Medical Effects and Extraction of Apis Mellifera. L in Southern Albania

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***Abstract*— Bee products have attracted the attention of consumers and researchers due to their curative properties and their chemical composition. Propolis (*Apis Mellifera, L*) is one of the bee products which are formed by mixing the wax and resinous substances with glycosidase enzyme present in the spit of bees. This product is used by bees to protect the hive from external inflows, pollution and keeps under control the temperature of the bees hive. Studies have shown that propolis presents a high number of biological and pharmacological properties as antimicrobial, antioxidant, anti-inflammatory, anticancer. In this study the extractions of propolis are carried out by liquid CO_2 under pressure at 0^0C, 20^0C and 40^0C. Propolis is collected in the southern Albania and the amount used for every the extractions is about 16 grams. The extraction carried out at 40^0C gave the best yield of 9.5 %, whereas the extractions at 0^0C and 20^0C gave rise to lower yields of 1.9 % and 4.9 % respectively.**

***Index Terms*— propolis, extraction, anti-oxidant activity, antimicrobial activity.**

I. INTRODUCTION

Propolis is extensively used in foods and beverages because it improves human health. It contains more than 300 natural compounds such as polyphenols, phenolic aldehydes, sequiterpene-quinones, coumarins, amino acids, steroids and inorganic compounds. Propolis is one of the most abundant sources of polyphenols, mainly flavonoids and phenolic acids [1]. The antimicrobial activity was showed to be related to the polyphenolic contents, as in [2] while the strong anti-oxidant activity, is accompanied by high phenolic and flavonoids content [3]. Propolis is one of the bee products with curative properties, which cannot be used as food due to the resinous composition. It is a red or brown resinous substance which is collected by honeybees from tree buds and it is formed by mixing resinous substances with wax and glycosidase enzyme present in the spit of bees [4], [5]. Propolis has been known from the Egyptian clerics for thousand years and it has been used as a mummification product in the ancient Egypt. It has been known even from the Persians and ancient Greeks for the reparation of clothes and treatment of skin diseases. At room and high temperature propolis is sticky. At low temperature propolis is solid. The chemical composition varies depending on the botanic source. Full analyses of propolis have indicated a composition of about 55 % resinous substance and balsamic components, 30 % bee wax, 10 % etheric and aromatic oils, alcohol and vanillin [6], [7]. Propolis can cure many diseases owning to its strong curative effects like urinary infections, throat infections, bronchitis and intestinal infections. The aim of the present work is to study the extraction of propolis by means of liquid CO_2 (near critical conditions) under pressure at different temperatures, i.e. 0^0C, 20^0C and 40^0C. CO_2 is extensively used solvent in extractions. It is harmless, environmentally safe and non-explosive and it can be easily removed from products. The kinetic data of this work indicated that the extraction carried out at 40^0C gave the best yield of 9.6 %, whereas the extractions at 0^0C and 20^0C gave lower yields of 1.9 % and 4.8 % respectively.

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II. MATERIALS AND METHODS

All the samples of *Apis mellifera*, L. (Propolis) are collected in October in south of Albania. Propolis was stored at low temperatures, 4 – 8^0C. The samples of propolis were beforehand crushed up in order to crumble them prior to extraction with liquid CO_2. The amount of propolis for every extraction was about 16 g.

Extraction method: all the extractions were carried out with liquid CO_2 (subcritical conditions). At temperatures between 55^0C to 31^0C and pressures 5 to 74 bars CO_2 behaves like non polar solvent. CO_2 is a selective solvent at temperature 31^0C and pressure 50-70 bar. It dissolves mainly non polar components and to some extent polar components with molecular weight over 400 g/mol. The extractions in this study are carried out in a homemade autoclave, as shown in Figure 1, within which a Soxhlet apparatus is assembled. The autoclave is a model improved by Lentz [8], which provides a visual control of condensation and the level of liquid in the siphon through a glass window in the upper part of autoclave scheme in Figure 1.

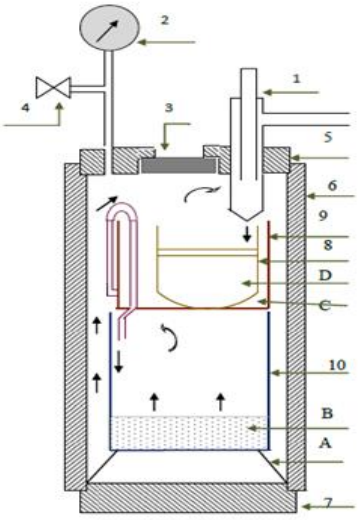

Fig. 1: Schematic representation of the autoclave used for all the extraction by means of liquid CO_2. 1. Cooler, 2. Manometer, 3. Glass window, 4. Valve, 5. Expansion adapter, 6. Steel cylinder, 7. Basement, 8. Sample container, 9. Soxhlet glassware with siphon, 10. Extract collector, A.

Liquid gas-extract, B. Extract in collector, C. Extract solution D. Material of extraction.

