PRELIMINARY STUDIES RELATED TO MICROSCOPY AND THE SEDEM EXPERT SYSTEM PROFILE ON FREEZED-DRIED EXTRACT OF *LYTHRI HERBA*

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ABSTRACT

The floral tips of the plant species Lythrum salicaria L. represent a rich source of total polyphenols, among which with the largest share we mention tannins, and this is why this plant material has a standardized monograph in the European Pharmacopoeia 10.0th edition.

According to the literature accessed so far, the plant material has antioxidant, anti-inflammatory, hemostatic, antibacterial and antifungal properties, along with modulatory action on carbohydrate metabolism.

Powder microscopic examination is an important step in establishing the identity of the plant species used, highlighting elements specific to the aerial part such as spiral vessels of the stem, fragments of the spongy mesophyll with calcium oxalate clusters cells and anomocytic stomata.

The application of the SeDeM method on dried plant extracts represents an innovative trend in pharmaceutical technology and contributes to the collection of data in a structured and standardized form.

In this paper, the functions and applications of the SeDeM expert system are illustrated upon the freeze-dried extract of Lythri herba for the purpose of easier identification and standardization. Future applications may include obtaining chewable gums or tablets by direct compression.

Keywords: *Lythri herba, microscopic examination, SeDeM expert system, direct compression*

INTRODUCTION

Lythrum salicaria L. is a plant known in traditional medicine due to its astringent and hemostatic properties in gastrointestinal diseases such as diarrhea and dysentery. This plant species proved its effectiveness during the First World

War and was successfully used as an infusion in the treatment of soldiers with dysentery [1].

The composition rich in total polyphenols and especially in ellagic tannins of the *Lythri herba* plant material highlights the importance of this species extracts in phytotherapy [1], [2], [3].

The first important step in establishing the identity of the *Lythri herba* plant material is the microscopic examination in powder, being known as a standard technique in determining plant species [4].

The SeDeM Expert Diagram System is a galenic pre-formulation system, which evaluates the suitability of excipients and active pharmaceutical ingredients (API's) for direct compression (DC) into tablets as well as predicting possible formulations to obtain acceptable direct compressible tablets. Until now, the SeDeM expert system has become one of the most successful preformulation methods, since it gathers almost all the frequently used physical parameters to fully characterize the properties of pharmaceutical powders.

Practical applications of SeDeM includes determination of the suitability of an API to be subjected to direct compression technology, determine the amount of excipient required for the compression of an API that is not apt for direct compression, practical application of the mathematical equation to calculate the amount of excipient required for a deficient API to be subjected to direct compression technology, quality control of batches of a single API or excipient used for direct compression, to differentiate the excipient in the same chemical family, to differentiate excipients of the same functional type, to develop orally disintegrating tablets by direct compression using SeDeM-ODT experimental program [5].

Also, the SeDeM expert system contributes to the manufacturing classification system (MCS) and Quality by Design Development. Consequently, this innovative tool is consistent with the current requirements of regulatory health authorities such as the FDA and ICH.

MATERIAL AND METHOD

The materials used in this study were the freeze-dried aqueous extract of *Lythri herba*, the powdered aerial parts of the dried *Lythrum salicaria* L. plants harvested in August 2019 from Năvodari area, Dobrogea, Romania, Novex-Holland microscope, Canon 710 digital camera. Freeze-drying method of aqueous extract of *Lythri herba* plant material increase the stability in time of the aqueous extract and facilitates subsequent retesting [3].

SeDeM expert system is based upon ICH guidelines and is comprised of 12 parameters divided into six factors, covering all the characteristics related to flow, compressibility and disintegration behavior of powder. Based on physical characteristics and functionality of the ingredients, these parameters are grouped and described as follows [6]:

A. Dimension factor

Parameters included in this factor affect the size of the tablet and its ability to pile up. Measurements of these parameters also include particle volume, inter-particle void volume and internal pore volume. Parameters included in this group are:

1) Bulk density (Da)
2) Tapped density (Dc)

B. Compressibility factor

The factor comprised of the parameters related to compressibility of powder and includes the following:

