

EXTRACTION OF BIOACTIVE COMPOUNDS FROM WITHANIA SOMNIFERA (ASHWANGANDHA) FROM ODRI VILLAGE: COMPARISON OF METHODS AND INFLUENCE OF SOLVENTS ON EXTRACT YIELD

Mahi LATIFI¹, Ejup LATIFI²

¹Max Zeller Söhne AG pflanzliche Heilmittel Romanshorn

²Roads of the Republic of Macedonia-- University of Tetova

Contact:mahi.latifi@hotmail.com

Abstract

Withania somnifera, commonly known as Ashwagandha, is a medicinal plant of great importance in traditional medicine and modern pharmaceutical research, as the interest in this plant is closely related to the presence of bioactive components, mainly withanolides, which are responsible for biological activity. The extraction efficiency of these compounds depends on many factors, including the type of solvent, temperature, extraction time, particle size, and the extraction method used. This paper presents an analysis of the impact of modern extraction methods of *Withania somnifera*, focusing on their impact on extract yield, secondary metabolite content, and biological activity. Modern methods such as ultrasonic extraction and microwave extraction using ecological solvents have shown potential for increasing efficiency and reducing solvent consumption compared to classical extraction methods. Two samples of the material will be analyzed. first plant *Withania somnifera* using two different extraction methods and two solvents with different concentrations in order to determine which of the methods brings a higher yield and the choice of the ideal concentration for extraction

Keywords: *Withania somnifera*, extraction, bioactive composition

Introduction

1.1 Bioactive composition of *Withania somnifera*

Medicinal plants have played a special role in the development of pharmaceutical products due to their high content of biologically active molecules. Recently, scientific research has been concentrated on the identification, isolation and characterization of these compounds for use in pharmacy for the discovery of new drugs. (Dagenais.Mishra,L,C,Singh,B.B.,(2000) *Withania somnifera* belongs to the Solanaceae family and is considered one of the most important plants in modern medicine. Its roots are the most used part for the preparation of extracts, although the leaves and other parts of this plant also contain metabolites of pharmacological interest. The main components of this plant are withanolides, a group of steroidal lactones with a structure similar to natural steroids. .(Dar ,N,Jand Hamid A(2019)) Experimental studies have shown that these compounds exhibit antioxidant, anti-inflammatory, immunomodulatory and neuro protective activities. For this reason, optimization of the extract is a critical step for providing extracts with standardized content. of active substances.(Chemat,F.Rambaut,N.N.Sicaire,A.G. Meullemistre,A.Fabiano Tixier A.S.and Akbert-VianM(2017)The chemical composition of *Withania somnifera* is complex and includes a wide range of secondary metabolites. The most studied group of components are Withanoids which consist of C28 steroidal lactones and are considered as chemical markers for the standardization of extracts of this plant. (J.Azmir .J.

Zaidul,I.S.M.,Rahman AM.M,Sharif,K.M,Mohamed,A Sahena,F Jahurul M.H.A,Ghafoor,K.Norulaini N.A.N and Omar A.K.M.(2013) Among the most important withanoids are withaferin A, withanosides and withanolide A. In addition to withanoids, this plant also contains alkaloids, flavonoids and biologically active sugars. The concentration of these components varies depending on the geographical origin and post-harvest processing conditions(Stalikas,C,D(2007). The biological activities reported for extracts of *Withania somnifera* are mainly related to the ability of the active compounds to modulate inflammatory pathways and the oxidative stress.(Mirjalili ,M.H,Moyano,E.Bonfil,M.Cusido,R.M.,and Palazon J.(2009))

1.2 Extraction methods of Withania somnifera

Extraction is a fundamental process in phytochemical research because it determines the quantity and quality of bioactive components obtained. The choice of extraction method directly affects the stability of active molecules and biological activity.(Dar,P.A.,Sofi,G.andJafiri,M.A.(2015),)

Acidification is one of the traditional extraction methods which is usually carried out in a closed apparatus which is fully controlled with adequate parameters.

Extraction with Soxhlet allows a continuous contact of the plant material with the hot solvent and usually provides a more efficient extraction compared to maceration, however the use of high temperatures can affect the degradation of some thermolabile compounds(F. Scaglione, F. Ferrara, S. Dugnani, M. Falchi, G. Santoro, und F. Fraschini, „Muley B.P.Khadabadi,S.S.and Banarase ,N.B(2009).

