

**LABORATORY REPORT**

SUBJECT: Determination of Total Hypericins, Total Phenolic Content and Total Flavonoid Content in an Hyperici Oleum sample

DATE: 21/10/2015

Sample name: HYPERICI OLEUM_HIPPOCRATES MEDICINE

Botanical species:	<i>Hypericum perforatum</i>
Description:	<i>Hypericum perforatum</i> extra virgin olive oil extract
Producer / Distributor:	KANELLOS KONSTANTINOS
Date of reception:	1/10/2015
Date of analysis:	8 - 12/10/2015
Packaging:	Glass bottle of 60 ml (4 x)

Comments and conclusions:

- **Total Hypericins Content : 90 µg / 100 g oil**
- **Total Phenolic Content (TPC): 15,3 ± 0,3 mg GAE / 100 g oil**
- **Total Flavonoid Content (TFC): 8,5 ± 0,1 mg QE / 100 g oil**

Validated report by:

Analytical Department

A. DETERMINATION OF TOTAL HYPERICINS IN HYPERICI OLEUM EXTRACT

The content of Hyperici Oleum sample in total hypericins was determined spectrophotometrically and expressed as µg of hypericin per 100 g of oil. For this purpose, the method described in the European Pharmacopoeia monograph of St. John's wort (Hyperici herba) was followed [1], with some modifications.

More specifically, 100 g of oil were extracted with methanol under a reflux condenser for 30 min. The mixture was then centrifuged and the supernatant was collected. The extraction procedure was repeated once more and the methanol extracts were combined and evaporated to dryness under vacuum. The residue was then diluted in methanol, introduced into a measuring flask and diluted to 100 ml with methanol.

The absorbance of the final methanol solution was measured at 587 nm by comparison with the compensation liquid (methanol). The percentage content of total hypericins, expressed as hypericin, was calculated using the following expression:

$$\frac{A \times 100}{m \times 780}$$

Where: A = the measured absorbance, m = mass of the oil to be examined in grams, 780 = the specific absorbance of hypericin at 587 nm.

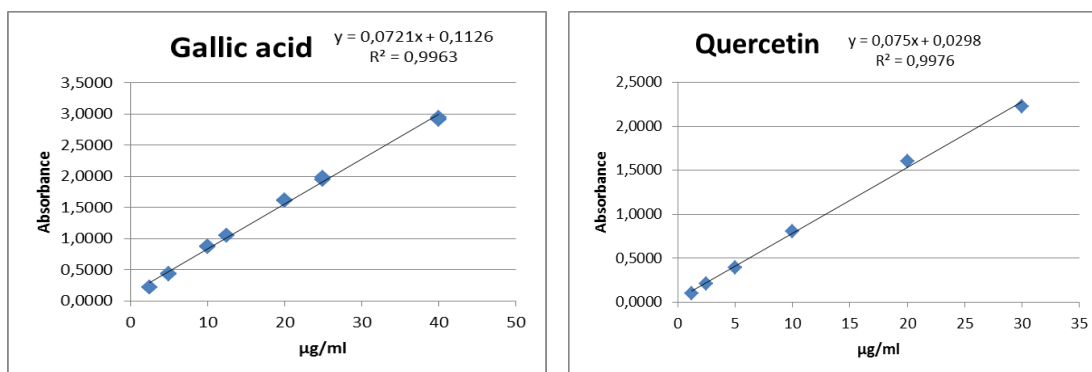
The result is given in **Table 1**.

B. DETERMINATION OF TOTAL PHENOLIC (TPC) AND TOTAL FLAVONOID (TFC) CONTENT

The total phenolic content (TPC) of the Hyperici Oleum sample was estimated by a colorimetric method based on the use of Folin-Ciocalteu reagent, which measures the total reducing capacity of a sample. The result (**Table 1**) was expressed as mg of Gallic acid equivalents (GAE) per 100 g of oil.

Similarly, the total flavonoid content (TFC) of the sample was estimated photometrically and the result (**Table 1**) was expressed as mg of Quercetin equivalents (QE) per 100 g of oil.

For the quantitative determination of total phenols and total flavonoids in the methanol extract of the Hyperici Oleum sample, standard calibration curves of Gallic acid and Quercetin were prepared and the analysis was performed at 765 nm and 415 nm respectively.



Calibration curves of Gallic acid (2.5-40 µg/ml) (left) and Quercetin (1.25-30 µg/ml) (right).

Table 1: Results from the analysis of the sample HYPERICI OLEUM_HIPPOCRATES MEDICINE.

Sample Name	HYPERICI OLEUM_HIPPOCRATES MEDICINE
Hypericin (µg/100 g Oil)	90
TPC (mg GAE/100 g Oil)	15,3 ± 0,3
TFC (mg QE/100 g Oil)	8,5 ± 0,1

References

- [1] European Pharmacopoeia, 3rd Edition, Suppl. **2001**. Hypericum Monograph. Council of Europe, Strasbourg, France.
- [2] Arsić I., Žugić A., Antić D.R., Zdunić G., Dekanski D., Marković G. and Tadić V., **2010**. Hypericum Perforatum L. Hypericaceae/Guttiferae Sunflower, Olive and Palm Oil Extracts Attenuate Cold Restraint Stress – Induced Gastric Lesions, *Molecules*, 15, 6688-6698.