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PREPARATION OF PHARMACEUTICAL BEE-WAX AND PHYSICOCHEMICAL ANALYSIS

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ABSTRACT

Regarding the importance of beeswax, we evaluate the preparation of the pharmaceutical beeswax in Iran. The raw beeswax was provided from the waxes prepared by *Appis Mellifera* L in two different seasons (fall and spring). In order to remove honey and other debris, wax pieces should be washed for several hours. Then, wax was melted by several methods by using hot water, steam water and organic solvents, in a controlled temperature. After filtering and cooling the wax sheets was analyzed according to British Pharmacopoeia. The same procedure was performed to prepare the white wax in the presence of a bleaching agent like hydrogen peroxide.

Some physicochemical values were out of standard limits by using ether for preparing spring and fall yellow wax. Using ethanol as a solvent for extraction was not suitable and decreased the values significantly. Regarding to those samples that were prepared by hot water we could get a suitable result. The spring white and yellow waxes which were prepared by hot water method in temperature below than 85° C is recommended as a suitable and economic method with the same results comparing with BP (British pharmacopoeia) standard beeswax.

Key words: *Appis mellifera*, beeswax, BP, Iran, extraction, pharmaceutical beeswax

INTRODUCTION

Origin natural waxes are mixture of various long-chain fatty acids and a variety of other constituents. Eight wax glands on the underside of the abdomens of the young bees secret small wax platelets. Beeswax has an extremely wide spectrum of useful applications and occupies a very special position among waxes. Beeswax has long found use in medicinal practices and in creams and lotions. The melting point of beeswax is not constant since the composition varies slightly with its origin (Crane, 1990). Beeswax is an inert material with high plasticity at a relatively low temperature (around 32^0C). By contrast, at this temperature most plant waxes are much harder and of crystalline structure. Beeswax is also insoluble in water and resistant to many acids, but is soluble in most organic solvents and after warming, in alcohol and fatty oils. Pure beeswax from *Appis mellifera* consists of at least 284 different compounds; over 111 are volatile (Tulloch, 1970, 1974, 1980). At least 48 compounds were found to contribute to the aroma of beeswax. Quantitatively, the major compounds are saturated and unsaturated monoesters, diesters, saturated and unsaturated hydrocarbons, free acids and hydroxy polyesters (Tulloch, 1980). There have been several methods for the beeswax preparation as a standard product (Coggshall and Morse, 1984; Abrutyn, 1980). Beeswax is considered safe for human consumption and has been approved as an ingredient in human food, cosmetic and pharmaceutical preparations (Chowdary and Sastry, 1987; Puleo, 1987). Pharmaceutical beeswax properties could be confirmed by the analysis as mentioned in BP (British pharmacopoeia, 1998). At the same time these properties can create a problem when wax is stored near toxic chemicals and pesticides or after treatment with various drugs inside the hive. Any fat soluble toxins can be absorbed and then released much later when the wax is consumed as food, used in cosmetics and pharmaceuticals or given to bees in the form of foundation sheets (Brenal *et al*., 1997; Nozal *et al*., 2002) Unfortunately because of expenses, detecting toxic compounds in beeswax has not included in pharmacopoeias but it is a goal of the researches now. The objective of this work was preparing standard pharmaceutical beeswax for the first time in Iran.

MATERIALS AND METHODS

Fall and spring beeswax combs were provided by Agricultural Research Centre of Mazandaran province. All the reagents and solvents such as xylene, hydrochloric acid, potassium hydroxide, acetic acid, Na- EDTA etc were purchased from Merck Company. In order to standardize the prepared beeswax BP method was used and all of the standard solutions were prepared according to BP.

Fall and spring yellow wax extraction:

It was prepared by 3 different methods. Separately 4 g of beeswax comb collected from 2 different seasons with 40 ml of distilled water was heated in temperature below 85° C until melting. The melted wax was filtered to

remove some impurities. Then melting and filtering were repeated several times. Yellow beeswax was obtained as a product. The other methods were carried on in a similar condition but the solvents were 90% ethyl alcohol and ether. Yellow wax was obtained after removing solvent by distillation under the pressure.

Preparation of white beeswax from yellow beeswax:

For bleaching, each kind of the yellow waxes prepared through the above procedures was treated by hydrogen peroxide solution separately on a steam bath for 30 min.

