**Introduction**

Rose oil is one of the most expensive essential oils, often referred as “liquid gold”. During its production from fresh rose flowers by hydrodistillation, significant amounts of waste water are produced and discarded in the fields. The waste water from rose oil distillation is actually a water extract of rose petals, which is reported to extend the life span of Drosophila flies (1) and to exhibit moderate anti-HIV activity (2). This extract is rich in phenolic compounds, possesses significant antioxidant activity and thus could be useful as a functional food ingredient (3-5). So it is expected that the by-product of rose hydrodistillation could be a readily available source of valuable compounds with potential to be applied in health nutrition and cosmetic industries. However, the data concerning the chemical composition of the waste water from rose distillation are very scarce.

Under the frame of the European project “EXANDAS”, several samples of the above-mentioned by-product were collected and their exploitation for the development of high-added value natural products was investigated. The aim of this work was to study the optimal processes in order to produce an extract rich in phenolic content.

**Plant material and applied methodologies**

The process for the recovery of a rose petals extract was designed in two stages: Firstly, samples of the residue that remains during the procedure of *Rosa damascena* hydrodistillation were collected in May 2016 (Galén company, Bulgaria). Then, the aqueous extract was filtered and treated with centrifugal liquid-liquid extraction using a solvent system of EtOAC/EtOH 10:1 in several flow rate ratios. The extracts were evaporated and were evaluated for their Total Phenolic Content (TPC), Total Flavonoid Content (TFC) while High Performance Thin Layer Chromatography (HPTLC) analysis was performed for their chemical profiling. The most promising extract, obtained by ratio of solvent system:extract 1:2, was subjected to centrifugal partition chromatography (CPC) and resulted to the isolation of nine pure compounds, which were identified by 1D and 2D NMR experiments (H, J, DEPT, COSY, HMBC, HSQC and NOESY).

**Liquid-liquid extraction**

In order to eliminate sugars and to obtain an enriched phenolic extract, liquid-liquid extraction was used. Different ratios aqueous extract/organic system had been subjected. In table 1, the volume of the organic phase and the recovery yield are presented for each trial. For the recovery of the organic phase, 100 ml of the initial aqueous extract were evaporated and resulted in 330,3 mg of dry extract.

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<th>Table 1: Liquid-liquid extraction trials</th>
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**Centrifugal Partition Chromatography**

The organic phase obtained from liquid-liquid extraction with ratio 1:2 was evaporated and the residue was subjected to CPC (Fig.1) using the stepwise gradient elution-extraction methodology and the following biphasic solvent systems. From the fractions collected (Fig. 2), nine pure compounds were isolated and identified as quercetin glycosides (1-3), kaempferol glycosides (4-7) and phenols (8, 9).

**Conclusions**

Rose is mainly processed for the production of rose oil and rose water. During the hydrodistillation, the aqueous extract remaining in the hydrodistillation unit, has been shown to contain several bioactive metabolites, mostly flavonoid glucosides, that could be effectively recovered as an enriched phenolic fraction using the liquid-liquid extraction technique. The final extract presents a significant phenolic content and antioxidant activity. The trial of the ratio 1:2 between the aqueous rose extract and the organic solvent system proved to be the most promising one, as in terms of yield, total phenolic content, total flavonoid content and the consumption of organic solvents. This phenolic mixture was subjected in CPC resulting to the isolation of nine pure compounds belonging to the categories of flavonoids and phenols. The phenolic fraction obtained from rose - hydrodistillation byproduct presents a strong interest in terms of composition and could be destined in the market of cosmetics and/or nutraceuticals and phytotherapeutics.

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**References**


**Fig.1 Centrifugal Partition Chromatography (CPC) instrument**

**Fig.2 HPTLC with the fractions collected using CPC**

(C) HPTLC of the fractions collected using CPC

1. R = 3-O-β-glucopyranoside
2. R = 3-O-β-D-galactopyranoside
3. R = 3-O-rutinoside
4. R = 3-O-β-glucopyranoside
5. R = 3-O-a-rhamnopyranoside
6. R = 3-O-a-L-rhamnopyranoside
7. R = 3-O-(6″-O-p-coumarosyl)-β-D-glucopyranoside

1: the pigments were identified as quercetin-3-O-glucoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside.