

POST-EXTRACTION WITH MACERATION, AN ADVANCED METHOD IN INCREASING THE EXTRACTION COEFFICIENT FOR AGNUS CASTUS

Ejup LATIFI¹, Mahi LATIFI², Shefket DEARI³, Nazif JASHARI⁴, Neset IZAIRI⁵

¹ Roads of the Republic of Macedonia-- University of Tetova

¹Max Zeller Söhne AG pflanzliche Heilmittel Romanshorn

² University of Tetova- Tetove

³University of Tetova Tetove

⁴NPK Tetova –Tetove -- University of Tetova

⁵ University of Tetova Tetove

mahi.latifi@hotmail.com

Abstract

Agnus castus Fructus is a pharmaceutical plant that has been used since ancient times in the form of fruits, but since the beginning of the 21st century, when this pharmaceutical plant was declared of particular interest due to its healing properties, it has also begun a very wide application in almost all pharmaceutical industries. The choice of the appropriate extraction method is a very important factor for the extraction coefficient. The maceration extraction method is quite practical and is applied quite successfully since the raw material for extraction is its fruits, since the carrier substances of Agnus Castus are concentrated in the fruits. It is worth noting that all maceration factors play a special role in the extraction coefficient, starting from the size of the granules, temperature, degree of mixing, concentration of the solvent and the duration of the extraction. Although single-stage extraction with maceration gives very good results, it has been tested with an extraction addition to increase the extraction coefficient for the same maceration parameters. This means an additional extraction for the same extraction conditions by adding an additional amount of fresh solvent so that the must allows the extraction process to reach equilibrium and the extraction process to be interrupted.

Keywords: extract, maceration, extraction coefficient

Introduction

Although the plant has been known as a pharmaceutical raw material since antiquity, its use in pharmacy only increased after the University of Würzburg designated it a medicinal plant of the future due to its carrier substances. . (Donmez, AA, 2004) This also led to increased interest in selecting suitable extraction methods and optimizing extraction parameters to increase the extraction coefficient and obtain extracts of the highest quality. Leaves and flowers are used for extraction, although in most cases only the fruit is used. The plant grows in Africa, Asia, and throughout Europe. It is a small tree that flowers in June and is harvested in September and October. (Blumenthal, M., Brinckmann, J.A., Wollschlaeger, B., 2003.) This medicinal plant is so attractive in pharmacy because of its flavonoid content in the form of rutin, hyperoside, and chlorogenic acid, which possess antioxidant properties. In the past, the fruits were eaten raw in Asian and African countries and used to treat heart disease and improve blood flow to the heart. It has been found that taking an extract of this plant is very beneficial to the body after a heart attack; this extract is patented. (Kumar, D., Kumar, P., Sharma, U., 2018)The plant has been and still is traditionally used in Asia and Africa as a tea, prepared by steeping it in hot water. It reaches a height of 2–10 meters and is characterized by white flowers and reddish fruits. In the food industry, it is used in the form of syrups, jams, or tinctures that require no further processing. Special extracts are also available, but these are used as pressed products. Glycosylflavones such as vitexin and vitexin harmnoside are also carrier substances that are particularly important in herbal medicines for hormonal disorders in women during the menstrual cycle (Suerken CK , Grzywacz JG, Bell RA, Lang W., Quandt SA (2006). They relieve pain and have no contraindications. It is very important to emphasize that the extraction process should aim to obtain as many flavonoids as possible as the main carrier compounds. If

the levels are low after extraction, combining it with other flavonoid-containing medicinal plants is recommended to achieve a stronger therapeutic effect. A successful combination is *Passiflora Herba*, which also contains flavonoids, in an extract ratio of 3:1 to *Agnus Castus*. . (Hernandez-Perez, A., Bah, M., Ibarra-Alvarado, C., Rivero-Cruz, JF, Rojas-Molina, A., Rojas-Molina, JI, Cabrera-Luna, JA, 2014) Since *Agnus Castus* is used in various medical fields, the highest possible extraction of the carrier compounds with therapeutic effects is particularly important for certain conditions. It should be noted that this medicinal plant is quite temperature-sensitive as a raw material due to the oils it contains as carrier compounds. coefficient (. Kumar, D., Arya, V., Bhat, ZA, Khan, NA, Prasad, DN, 2012). The material becomes a serious source for the formation of aflatoxins B1 and B2, which multiply very rapidly, are toxic, and render the raw material unusable. This occurs mainly at the temperatures that can be reached during grinding in laboratory mills. Therefore, it is significantly better to carry out the grinding process in two stages: first, a pre-grinding, followed by a fine grinding of the ground material. Grinding the granules therefore requires more careful and specialized handling. Although aflatoxin can be removed under laboratory conditions by treatment with diatomaceous earth or other reagents, this is impossible under industrial conditions (**Mahi Latifi, Arianit Reka, Ejup Latifi 2019**)

