International Food Research Journal 24(3): 1233-1238 (June 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Green extraction and simultaneous inclusion complex formation of *Sideritis scardica* polyphenols

[1]Korompokis, K., [1]Igoumenidis, P. E., [2*]Mourtzinos, I. and [1]Karathanos, V.T.

[1]Laboratory of Chemistry - Biochemistry - Physical Chemistry of Foods, Department of Nutrition and Dietetics, Harokopion University, 70 El. Venizelou Str., Kallithea, 17671, Athens, Greece
[2]Department of Food Science and Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

Article history

Received: 24 March 2016
Received in revised form: 28 June 2016
Accepted: 1 July 2016

Keywords

Tea
Cyclodextrin
Antioxidants
Thermal analysis

Abstract

Sideritis spp. (also known as Mountain tea), is a very common herb in Mediterranean basin, rich in bioactive polyphenolic ingredients. In this study, extraction from *Sideritis scardica* (SS) and concomitant inclusion complex formation of polyphenols in aqueous solutions of cyclodextrins (CDs) was investigated, by estimating the total phenolic content (TPC) and the antioxidant capacity of the extracts. Our results showed that the utilization of cyclodextrins boost the extraction yield of polyphenols. Both TPC and antioxidant capacity of SS aqueous extracts, containing CDs, presented greater values compared to pure SS aqueous extracts. Furthermore, the implementation of differential scanning calorimetry (DSC), conducted under inert and oxidative conditions, suggested the potential formation of inclusion complexes. The green extraction of Sideritis using cyclodextrins could pave the road for herb's more effective extraction and use in food and nutraceutical industry.

Introduction

Cyclodextrins (CD) (cyclic glucose oligomers having six, seven or eight glucose units, linked by 1,4-α-glucosidic bonds and called respectively α-, β- and γ-cyclodextrin) are formed by the enzymatic modification of starch. β-cyclodextrin (β-CD) is one of the most commonly used cyclodextrins due to its low cost production and the non-toxic character (Kurkov and Loftsson, 2013). As cyclodextrins are derived from starch, they are generally regarded as essentially non-toxic materials. However, β-cyclodextrin can form insoluble complexes with cholesterol that disrupt the function of the kidneys so it should not be used in parenteral applications and its oral use should be limited to a daily maximum of 5 mg/kg. Both α-cyclodextrin, γ-cyclodextrin and hydroxylpropyl-β-cyclodextrin are suitable for oral applications. The acceptable daily intakes (ADIs) are given as "non-specified" by JECFA (Joint FAO/WHO Expert Committee on Food Additives) for α-cyclodextrin and γ-cyclodextrin (Astray et al., 2009). Oral bioavailability is very low and β-CD is mainly metabolized by gut microbiota (Kurkov and Loftsson, 2013). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) is a β-CD derivative, which presents enhanced water solubility and lower toxicity (Hsu *et al*., 2013).

β-CD and HP-β-CD can form inclusion complexes with a variety of bioactive molecules. In particular, CDs are shaped like a truncated cone and their central cavity is rather lipophilic, while their exterior surface is hydrophilic, enabling the encapsulation of hydrophobic compounds of proper size and geometry in aqueous environments (Mura, 2014). Owning to their unique physicochemical properties, CDs can be used as potent encapsulation agents for labile compounds such as polyphenols and other plant bioactive components (Pinho *et al.*, 2014).

Green extraction is considered as an innovative, eco-friendly and effective way to extract natural and valuable compounds. (Chemat *et al.*, 2012). The use of aqueous solutions of CDs as extraction media can be considered as green extraction technique as water is the main solvent and the existence of cyclodextrin hydrophobic cavity enhances the extraction of less polar molecules such as polyphenols due to inclusion complex formation (Ratnasooriya and Rupasinghe, 2012; Alexandru *et al*., 2014).

Sideritis scardica belongs to *Sideritis* spp. (also known as Mountain tea) and is grown around the Mediterranean area. SS is known for its significant role in folk medicine, as its tea is used for the treatment of inflammation, gastrointestinal disorders, coughs and common cold (Tadic *et al*., 2012; Koutsaviti *et al.*, 2013; Todorova and Trendafilova, 2014).

*Corresponding author.
Email: *mourtzinos@agro.auth.gr*
Tel: +30 2310991129

Recent studies have reported anti-inflammatory, gastroprotective and cytotoxic effects *in vivo* (Tadic *et al*., 2012), induction of cellular antioxidant defenses and prevention of oxidative stress (Danesi *et al*., 2013). All these pharmacological properties coupled with its pleasant taste and flavor, could promote mountain tea as an alternative to tea preparations based on camellia sinensis.

Several methods have been applied for the extraction of phytochemicals from *Sideritis* spp. due to their pharmacological and nutritional interest. Most of them are conventional, such as maceration, ultrasound-assisted and microwave assisted extraction (Alipieva *et al.*, 2010). Alternative techniques, using non-organic solvents, such as supercritical fluid extraction have been applied to *Sideritis* species (Tadić *et al.*, 2012). The usage of cylodextrins for the effective preparation of extracts from plant materials have been recently studied (Kyriakidou *et al*., 2016).

The aim of the present study was to examine the simultaneous extraction and inclusion complex formation of *Sideritis scardica* using β-CD and HP-β-CD solutions. The parameters which were investigated were total phenolic content, antioxidant capacity, thermal stability and protection against oxidation. To the best of our knowledge extraction of Sideritis scardica in presence of cyclodextrins has not been reported in the literature so far.

Materials and Methods

Plant material

Sideritis scardica used in this study, was cultivated on mount Olympus in Greece and was collected during the time of flowering (second week of June). The aerial flowery parts of the plant were stored in a dry, cool, dark place until further handling.

Reagents and chemicals

Folin–Ciocalteu reagent, (2N) DPPH (2,2-diphenyl-1-picryhydrazyl) stable radical and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (97%) were obtained from Sigma–Aldrich. β-CD (pharmaceutical secondary standard; traceable to USP) and HP-β-CD (average degree of substitution was 0.5-1.3) were purchased from Aldrich Chemie GmbH (Steinheim, Germany). Methanol was of analytical grade and was obtained from Merck (Darmstadt, Germany). Deionized water was used for the preparation of the extracts.

Preparation of Sideritis scardica *extracts*

All kinds of SS extracts were prepared in the same way, using 100 g of aerial parts of the plant and 1000 mL of solvent (plant/solvent ratio was 1:10 w/v) The solvents used for the preparation of each extract were: a) deionized water, b) aqueous β-CD solution (8 mM), c) aqueous β-CD solution (16 mM) and d) aqueous HP-β-CD solution (27 mM). Different concentrations of CDs were used in order to find the optimum, as the total amount of encapsulated ingredients of the extracts was not known. 16mM is the maximum concentration of β-CD that can be diluted in water. HP-β-CD due to higher water solubility enables its usage in higher concentrations. The herb was boiled for 3 min, with or without β-CD and HP-β-CD, and remained in warm water for extra 5 min. All extracts were filtered through filter paper. Part of the extracts was dehydrated by freeze drying for further analysis (Heto Lyolab 3000, Heto-Holten, Allerod, Denmark). All types of herb extractions were carried out in triplicate.

Determination of total phenolic content

Total phenolic content (TPC) of extracts was assayed spectophotometrically using the Folin-CIocalteau method, adapted to a micro scale (Arnous *et al*,. 2002). The results were expressed as gallic acid equivalents (mg GAE/mL of extract), using a standard curve which showed linear correlation $R^2 > 0.99$.

Scavenging activity on DPPH· radical (antioxidant activity)

The antioxidant properties of the tested extracts were evaluated in terms of radical scavenging activity by applying the DPPH· free radical assay, as previously described by Arnous *et al.* (2002). The final results were calculated based on the standard curve (linear correlation $R^2 > 0.99$) and were expressed as Trolox equivalents (mg TE/mL of extract).

