Microspheres containing an extract of *Crataegus monogyna* Jacq. for oral administration

Bucco M.[1], Gaggeri R.[1], Lucconi G.[1], Chlapanidas T.[1], Martino E.[2], Rossi D.[1], Faustini M.[3#], Vigo D.[3], Collina S.[1] and Torre M.L.[1]

[1]Dipartimento di Chimica Farmaceutica, Università di Pavia. [2]Dipartimento di Ecologia del Territorio, Università di Pavia. [3]Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università di Milano.

[#]massimo.faustini@unimi.it

INTRODUCTION

Autochthon plant extracts with reactive-oxygen species (ROS) scavenging activity are promising candidates for the pharmaceutical industry (Gavini E. 2005). Antioxidants are emerging as prophylactic and therapeutic agents, but their bioavailability depends on several factors like solubility and storage instability (Chang Q. 2005). Therefore the practical use of ROS-scavenging extracts in oral administration is limited. Microencapsulation technology could help to avoid these pitfalls: given this framework, the aim of this work was to prepare and characterize microspheres containing *Crataegus monogyna* Jacq. extract with antioxidant activity to be employed in the pharmaceutical field for oral administration.

MATERIAL AND METHODS

Plant: *Crataegus monogyna* Jacq. was collected in May 2007 from natural populations. The freshly cut plant was sorted and dried under active ventilation at room temperature until achieving constant weight. Plant materials were ground with a blade-mill to obtain a homogenous drug powder, stored in airtight glass jars at 4°C, in dark conditions. The humidity value of the samples did not change during the storage.

Extraction: extraction was performed by maceration keeping the ratio sample weight/solvent volume constant at a value of 0,2 g/ml. Plant materials were macerated in aqueous ethanol 95% under mechanical stirring for 3 hours. After filtration, a second extraction was performed with fresh solvent in the same condition for 3 hours (Tadic V.M. 2008). The two extracts were combined and dried *in vacuo* to obtain a dry extract.

ROS-scavenging activity: methanol solutions at different extract concentrations were mixed with 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) solutions, kept in the dark for 20 min at room temperature and then absorbance was measured at 515 nm. Absorbance of a blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily as blank (Tadic V.M. 2008). Propofol was used as control, and the test was carried out in triplicate. Radical scavenging activity was calculated by the following formula: % activity=(Ab-Aa)/Ab*100, where: Ab: absorbance of blank sample; Aa: absorbance of tested extract solution. Extract and microsphere ROS-scavenging activity was also calculated after treatment with HCl pH1 for 2h at 37°C. The ROS-scavenging activity for the treated extract was evaluated on both filtered residue and the whole treated sample (residue and supernatant) after *in vacuo* drying. All reagents were purchased from Sigma-Aldrich.

Microencapsulation: three different microsphere types containing *C. monogyna* Jacq. extract were obtained by spray drying (Büchi Mini Spray Dryer) of aqueous solutions as previously described (Vigo D. 2004); microsphere compositions are reported in Table 1. Granulometric analysis of microspheres was performed by a laser light scattering granulometer (Beckman Coulter LS230), equipped with a small volume cell, 120 mL volume with refractive index set at 1.330 for water,

obscuration 5%. Acid aqueous suspension of microspheres were placed in the measurement cell and ran in 5 replicates of 90 seconds each.

HPLC analysis of *Crataegus monogyna*: hyperoside (HY) was chosen as a molecular marker for titration, and quantified either before or after the microencapsulation process. An HPLC-PAD system (Jasco Europe s.r.l, Cremella, Lecco, Italy, λ=340nm), equipped with Chromolith SpeedROD RP-18 column was used (Martino E. 2008). Each determination was carried out in triplicate. Results are reported as milligrams of HY / gram of extract or microspheres.

SEM-EDX analysis: SEM-EDX analysis was performed both on extract and microspheres. For SEM analysis samples were placed on small aluminum cylinders using graphite glue and coated with gold 99% pure (Sputter coater) with Cressington Sputter 108 auto. Morphological analysis was performed by a JEOL JSM-6380LV electron scanning microscope. EDX analysis was carried out with iXRF system and EDS-2004 system for revelation.

Statistical analysis: ROS-scavenging activity was evaluated by the analysis of covariance (ANCOVA), considering the extract or the reference molecule as a fixed factor, and the concentration of the plant extract or the reference molecule as covariate. Differences between groups were assessed by Tukey test for multiple comparisons. Statistical significance was set at p=0.05. All analyses were performed with JMP 7 for Windows (SAS Institute).

RESULTS AND DISCUSSION

Extraction yield ranging from 25% to 30% was obtained according to previously reported results (Martino E. 2008). The extraction method here described was chosen because of its ease of scalability, although its yield was suboptimal. The encapsulation process was flexible to permit the loading of variable extract quantities (Micro A: 6.1%; Micro B: 11.1%; Micro C: 22.2%) with high encapsulation efficiency (Micro A: 99%; Micro B: 88%; Micro C: 98%). Microspheres have a round shape with a wrinkled surface, due to the presence of alginate (Figure 1).