Microwave extraction is based on the rapid heating of the plant matrix through microwave energy. This enables a more efficient release of intracellular compounds and often results in higher yields.(L..Vandem Berghe,W Sabbe,L.Kaileh,M Haegeman,GandHeyninck,K(2012).

1.3 Factors affecting the extraction process

The efficiency of extraction is affected by several factors such as the type of solvent, temperature, extraction time, particle size of the plant raw material, mixer settings, solvent concentration, and the ratio of the amount of solvent used to the plant raw material. Therefore, in order to have a proper extraction, adequate tuning of these parameters is also required.(Kumar A Kaushik,P.andKumar P. 2021))

Body of Manuscript

In this paper, the application of two extraction methods and the use of solvents with different concentrations was examined. In the first sample, the classical method for extraction by maceration was used, while in the second sample, the ultrasonic extraction method was used, also with different solvent concentrations, so analyses were made for four samples of the pharmaceutical plant material *Withania somnifera*. Before the extraction process was carried out, the plant material was prepared. First, the *Withania somnifera* roots were harvested, which after harvesting was subjected to a washing process due to the presence of soil in the *Withania somnifera* roots. The roots of the raw material before starting the extraction were subjected to drying after a high presence of moisture was observed in the *Withania somnifera* roots. The sample was placed in a dryer at a temperature of 105 °C for 10 min. for two hours. The dried sample was subjected to grinding in the Retch 1000 electric mill using a 1.0 mm sieve in order to create finer fractions. From the ground sample, 50.7 g were weighed on a Bzerba 2000 balance and placed in the Retsch

5000 sieve analysis apparatus for 10 min. By measuring the amount of fractions on different sieves, the sieve analysis was completed, constructing the distribution of the fractions of the ground raw material on different sieves, Table 1 Figure 1.

In a 2000 ml beaker, 100 g of the ground raw material were weighed and 800 ml of 50% ethyl alcohol were added. The beaker with this mixture was placed on a rack where the temperature was adjusted to 50°C. A mixer which is set to 380rpm. From here the maceration process begins for a duration of 60 min. For certain extraction times, a sample of the solution is taken and filtered with a 0.2 µm microfilter, and the filtered samples are subjected to dry mass measurement in the Toledo 5000 apparatus and from the results obtained, the extraction curve is constructed Table 2 Figure 2. In another 2000 ml chemical beaker on the Bizerba scale, 100 g of ground raw material are also weighed and 800 ml of 70% ethyl alcohol are added. The chemical beaker is placed in a rack where the temperature is set to 50°C. A mixer is placed on the chemical beaker which is set to 380rpm where the maceration process begins. For different maceration times, a sample of the mixture is taken and subjected to filtration. in a 0.2 µm microfilter and for the filtered solutions, the dry mass was measured in the Toledo 5000 apparatus and based on the obtained results, the extraction curve was constructed Table 2 Figure 2.

For the third sample which is prepared for extraction with an ultrasonic bath in a 1000 ml chemical beaker, 100 g of the blue *Withania somnifera* plant raw material were weighed on a Bizerbar scale and 800 ml of 50% ethyl alcohol was added..With the start of the apparatus, the extraction process begins in a duration of 60 min. For certain extraction times, a sample is taken from the mixture and filtered through a 0.2 µm microfilter and for the same, the dry mass was measured in the Toledo 5000 apparatus and from the obtained values, the extraction curve was constructed Table 3 Figure 3.

Also for the fourth sample in In a 1000 ml beaker, 100 g of the raw material *Withania somnifera* were weighed, to which 800 ml of 70% ethyl alcohol were added, and after starting the apparatus, the extraction process began. For certain extraction times, a sample was taken, which was filtered in a 0.2 µm microfilter, and the dry mass was measured in the Toledo 5000 apparatus, and from the values obtained, the extraction curve was constructed. Table 3 Figure 3

Table and Figures

Table 1 Granulometric analysis of *Withania somnifera*

Size of strainer	Measuring vessel (gr)	Vessel + raw material (gr)	Netto (gr)
8.00 mm	448.1	448.7	0.6
4.00 mm	430.82	432.62	1.8
2.00 mm	399.7	405	5.3
1.00 mm	362.8	374.1	11.3
0.50 mm	322.5	333.7	11.2
0.25 mm	290.1	298.6	8.5
0.125 mm	279.48	286.28	6.8
Sludge	400.88	405.78	4.9

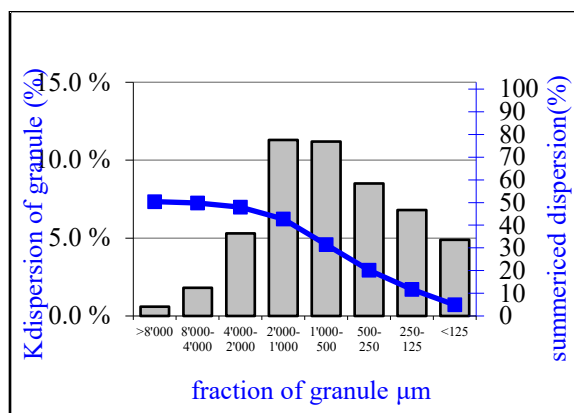


Figure .1 Fraction of granule *Withania somnifera*

Table.2 results of dry mass in relation with the extraction time of *Withania somnifera* Sample 1 ,2

Sample 1	
Time(min)	Dry content (%)
0	0
1	0.56
10	0.98
25	1.42
30	1.56
45	2.01
60	2.12
Sample 2	
Time (min)	Dry content (%)
0	0.11
1	0.66
10	1.29
25	2.03
30	2.32
45	2.96
60	3.03

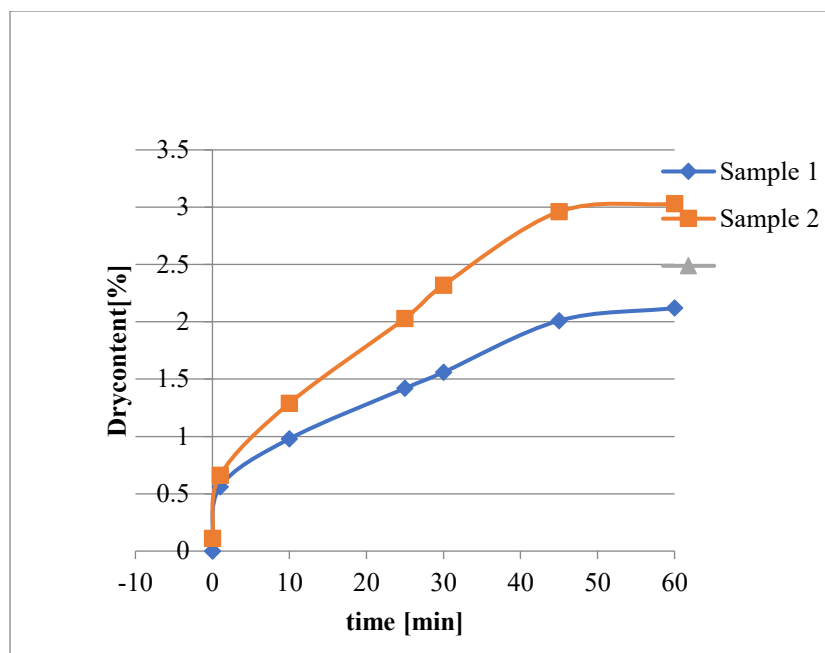


Figure.2 Outline of extraction Withania somnifera 1 ,2

Table.3 results of dry mass in relation with the extraction time of Withania somnifera Sample 3,4

Sample 3	
Time(min)	Dry content (%)
0	0
1	0.35
10	0.66
25	1.35
30	1.58
45	2.13
60	2.36
Sample 4	
Time (min)	Dry content (%)
0	0
1	0.66
10	1.36
25	1.96
30	2.35
45	2.78
60	3.15

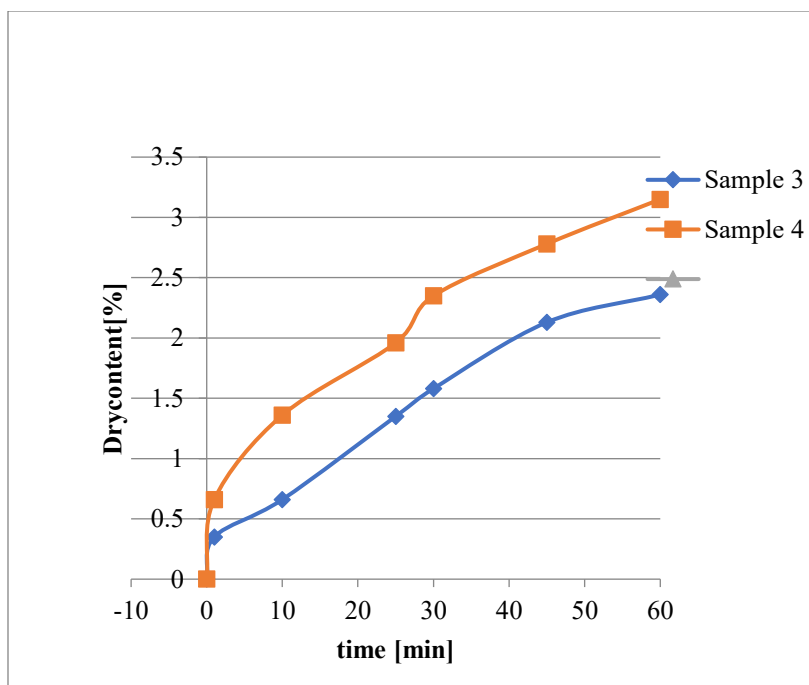


Figure.2 Outline of extraction *Withania somnifera* 3,4

Conclusion

Based on the results obtained, it is noted that the concentration of the solvent is of particular importance. It is noted that 50% ethyl alcohol is not suitable for both extraction methods because the dry mass values are lower and the extract yield is lower. The concentration of the solvent 70% ethyl alcohol gives very good results for both extraction methods and the extract has a higher yield. Regarding the selection of the extraction method, it can be concluded that the ultrasonic extraction method gives slightly more encouraging results and can be found to be the most suitable method for the extraction of this pharmaceutical raw material.

References

- [1] Dar ,N,Jand Hamid A(2019) pharmacologic overview of *Withania somnifera*,the Indian ginseng.Journal of pharmacognosy and Phytochemistry 8,1-7
- [2] Dagenais.Mishra,L,C,Singh,B.B.,.(2000)Scientific basis for the therapeutic use of *Withania somnifera*(Ashwagandha).A review.Alternative Medicine Review 5 334-346
- [3] F. Scaglione, F. Ferrara, S. Dugnani, M. Falchi, G. Santoro, und F. Fraschini, „Muley B.P.Khadabadi,S.S.and Banarase ,N.B(2009).Phytochemical constituent and pharmacological activities of *Withania somnifera*;A review.International Journal of pharmaceutical Sciences,Review and research 1(19-24)
- [4] Mirjalili ,M.H,Moyano,E.Bonfil,M.Cusido,R.M.,and Palazon J.(2009)Stereooidal lactones from *Withania somnifera*,an ancient plant for novel medicine,Molecules,14,2373-2393
- [5] Dar,P.A.,Sofi,G.and Jafiri,M.A.(2015),*Withania somnifera*;A review of the phytochemistry and pharmacology,International Journal of Pharmaceutical Science and Research 6 1204-1215
- [6] I.Vandem Berghe,W Sabbe,L.Kaileh,M Haegeman,Gand Heyninck,K(2012).Molecular insight in the multifunctional activities of *Withania somnifera*,International Journal of molecular Sciences 13 ,11131-11145K.
- [7] Kumar A Kaushik,P.and Kumar P. 2021)Recent advances in extraction techniques of bioactive compounds from medicinal plants.A review,Journal of applied research on medicinal and Aromatic Plants, 24.

- [8] J.Azmir .J. Zaidul,I.S.M.,Rahman AM.M,Sharif,K.M,Mohamed,A Sahena,F Jahurul M.H.A,Ghafoor,K.Norulaini N.A.N and Omar A.K.M.(2013) Techniques for extraction of bioactive compounds from Plant materials.Jornal of food Engineering 117, 426-436
- [9] Chemat,F.Rambaut,N.N.Sicaire,A.G. Meullemistre,A.Fabiano Tixier A.S.and Akbert-VianM(2017) Ultrasound assisted extraction of Foodund natural prrodukts.Mechanism,techniques ,combinations and aplications.Ultrasonic Sonochemisrey
- [10] Stalikas,C,D(2007)Extraction separation anddetection methods for phenolic acids and Flavonoids.Journal of separation science30.3268-3274