Study of complex formation by differential scanning calorimetry (DSC)

The differences between each extract's thermal stability and the confirmation of inclusion complex formation was carried out using DSC, as previously described by Mourtzinos *et al.* (2007) with slight modifications. 10 ± 0.1 mg of each lyophilized extract was placed in aluminum pans which were hermitically sealed and put in Perkin-Elmer DSC oven (DSC-6, Boston, MA). Nitrogen flow was predefined to 20 mL/min. The temperature remained stable to 70°C for 1 min, and then increased gradually to 230°C, with a screening rate of 5°C/min. Lastly, the temperature remained stable to 230°C for 1 min, before it started to cool. This procedure was applied in triplicate for each extract, as well as for pure β-CD

and HP-β-CD, in order to check the reproducibility.

Study of oxidation by DSC

Oxidative stability was evaluated for all types of SS extracts. 10 ± 0.1 mg of each sample were weighed and put in open aluminum pans, as contact with oxygen was required. Afterwards, the pans were placed in Perkin-Elmer DSC instrument (DSC-6, Boston, MA). Oxygen flow was set to 20 mL/min. Temperature was rapidly increased from room temperature to 120°C, at a heating rate of 90°C/min, and remained stable for 1 min in order to ensure better temperature contribution. Then, the temperature continued to increase with a rate of 5°C/min until 380°C. Once again, the temperature remained stable for 1 min, and afterwards started to dwindle. All thermographs for each specimen were obtained in triplicate.

Statistical analysis

All experiments were conducted in triplicate. (n=3). Descriptive statistics and statistical differences were calculated implementing one-way analysis of variance (ANOVA) followed by Tuckey's post hoc tests using statistical software SPSS 21.0. All data is reported as mean ± standard deviation (SD) and $p<0.05$ was chosen as the threshold for statistically significant differences.

Results and Discussion

Determination of total polyphenols

The TPC of four extracts are presented in Figure 1, where quantitative variations can be observed (however $p > 0.05$ in all cases). The aqueous extract of SS presented TPC equal to 3.26 ± 0.884 mg GAE/mL of SS extract, which is considered rather increased due to high plant/solvent ratio. Further comparison with similar plant extract was not possible since environmental factors such as the climate, the place and time of herb collection, as well as the method, the time of extraction and the amount of herb used for the extract preparation may hinder the comparison (Shan *et al*., 2005; Kratchanova *et al*., 2010). Thereafter, an increment of phenolic content was presented in parallel with the increase of cyclodextrins concentration. In the case of extraction with HP-β-CD, the highest TPC was shown (4.28 ± 0.32 mg GAE/mL of SS extract). This concentration-dependent increase implies that the presence of cyclodextrins could enhance the extraction yield leading to higher polyphenolic amount, probably, due to inclusion complex formation between SS ingredients and β-CDs cavities. Main polyphenols

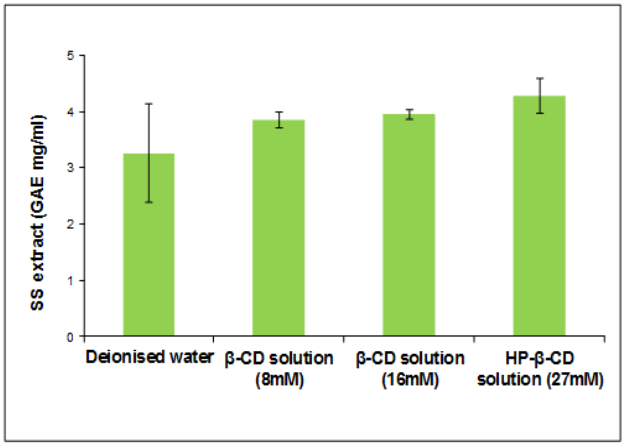

Figure 1. Total phenolic content of extracts with different extraction media: a) deionised water, b) β-CD solution (8 mM), c) β-CD solution (16mM) and d) HP-β-CD solution (27 mM). No statistically significant differences were detected between different extraction media ($p > 0.05$)

present in aqueous solution of SS are epigallocatechin gallate, chlorogenic acid and myricetin; all of them have been encapsulated in CDs in previous studies (Ishizu *et al*. 2006; Lucas-Abellan *et al*., 2008; Zhao *et al.*, 2010; Samanidou *et al.*, 2012;). The Folin-Ciocalteau method was carried out for the pure aqueous solutions of β-CD (8 and 16 mM) and HP-β-CD (27 mM) and showed almost no absorbance, indicating that the presence of cyclodextrins do not affect TPC measurement. The increase notified in Figure 1 could be partially attributed to the improved water solubility of polyphenols when encapsulated in β-CD and HP-β-CD, as was previously reported by Kalogeropoulos *et al.* (2009) and Nguyen *et al.* (2013), respectively. It has been referred that aqueous solutions of β-CD can lead to higher phenol recovery from slurry of grape pomace compared to pure water as extractant (Ratnasooriya and Rupasinghe, 2012).