3) Inter-particle porosity (Ie)
4) Carr index (IC%)
5) Cohesion index (Icd)

C. Flow ability / powder flow factor

This factor governs flow ability of the powder during compression and includes the following:

6) Hausner ratio (IH)
7) Angle of repose (α)
8) Flowability (t")

D. Lubricity / stability factor

Lubricity during compression and stability of the compressed tablets are affected by the parameters included in this factor. These are the following:

9) Loss on drying (%HR)
10) Hygroscopicity (%H)

E. Lubricity / dosage factor

Parameters included in this factor affect the lubricity and dosage of the tablet and comprised of the following:

11) Percentage of particles measuring < 50 μ (%Pf)
12) Homogeneity index (Iθ)

Acceptable limit values for each parameter of SeDeM expert system

Certain limit values are set for each parameter included in SeDeM expert system on the basis of experimental results and values described in the Handbook of Pharmaceutical Excipients [7].

Graphical presentation of results from SeDeM expert system

Results of SeDeM expert system are graphically presented as SeDeM diagram built on the basis of basic parameters. Values obtained from the experimental determination or calculations of various parameters are converted to *r* values by applying specific factors, representing radii of the diagram. The diagram is formed by connecting radius values with linear segment, having 0 as a minimum value, 10 as maximum value, and 5 as minimum acceptable value. [6]

***Table 1.** Parameters and methods included in the expert system SeDeM*

Incidence factor	Parameter (Symbol)	Methods	Unit	Equation	Limit value (v)	Radius (r)
Dimension	Bulk density (Da)	Sect. 2.9.15 Eur.Ph.	g/ml	$Da = P/Va$	0-1	10v
	Tapped density (Dc)	Sect. 2.9.15 Eur.Ph.	g/ml	$Dc = P/Vc$	0-1	10v
Compressibility	Interparticle porosity (Ie)	Non-compendial / computed	-	$Ie = \frac{Dc-Da}{Dc \times Da}$	0-1.2	10v/1.2
	Carr index (IC)	Non-compendial / computed	%	$IC = \frac{Dc-Da}{Dc} \times 100$	0-50%	v/5
	Cohesion index (Icd)	Non-compendial	N	Experimental	0-200	v/20
Flowability / Powder flow	Hausner ratio (IH)	Sect. 2.9.36 Eur. Ph	-	$IH = Dc/Da$	3-1	(30-10v)/2
	Angle of repose (α)	Sect. 2.9.36 Eur. Ph	-	$\alpha = \tan^{-1} H/r$	50-0	10-(v/5)
	Flowability (t″)	Sect. 2.9.36 Eur. Ph.	s	Experimental	20-0	10-(v/2)
Lubricity / Stability	Loss on drying (%HR)	Gen. meth. 2.9.32 Eur.Ph.	%	Experimental	10-0	10-v
	Hygroscopicity (%H)	Gen. meth. 5.11 Eur Ph.	%	Experimental	20-0	10-(v/2)
Lubricity / Dosage	Particles < 50μ (%Pf)	Gen. meth. 2.9.12 Eur. Ph.	%	Experimental	50-0	10-(v/5)
	Homogenity index (Iθ)	Gen. meth. 2.9.12 Eur. Ph.	-	$I\theta = \frac{Fm}{100 + \Delta Fmn}$	0-2 x 10^{-2}	500v

RESULTS AND DISCUSSIONS

Powdered aerial parts of dried Lythri herba and freeze-dried aqueous extract of Lythri herba

Aerial parts of *Lythrum salicaria* L. (*Lythri herba*) were collected in August 2019 from Năvodari area, Constanța city, Romania and one specimen has been deposited in the “exicata” collection of the Pharmacognosy discipline within the Faculty of Pharmacy, Ovidius University in Constanța, Romania.

The freeze-dried aqueous extract of *Lythri herba* was obtained by the concentration method with rotavapor and lyophilization of the aqueous extract from the floral tips of *Lythrum salicaria* L. (Fig. 1D).This obtaining method The dried floral tips from the *Lythrum salicaria* L. (Fig. 1A-1B) species was milled obtaining a brown-yellow powder (Fig. 1C).