Once the sample container is filled with the granular material then the autoclave is allowed to be filled with CO_2 gas up to a constant weight (230 - 235 g CO_2). The amount of CO_2 is controlled by weighting the autoclave before and after filling with CO_2 gas. The autoclave is sunk afterwards into a water bath. The extraction process occurred at three different temperatures (0^0C, 20^0C, 40^0C). Therefore, the water bath was kept at a desired temperature prior to extraction process. All the obtained extracts were stored in vials and kept at a dark place.

III. Results and Discussions

The extraction process was carried out for a certain number of cycles. The latter accounts for the time of extraction process (in minutes). Table 1 displays overall results for the extractions carried out at 0^0C, 20^0C and 40^0C. It is obvious from Table 1 that highest yield is obtained when the extraction was carried out at 40^0C (temperature of water bath) as expected.

Table 1: Overall results of extractions yield (%) carried out with liquid CO_2 at different temperatures

Temperature of water bath (^{0}C)	Time of extraction (min.)	Amount of propolis (g)	Yield (%)
0	**648**	0.28	**1.90**
20	**1008**	0.73	**4.80**
40	**1026**	1.01	**9.60**

In order to obtain the highest yield at different temperatures, all the extraction were allowed to run overnight. For the extraction carried out at 0^0C, the best result of the yield was obtained after ~ 11 hours (Table 2). It is apparent from Table 2 that by increasing the time of extraction the number of cycles rises as well.

Table 2: The overall kinetic data for the extraction of propolis with liquid CO_2 at 0^0C

Number of cycles	Time of extraction (min.)	Amount of extract (g)	Yield (%)
2	108	0.11	0.71
6	324	0.15	1.09
8	432	0.21	1.44
10	540	0.23	1.72
12	**648 (~11 h)**	0.28	**1.90**

The data of Table 2 are plotted in Figure 2. It is obvious from Figure 2 that upon increasing the time of extraction the yield of extraction goes up. Upon increasing the time of extraction (longer than 11 hours) the amount of the extract obtained did not change compared with the amount obtained after ~ 11 hours.

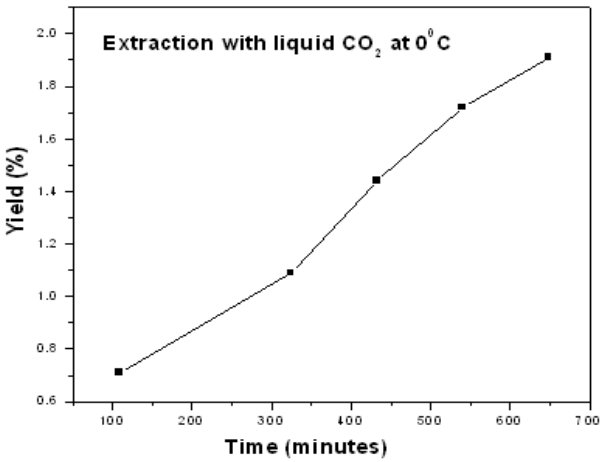

Figure 2: Plot of yield versus time of extraction. Temperature of extraction used was 0^0C.

For the extraction carried out at 20^0C, the best result of the yield was obtained after ~ 16 hours (Table 3). It is apparent from Table 3 that by increasing the time of extraction the number of cycles rises as well.

Table 3: The overall kinetic data for the extraction of propolis with liquid CO_2 at 20^0C

Number of cycles	Time of extraction (min.)	Amount of extract (g)	Yield (%)
3	84	0.24	1.62
7	196	0.30	1.95
10	280	0.45	2.99
12	336	0.49	3.29
16	448	0.56	3.72
20	560	0.64	4.25
26	728	0.68	4.56
36	**1008 (~16 h)**	0.73	**4.80**

The data of Table 3 are plotted in Figure 3. It is noticeable from Figure 3 that upon increasing the time of extraction the yield of extraction ascends too. Upon increasing the time of extraction (longer than 16 hours) the amount of the extract obtained did not change compared with the amount obtained after ~ 16 hours.

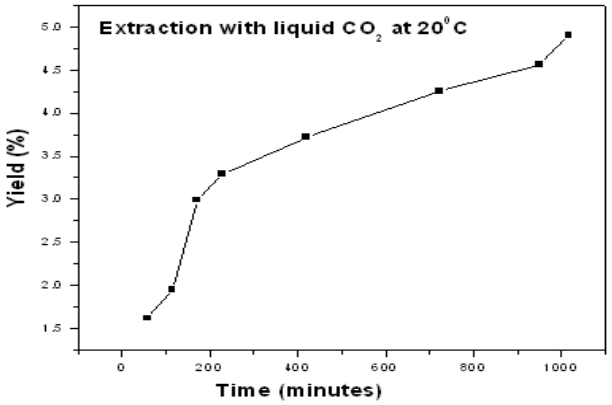

Figure 3: Plot of yield versus time of extraction. Temperature of extraction used was 20^0C.