	A	B	C
Polymethacrylate	75.8	69.4	55.6
Na Alginate	15.2	13.9	11.1
Poloxamer	1.5	2.8	5.6
Na lauryl sulphate	1.5	2.8	5.6
Extract	6.1	11.1	22.2

Table 1: % composition of Micro A, Micro B and Micro C

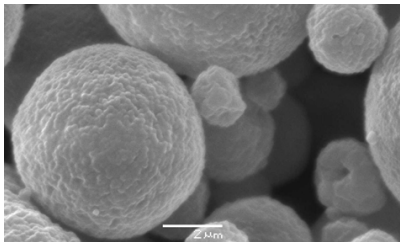

Figure 1: SEM analysis of Micro C

Also the size of the microspheres (Figure 2) were suitable for pharmaceutical use: all the batches have d90 less than 2.5µm.

ROS-scavenging activity results (Figure 3) indicate that *C. monogyna* Jacq. presents an antioxidant activity similar to control (propofol). Antioxidant activity was maintained in all microspheres in acceptable levels. The best formulation (Micro B) presents the same ROS-scavenging activity in respect to the extract. SEM-EDX analysis emphasizes the absence of heavy metals, both in the extract (Figure 4) and in the microspheres (Figure 5); heavy metals are considered contaminants of herbal products, and it is imperative to avoid their presence. After treatment with HCl pH1 an important reduction in antioxidant activity can be observed for the extract (Figure 6) whereas microspheres maintain their ROS-scavenging activity (Figure 7).

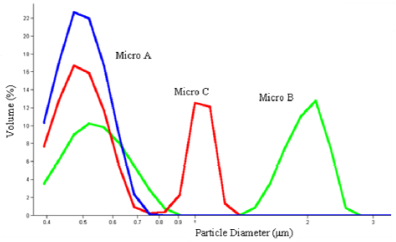

Figure 2: Granulometric analysis of microspheres

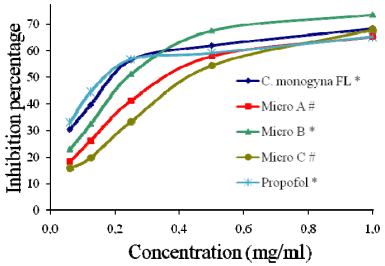

Figure 3: ROS-scavenging activity of *C. monogyna* Jacq. extract and microspheres. Different symbols indicate a p<0.05 difference between groups.

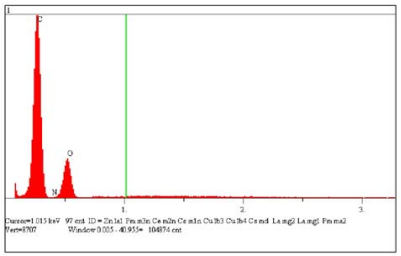

Figure 4: EDX spectrum of extract

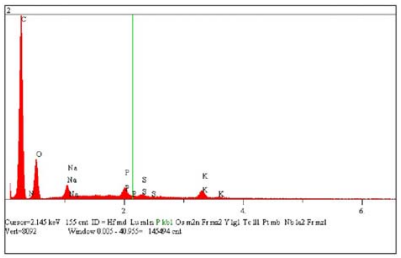

Figure 5: EDX spectrum of Micro C

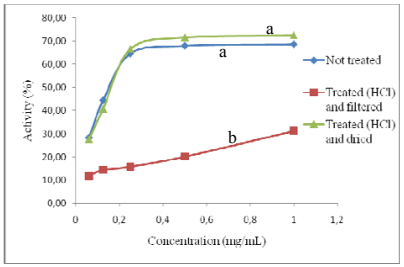

Figure 6: Extract ROS-scavenging activity before and after treatment with HCl pH1. Different letters indicate a p<0,0001 difference between groups.

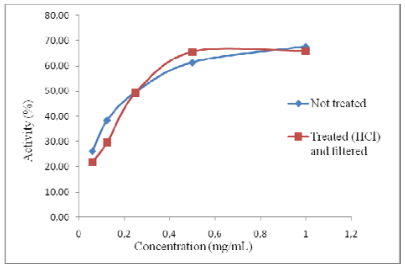

Figure 7: Micro A ROS-scavenging activity before and after treatment with HCl pH1 (significant difference between groups, p=0,002)

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CONCLUSIONS

Encapsulation of *C. monogyna* extract by a spray-drying technique enables the production of microspheres with high ROS-scavenging activity, exhibiting properties suitable for oral administration in human and veterinary medicine. Therefore this is as a promising new approach for improving the stability of extracts with ROS-scavenging activity.

REFERENCES

• Gavini E. et al. (2005). *Tabletted microspheres containing Cynara scolymus (var. Spinoso sardo) extract for the preparation of controlled release nutraceutical matrices.* Journal of Microencapsulation 22(5) 487–499.

• Chang Q. et al. (2005) *Comparison of the Pharmacokinetics of Hawthorn Phenolics in Extract Versus Individual Pure Compound.* The Journal of Clinical Pharmacology 45(1) 106-112.

• Tadic V.M. et al. (2008) *Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract.* J. Agric. Food Chem. 56(17) 7700–7709.

• Vigo D. et al. (2004) *Microparticulate systems for the oral administration of biologically active substances.* European Patent EP1778192. Priority number: IT2004MI01255/ 20040622.

• Martino E. et al. (2008) *Influence of the Extraction Mode on the Yield of Hyperoside, Vitexin and Vitexin-2''-O-Rhamnoside from Crataegus monogyna Jacq. (Hawthorn).* Phytochemical Analysis 19(6) 534–540.

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