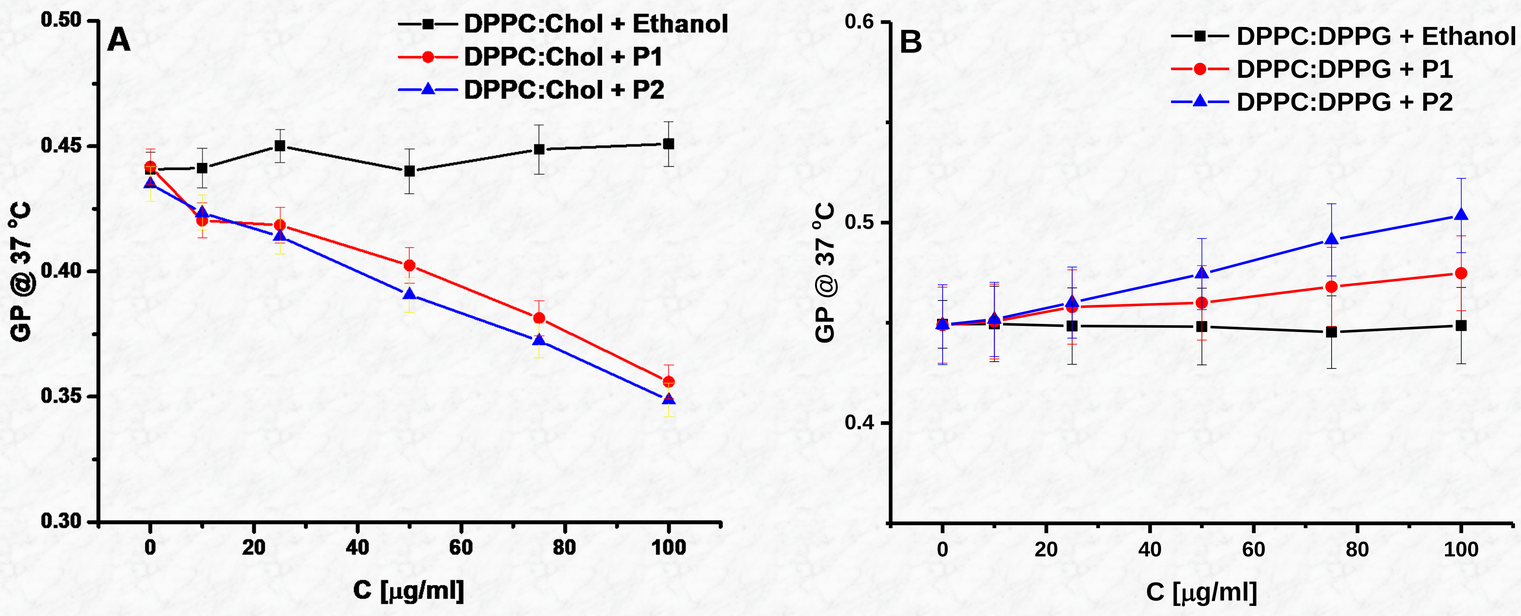


**Figure 3. GP variation for each combination propolis extract - membrane type: (A) black - DPPC:Chol + Ethanol (70%) – used as control, red – DPPC:Chol + P1, blue – DPPC:Chol + P2; (B) black - DPPC:DPPG + Ethanol (70%) – used as control, red – DPPC:DPPG + P1, blue – DPPC:DPPG + P2. Final concentrations of propolis in suspension were: 10, 25, 50, 75 and 100 µg/ml.**

## Conclusions

- **In case of neutral lipid membranes (Figure 3 – A) addition of propolis in suspension lead to a decrease of GP parameter, being an indication that the Laurdan molecules are more accesible to water molecules, thus the lipid membrane is more fluid.**
- **In case of negatively charged membranes (Figure 3 – B) addition of propolis in suspension lead to a increase of GP parameter, thus the lipid membrane is more rigid, Laurdan molecules being less accessible to water molescules.**
- **The differences that appear at the interaction between the two types of lipid membranes and propolis are due to the constituents of the latter (different proportions of hydrophobic molecules and / or that have electric charge).**


## Acknowledgments

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