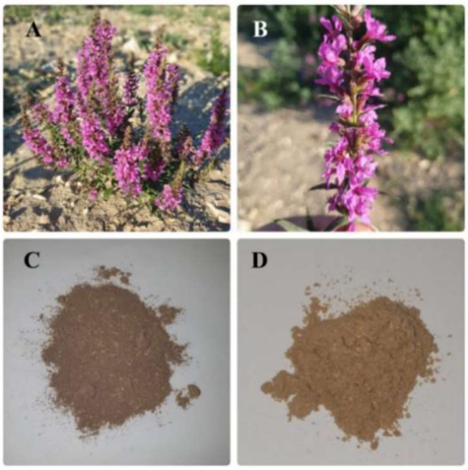

Fig. 1. *A. Lythrum salicaria L. species, B. Lythri herba plant material, C. Powdered aerial parts of Lythri herba, D. Freeze-dried aqueous extract of Lythri herba*

Microscopic characterization of the Lythri herba powder

The *Lythri herba* plant material has the following characteristic elements derived from stems consisting of spiral and ringed small-caliber stem vessels (Fig. 2a), accompanied by fiber bundles (Fig. 2b) and unicellular or multicellular covering trichomes (Fig. 2c).

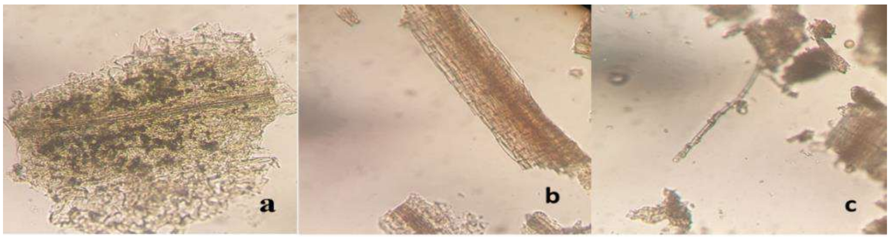

Fig. 2. *Microscopic characterization of the Lythri herba stem powder (160X)*

In the powder of the plant material *Lythri herba* we noticed the following leaf characteristic elements such as small spiral vessels (Fig. 3a), unicellular or bicellular covering trichomes (Fig. 3b), anomocytic stomata (Fig. 3c) and spherical cells, thin-walled and with calcium oxalate crystals (Fig. 3d).

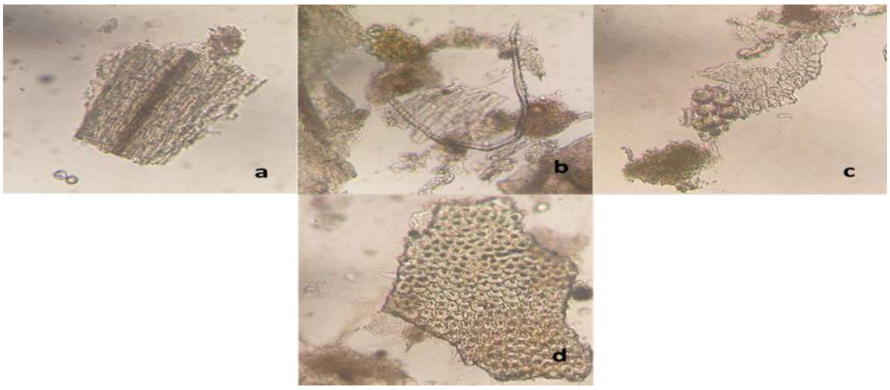

Fig. 3. *Microscopic characterization of the Lythri herba leaf powder (160X)*

Microscopic elements characteristic of the flower were noticed in the powder of the plant material *Lythri herba* such as petal epidermal cells with sinuous walls and granular cuticles (Fig. 4a), unicel-lular or bicellular trichomes covering the calyx of the flower (Fig. 4b) and numerous spherical pollen grains, with three germinating pores and thin exine (Fig. 4c).