Physicochemical properties of yellow and white wax according to BP:

Melting point:

The simplest method is to determine the melting point, by measuring the temperature at which the first liquid wax appears during very slow heating. It should be between 61 and 66°C or preferably between 62 and 65°C. For this purpose some white or yellow wax in a flask was heated though the temperature increases 10° C every minute. The melting point recorded when the first drop observed due to melting.

Acid value:

In a flask, 2 g of beeswax in 40 ml of xylene was added. While the mixture heated ethyl alcohol 96% (60ml) and phenolphthalein solution (0.5 ml) were added. And titrated with 0.5 M potassium hydroxide solution until the solution remains pink after shaking for 10 seconds (the volume of KOH un this stage = n_1) and the titration was continued until a constant color was observed (n_2). Acid value was calculated by the formula: $28.5 (n_1-n_2)/W$ (W refers to the sample weight in grams). This titration was performed on yellow and white wax obtained from different seasons separately.

Saponification value:

The test measures the amount of hydrocarbons, which saponify (turn into soap) and give a clear solution. Saponification value was calculated by the formula: $28.5 (n_1-n_2)/W$ (W refers to the sample weight in grams). In a flask containing 30 ml of xylene, 2 g of beeswax was added and heated. The mixture was treated with 0.5 M potassium hydroxide solution and refluxed for 3 h and then phenolphthalein solution (0.5 ml) was added. The titration of the hot mixture was carried out by 0.5 M hydrochloric acid (n_2). The n_1 volume was obtained by a titration of 25 ml of an alcoholic KOH solution by a 0.5 M hydrochloric acid (n_1). This titration was performed on yellow and white wax obtained from different seasons separately. Ester value was calculated by subtracting acid value from saponification value and ratio number (ratios between ester and acid values) calculated by ester value divided by acid value.

RESULTS AND DISCUSSION

Data are shown in the Tables 1 and 2. As it shows the spring yellow and white waxes extracted by water were obtained in 95% and 95.5% yields.

Quality standards for wax are set in most countries according to their pharmacopoeias. Physiochemical values in the tables are determined according to BP. Melting point values within the standard range are not a guarantee of purity and determination of other values are necessary. Ester and saponification values of fall yellow wax are within the limits of BP, but acid and ratio number are out of BP limits. Spring yellow wax extracted by water shows values in Bp levels. Fall white wax extracted by water shows melting point and saponification value in agreement with BP. Melting point, acid, saponification, ester values and ratio number of spring white wax extracted by water gives a standard range as BP. In this study the physicochemical values are in standard range for white and yellow (spring and fall both) waxes that were extracted by water. The results shows water is the most suitable solvent for extraction of beeswax, since the waxes were prepared with water extraction demonstrates standard physicochemical values. Ethanol and ether cannot be a suitable solvent for the extraction; the values are out of BP standard range. One of the extraction methods for beeswax is boiling in solar wax melters, and then filtering, but this method did not show a favorable yield. Also extraction by using steam, boiling water and hot water in metallic containers such as Al, Cu and stainless steel gives higher yields. On the other hand some metals react with wax and discolorination happens. Direct exposure of wax to hot steam results in partial saponification (Crane 1990: Coggshall and Morse, 1984). Extraction of beeswax in hot water by using centrifuge is expensive. Using chemical solvents such as benzene, acetone, and toluene have been used in laboratories and industry but the solvents remains in the final product in

some extent. We decided to prepare pharmaceutical beeswax from wax secreted by Appis mellifera collected in 2 seasons of spring and fall. In this study using water below 85° C is the most suitable solvent of extraction and the products show standard values within set limits as mentioned in BP and it is also economically valuable. The standard values can change after excessive heating. Also yellow wax was prepared in water and collected in spring had the best results since the nature in the spring is more suitable for bees to produce wax. White wax in the similar condition shows good results (collected in spring, extracted by water). Ethanol and other chemical solvents change the standard values from the standard levels. Ethanol removes free fatty acids of beeswax and changes the values into the out of standard. Recent researches have shown that bee's combs might be contaminated by environmental contaminants like insecticides (Brenal *et al*., 1997; Nozal *et al*., 2002). So it seems to detect these contaminants should be considered more in order to get standard bee products such as beeswax.

Table 1: Results of BP standard tests on prepared yellow wax collected in spring and fall.