Radical activity scavenging

The antioxidant capacity of four SS extracts is presented in Figure 2 where a similar pattern with TPC was followed ($p > 0.05$). Pure SS aqueous extract exerted very high antioxidant capacity (4.11 ± 0.45 mg TE/mL of SS extract). No notable antioxidant ability was recorded, when β-CD and HP-β-CD aqueous solution were tested, indicating that cyclodextrins do not contribute independently to the antioxidant activity. The presence of β-CD and HP-β-CD during extraction resulted in higher antioxidant ability in a concentration-dependent way. In general, the highest antioxidant activity was observed when HP-β-CD (27 mM) was used. As it has already been referred, polyphenols are much more efficiently solubilised in water after being incorporated in the hydrophobic cavities of the cyclodextrins. Therefore, the enhanced

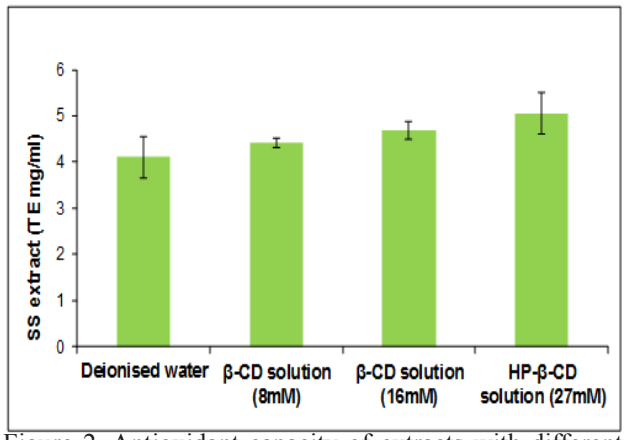

Figure 2. Antioxidant capacity of extracts with different extraction media: a) deionised water, b) β-CD solution (8 mM), c) β-CD solution (16 mM) and d) HP-β-CD solution (27 mM). No statistically significant differences were detected between different extraction media ($p > 0.05$)

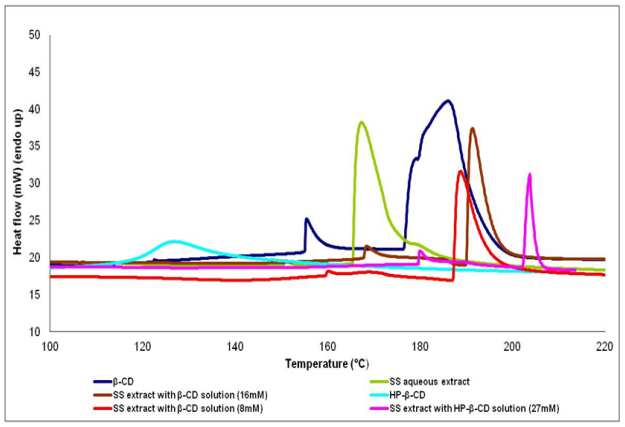

Figure 3. Characteristic DSC thermographs of extracts under inert conditions

solubility of polyphenols may be responsible for better antioxidant ability, as it is easier to quench free radicals. Furthermore, as the phenolic content was presented richer, improved antioxidant ability was expected as the antioxidant capacity of extracts of SS is positively correlated with their phenolic content (Tadic *et al*., 2012). Finally, as described by Mantegna *et al.* (2012), the presence of β-CD during ultrasound-assisted extraction of resveratrol and other polyphenols from the roots of Polygonum cuspidatum led to significantly enhanced antioxidant profile, which was found similar to methanol-extracted *P. cuspidatum*. This finding indicates that the green method using cyclodextrins solutions as extractants is successful enough to replace organic solvents.