Lastly, for the extraction carried out at 40^0C, the best result of the yield was obtained after ~ 17 hours (Table 4). It is also apparent from Table 4 that by increasing the time of extraction the number of cycles increases as well, as already indicated with the extractions at 0^0C and 20^0C.

Table 4: The overall kinetic data for the extraction of propolis with liquid CO_2 at 20^0C

Number of cycles	Time of extraction (min.)	Amount of extract (g)	Yield (%)
3	57	0.26	1.76
6	114	0.57	3.84
9	171	0.82	5.46
12	228	0.96	6.39
22	418	1.09	7.25
38	722	1.26	8.37
50	950	1.43	9.18
54	**1026 (~17 h)**	1.01	**9.60**

In comparison to the graphs plotted in Figures 2 and 3, the graph plotted in Figure 4 (plotted data of Table 4) also indicates that upon increasing the time of extraction the yield of extraction goes up too. Increasing the time of extraction up to ~20 hours did not give rise to higher yields.

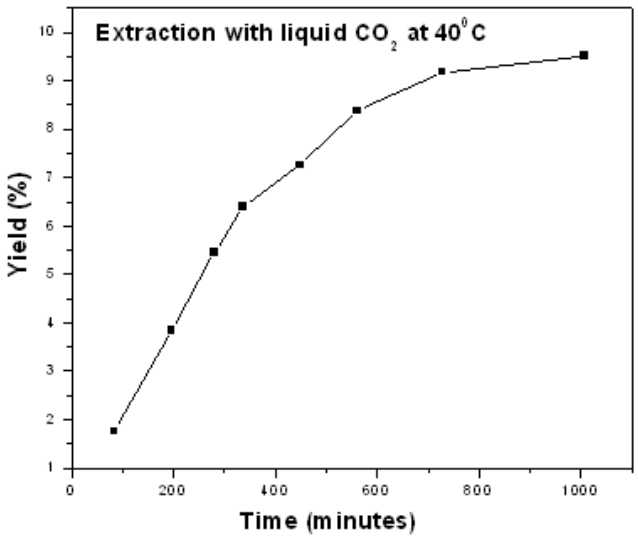

Figure 4: Plot of yield versus time of extraction. Temperature of extraction used was 40^0C

IV. Conclusions

To this end a summary of the kinetic data for propolis extracted at three different temperatures are plotted in Figure 5 in a 3-D graph. Yield of extration is ploted as function of temperature and time of extraction. As noted upon increasing the temperature and the time of extraction the yield of extracts enhances as well. The best yield is obtained at 40^0C. Application of the lower temperatures gave rise to lower yields.

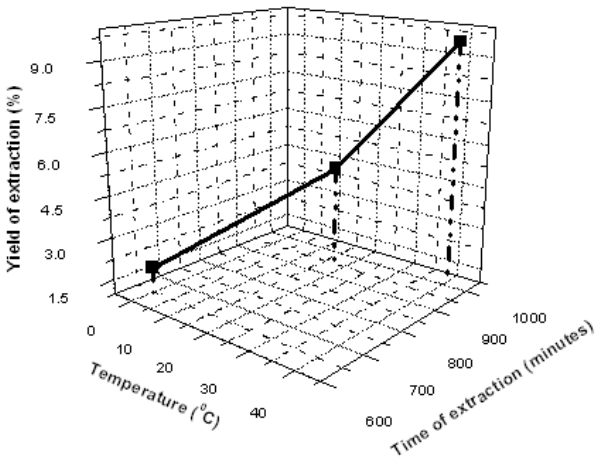

Figure 5: 3-D Plot of the of the kinetic data reported in this work (from Table 1).

The maximum yield of 9.6 % was reached after 1026 minutes or ~17 hours for the extraction carried out at 40^0C. The maximum yield of 4.8 % was reached after 1008 minutes or ~16 hours for the extraction carried out at 20^0C, whereas the maximum yield of 1.9 % was reached after 648 minutes or ~11 hours for the extraction carried out at 0^0C.

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I am a Pharmacist and I finished my studies in Master of Science Degree in Pharmacy at Florence University in Italy in 2008. During all my years of study I obtained grants from Italian Government. Consequently held

successfully the state professional exam of Pharmacists. Actually I am a PhD student at Department of Life, Health and Environmental Sciences at University of L'Aquila. I work as a Coordinator of Master in Science Degree in Pharmacy at Albanian University in Tirana. As a Coordinator have a special set of responsibilities to ensure that faculty of Pharmacy is fully supported in teaching efforts and that all students in the course have equal learning opportunities. I am a Lecturer in "Toxicological Chemistry" teaching pharmacological nature of drugs, toxic compounds, pesticides, drug-receptor interactions on the molecular site of action, structure-activity relationships, drug absorption, distribution, metabolism, elimination, and toxicology. Since 2013 I am also a lecture in "Albanian Pharmaceutical Law, regulations and guidelines" teaching pharmaceutical ethics, Albanian Pharmaceutical Law, regulations and guidelines, emerging and safety medications. I have excellence proficiency in Italian, English and German Languages.

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