Physico chemical values and yields Samples	mp° C	Acid value	Saponification value	Ester value	Ratio number	% Yield
Standard white wax[a]	61- 65	17-24	87-104	70-80	3.3-4.3	
Fall yellow wax extracted by water	62.9±0.37[b]	16.71±0.41	97.02±1.34	80.33±1.29	4.80±0.14	95
Fall yellow wax extracted by ether	61.5±0.50	17.05±0.63	87.79±1.90	70.74±1.41	4.15±0.33	87
Fall yellow wax extracted by 90% ethanol	60.5±1.58	11.44±1.07	83.30±1.59	71.86±1.65	8.87±1.83	92.2
Spring yellow wax extracted by water	62.7±0.47	16.94±0.54	95.93±1.11	78.87±0.87	4.65±0.12	95.5
Spring yellow wax extracted by ether	61.7±0.27	16.80±0.57	84.14±1.21	67.34±1.24	4.60±0.21	91
Spring yellow wax extracted by 90% ethanol	61.6±0.65	11.78±1.19	76.01±1.89	64.23±1.26	5.48±0.48	93.7

[a] all data shows mean±SD. [b] the physicochemical values for standard beeswax according to BP

Table 2. results of BP standard tests on prepared white wax collected in spring and fall

Physicochemical values and yields Samples	mp° C	Acid value	Saponification value	Ester value	Ratio number	%Yield
Standard yellow wax[a]	61- 65	17-22	87-102	70-80	3.3-4.3	-
Fall yellow wax extracted by water	62.5±0.44 [b]	16.6±0.27	90.60±0.68	74.11±0.54	4.46±0.06	92.5
Fall yellow wax extracted by ether	60.7±0.57	17.27±1.33	83.64±1.68	66.36±1.01	3.86±0.34	89.5
Fall yellow wax extracted by 90% ethanol	59.2±1.04	8.75±1.50	81.90±1.56	73.15±1.8	8.66±1.14	89.5
Spring yellow wax extracted by water	62.4±0.37	17.16±0.67	90.88±0.56	73.71±1.09	4.30±0.22	95
Spring yellow wax extracted by ether	61.4±0.42	17.27±0.92	80.50±1.71	63.22±5.40	3.67±0.48	90
Spring yellow wax extracted by 90% ethanol	60.1±0.79	11.20±1.16	72.93±1.21	61.71±1.26	5.53±0.65	90

all data shows mean±SD. [b] the physicochemical values for standard beeswax according to BP

REFERENCES

Abrutyn E. (1981). Beeswax: vanishing cosmetic raw material. *Drug Cosmet Ind*., 127: 136- 138.

Brenal, J.L., M.J. Nozal, L. Toribio, J.J Jimenez, J. Atienza (1997). High- performance liquid chromatography determination of benomyl and carbendazim residues in apiarian samples. *J. Chromatography* A, 787: 129- 136.

British Pharmacopoea, (1998). *1st international* Ed, London, HMSO, pp: 58.

Chowdary, K. P. and V. H. Sastry (1987) Microencapsulation of diclofenac- comparison of drug release from various microcapsules. *Eastern- Pharmacist*, 40: 119- 122.

Coggshall, W. L. and R. A. Morse (1984). *Beeswax - Production, harvesting, processing and products*. Cheshire/CT: Wicwas Press, P.O. Box 817.

Crane, E. (1990).. *Bees and beekeeping. Science, Practice and World Resources*. Ithaca/NY: Comstock Publishing Associates, ISBN:0-8014-2429-1.

Nozal, M. J., J. L. Brenal, J.J. Jimenez, M.J. Gonzalez, M. Higes (2002). Extraction of thymol, eucalyptol, menthol, and camphor residues from honey and beeswax. *J. Chromatography* A, 954: 207- 215.

Patel, S., D. R. Nelson and A.G. Gibbs (2001). Chemical and physical analyses of wax ester properties. *J.Insect Science*, 1.4: 1-7.

Puleo S. L. (1987). *Beeswax*. Cosmetics & Toiletries, 102: 57-58.

Tulloch, A. P. (1970). The composition of beeswax and other waxes secreted by insects. *Lipids*, 5: 247- 258.

Tulloch, A.P. (1974). *Composition of some natural waxes*. Cosmetics and Perfumery, 89: 53-54.

Tulloch A.P. (1980) Bees- wax- composition and analysis, Bee World, 61: 47 – 62.

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