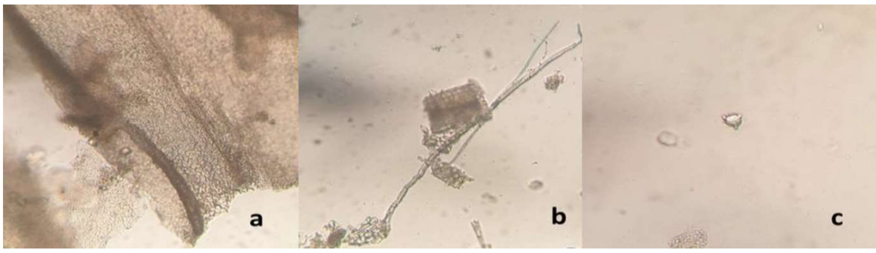

Fig. 4. *Microscopic characterization of the Lythri herba flower powder (160X)*

The initial experiments followed the analysis of the micrometric properties of the plant material in order to specify the flow and compression capacity. Determining the density of a powder is an important feature in the analysis of micrometric properties, as it is considered to be an indicator of powder processing by tasing and compacting. The higher the density of the powder, the greater its ease of forming tablets. The two micrometric parameters (IC and IH) are also important characteristics in the design, formulation and maintenance of the quality of any pharmaceutical product and research [5, 8]. Superior quality of the drug ensures its increased bioavailability in the body, an expected effect and limited side effects reported by pharmacovigilance studies [9].

By analysing the results obtained (Table 2) it can be observed that the values of the micrometric parameters for the dry extract fall into the category of low-flow powders, according to Carr [10, 11]. The low density of the powder, before and after tasing (Da = 0.15, Dc = 0.23) the advanced degree of finesse, the intermolecular links (Ie = 0.05) explain the adhering properties of the material, the poor flow and the difficulty of future direct compression.

Table 2. *Results from the SeDeM Expert Diagram System of the freeze-dried aqueous extract of Lythri herba plant material*

Parameter	Da (g/ml)	Dc (g/ml)	Ie	IC (%)	IH	α (º)	t'' (s)
Results	0.15	0.23	0.05	34.5	1.53	-	-
Radius *(r)*	1.5	2.3	0.42	6.9	7.35	0	0
Incidence factor (mean value)	Dimension		Compressibility		Flowability/ powder flow		
	1.9		-		2.45		

The experiments for the angle of repose and the flow time did not returned any results because the powder analysed did not flow through the funnel. This negative aspect is attributed to the low density, electrostatic character, adhesion to the walls of the flow paths and underlines the poor flow of plant material.

The mean values for incidence factors dimension (1.9) and flowability (2.45) are lower than the acceptable value of 5, which concludes that the physical properties of the powder (particle geometry, surface texture, cohesion forces) negatively influence the flow of plant material.

Judicious analysis of compression capacity requires future experiments for the cohesion index, hygroscopic character, selection of directly compressible excipients.

In order to achieve the complete profile obtained by applying the SeDeM expert system, we aim to continue the experiments related to compression, dosage and stability. Moreover, by obtaining the SeDeM diagram, the profile of the dry extract, with its advantageous properties but also deficient ones, can be seen more clearly.

For future studies, directly compressible excipients may be selected in order to compensate the deficiencies of the API and theoretically provides the final mixture the characteristics to be compressed. In this way, the information provided by the SeDeM system allows the formulator to start working with excipients that have a high probability to provide suitable formulations, thus reducing the lead time of formulation.

CONCLUSION

The microscopic characterization in powder helped to determine the identity of the species *Lythrum salicaria* L. from Romania, because all the elements highlighted in this study corresponded to the data from the monograph *Lythri herba* of the European Pharmacopoeia 10^{th} edition.

Application of the SeDeM expert system contributes to the realization of a complete pharmaco-technical profile for the plant material powder that includes: dimensional properties, compression capacity, reology, stability and dosing. The application of galenic correction formulas and the visual interpretation of the results facilitates the selection of the appropriate excipients for compression and reduction of the time of formulation.

Moreover, the SeDeM expert system contributes to the collection of data in a structured and standardized form, facilitating the exchange of knowledge between researchers. In this respect the iTCM database contains 73 botanical extracts alongside 91 pharmaceutical excipients in solid-state.

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