Body of Manuscript

Agnus castus is a pharmaceutical plant that requires special treatment both during the preparation of the raw material for extraction and during the extraction process. Like any other pharmaceutical raw material, *Agnus castus*, after harvesting and the drying process, undergoes a grinding process in order to increase the active surface area of contact of the granules with the solvent. As usual for other pharmaceutical plants, it is enough for the raw material to be put into a laboratory mill using a special sieve, but in *Agnus castus* this is impossible due to the content of essential oils, which leads to an increase in temperature during grinding and the likelihood of the creation of Aflatoxin is high. Therefore, grinding also requires special treatment, therefore grinding is carried out in two stages, where in the first stage only grinding is done with a 3 mm sieve and in the second stage grinding is done using a 1 mm sieve. Under these circumstances, we do not have an increase in temperature. The grinding and milling were carried out in a Retsch 1000 laboratory mill. After the milling process, 50 g of the ground raw material were weighed and placed in a Retsch 5000 sieve analysis apparatus where granulometric analysis was performed. A higher amount of the 1.0 μm fraction was observed, which is very suitable for extraction. The values obtained during the sieve analysis are presented in Table 1 Figure 1. For the first sample, 105 g of the ground raw material were weighed in a 1000 ml laboratory chemical beaker, to which 850 ml of solvent was added, in this case ethyl alcohol with a concentration of 60%, which was previously heated to a temperature of 60°C. This amount of solvent is sufficient to cover the entire amount of the raw material with the solvent - 60% ethanol. Before the extraction process begins The chemical beaker is placed in a Bucher 2500 rack where the temperature is set to 60°C. A mixer is placed on the chemical beaker which is set to 180 rpm. From this moment the maceration process begins for a duration of 90 min. For different maceration times, a sample was taken from the solution, filtered through a 0.2 μm filter and for all samples the dry mass was measured in order to construct the extraction curve. Table 2 Figure 2. For the second sample, 105 g of the ground sample were also weighed in a 1000 ml laboratory chemical vessel to which 850 ml of ethyl alcohol with a concentration of 60% were added. Which has previously been tempered at a temperature of 60°C. Before the maceration process begins, the chemical beaker is placed in a Bucher rack which is set at 60°C. A mixer is installed above the chemical beaker which is set at 180 rpm. At this moment, the extraction process begins in 60 min. For different maceration

times, a sample is taken from the mixture and filtered with a 0.2 μm filter. The filtered samples are subjected to dry mass measurement in the Toledo 5000 apparatus to constitute the extraction curve. After extraction in 60 min, the mixing is stopped and the solution is left to stand quietly for 30 min so that the phase separation occurs where the extract solution is layered in the upper part while the *Agnus castus* remains in the lower part. Some fine particles of solid matter are observed that The bark of *Agni Castus* is crushed during maceration. With a Sepex 120 laboratory pump, the upper part of the extract is withdrawn using a 0.5 mm sieve until conditions exist for extract absorption so that only the solid phase remains. To this solid phase, 300 ml of pure ethyl alcohol solvent with a concentration of 60% is added, which has been tempered to a temperature of 60°C. and the mixing begins with a mixer which is tuned to 180 rpm. Here the post-extraction process begins for a duration of 30 min. For different post-extraction times, samples were taken from the solution in the beaker, filtration was done with a 0.2 μm filter and the dry mass was measured in the Toledo 5000 apparatus and based on the values obtained, the extraction curve was constituted Table 2 Figure 2. After the complete extraction process is completed - after 90 min the mixer is removed and the lye is turned off and for a period of 30 minutes the mixture remains quiet to create the phases. After 30 min the obtained extract which is located in the upper part of the beaker is removed from the beaker by means of the Sepex 120 pump into another 1000 ml beaker while the solid lower part is subjected to pressing in the Balg laboratory press. 2500 where the obtained extract is combined with the previously obtained extract and the solid part after pressing is discarded. The results of extraction and post-extraction are presented in Table2 Figure 2.

Conclusion

Based on the two analyses performed, it can be observed that for the first sample a satisfactory extraction coefficient is achieved, but it is also observed that after 60 min of extraction, it is observed that the digestion slowly passes into equilibrium due to the creation of a supersaturated solution, where very slow extraction begins until a complete cessation of extraction. From the analysis of the second sample, almost the same phenomenon can be observed up to a maceration time of 60 min, but after the addition of pure solvent, an increase in dry mass is observed up to the 90th minute, which is fully consistent with the rules of pharmacy, since there is still carrier material that has not yet been extracted, therefore after extraction it is seen that it has given much better results, for this reason it is preferable that the extraction be done twice, so post-extraction is of great importance for the extraction of *Agnus castus*.

Table and Figures

Table 1 Granulometric analysis of Agnus castus Fructus

Size of strainer	Measuring vessel (gr)	Vessel + raw material (gr)	Netto (gr)
8.00 mm	448.1	448.1	0
4.00 mm	430.82	430.92	0.1
2.00 mm	399.7	405.58	5.88
1.00 mm	362.8	383.77	20.97
0.50 mm	322.5	332.85	10.35
0.25 mm	290.1	297.06	6.96
0.125 mm	279.48	282.61	3.13

Sludge	400.88	403.23	2.35
--------	--------	--------	------

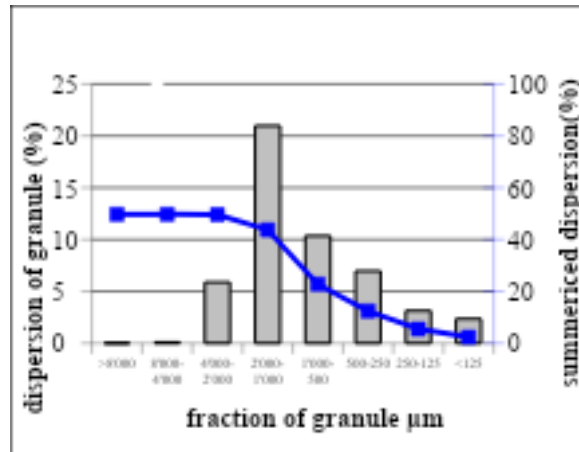


Figure 2 Fraction of granule Agnus castus Fructus

Table.2 results of dry mass in relation with the extraction time Crataegi Fructus sample 1,2

Sample 1	
Time(min)	Dry content (%)
0	0
1	0.23
10	0.96
15	1.26
30	1.58
45	1.93
60	2.45
90	2.96
Sample 2	
Time (min)	Dry content (%)
0	0.11
1	0.55
10	0.89
15	1.36
30	1.79
45	2.46
60	2.96
90	3.54

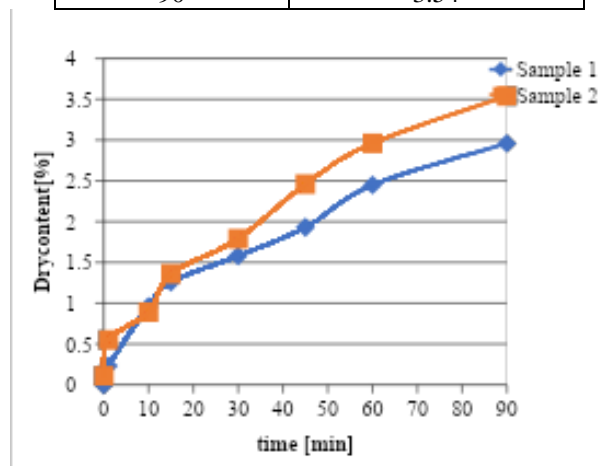


Figure 2 Outline of extraction Agnus castus Fructus sample 1,2

Reference

- [1] Blumenthal, M., Brinckmann, J.A., Wollschlaeger, B., 2003. The ABC Clinical Guide to Herbs. American Botanical Council, Austin, pp. 235–246. British Pharmacopoeia, 2009.
- [2] Donmez, A.A., 2004. The genus *Crataegus* L. (Rosaceae) with special reference to hybridisation and biodiversity in Turkey. *Turkish Journal of Botany* 28, 29–37.
- [3] Hernandez-Perez, A., Bah, M., Ibarra-Alvarado, C., Rivero-Cruz, J.F., Rojas-Molina, A., Rojas-Molina, J.I., Cabrera-Luna, J.A., 2014. Aortic relaxant activity of *Crataegus gracilior* Phipps. and identification of some of its chemical constituents. *Molecules* 19 (12), 20962–20974
- [4] Kumar, D., Kumar, P., Sharma, U., 2018. UPLC-DAD-MS based quality control and discrimination analysis of different aerial parts of *Crataegus rhipidophylla* Gand. found in Indian Western Himalaya. *Analytical Chemistry Letters* 8 (2), 177–187. <https://doi.org/10.1080/22297928.2017.1400919>.
- [5] Kumar, D., Arya, V., Bhat, Z.A., Khan, N.A., Prasad, D.N., 2012. The genus *Crataegus*: chemical and pharmacological perspectives. *Revista Brasileira de Farmacognosia* 22 (5), 1187–1200
- [6] Mahi Latifi, Arianit Reka, Ejup Latifi Removal of aflatoxin bacteria from the pharmaceutical raw material *Agnes Castus* using aluminum oxide as adsorbent, 3rd International Joint Science Congress of Materials and Polymers ISCMP2019, 12-14 September 2019 Kosovo, size 128
- [7] Suerken C.K., Grzywacz J.G., Bell R.A., Lang W., Quandt S.A. (2006) Complementary and alternative medicine use among older adults: ethnic variation. *Ethn Dis.* 16, 723-731