DSC thermographs interpretation

The thermal curves of β-CD, HP-β-CD, aqueous SS extract, SS extracts with β-CD solution (8 mM and 16 mM) and SS extract with HP-β-CD solution (27 mM) are comparatively presented in Figure 3. In the case of pure β-CD, an endothermic melting peak was observed at 185°C while the enthalpy of fusion was 286.99 J/g. Indeed, the endothermic peak of β-CD was detected to 189°C by da Rosa *et al.* (2013). The appearance of this peak could, possibly, be due to water molecules' elimination from β-CD cavity (Mourtzinos *et al*., 2007). The enthalpy of evaporation of water at 185°C is 1996 J/g. Thus, the enthalpy of 286.99 J/g could be attributed to the evaporation of 0.14 g of water from 1 g of β-CD. It has been reported that the crystal water content of commercial β-CD, determined by Karl-Fischer volumetric titration, is approximately 14.6%, which is in close agreement with the above calculation (Hădărugă *et al*., 2012). The broad endothermic peak of HP-β-CD was appeared at 126.74°C (ΔH = 62.38 J/g) due to elimination of the contained water, applying the same rationale. The endothermic peak at 169.39°C (ΔH = 167.26 J/g), observed for the aqueous SS extract, may be related to melting with decomposition of the compounds of SS extract.

DSC thermographs of the two extracts with β-CD solutions (8 and 16 mM) exhibited peaks at 189.25°C and 196.35°C, respectively. These endothermic peaks were characteristically different, compared to those of SS aqueous extract and β-CD individually. The clear disappearance of SS extract and β-CD peaks and the detention of a new peak at higher temperature indicate the formation of a supra-molecular structure between the components of SS extract and β-CD (Marques *et al*., 1990). A detection of a new peak at higher temperature has been used as indication of inclusion complex formation by others (Wang et al. 2011; Krishnaswamy *et al*., 2012).The same pattern was followed by the SS extract with HP-β-CD solution (27 mM). The new endothermic peak was observed at 203.76°C. Enthalpy of fusion at all three extracts was relatively lower than enthalpy of SS aqueous extract and β-CD. The higher rightward transposition of melting temperature and the lowest enthalpy was observed in the case of HP-β-CD as extraction medium, while the peak was quite sharp. This finding, probably, suggests that the majority of Sideritis compounds are steadily incorporated inside the cavity, while a small portion of them is less strongly attached. Therefore, when the temperature increases and reaches 203.76°C, some of the entrapped compounds are released, disassembling the inclusion complex, and degrade, demonstrating a rather smaller and sharper peak. Furthermore, the encapsulation of Sideritis ingredients inside the cavity of HP-β-CD seems to be more consistent compared to β-CD as encapsulation material. It has already been

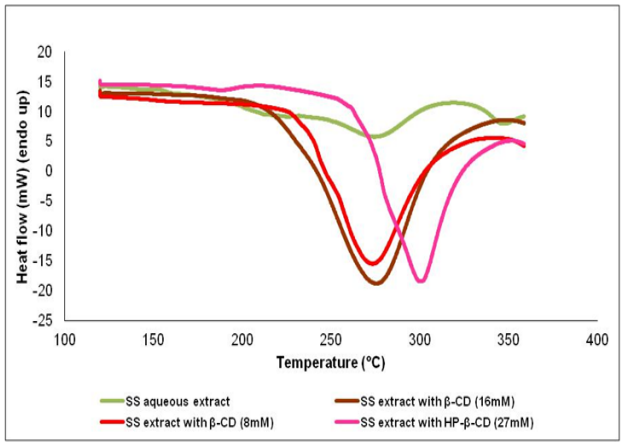

Figure 4. Characteristic DSC thermographs of extracts under oxidative conditions

shown that HP-β-CD possesses a greater capacity to form inclusion complexes than β-CD because of substitution by hydroxypropyl groups, which further improves the binding capacity (Liu *et al*., 2012).

DSC scanning under oxidative environment

DSC was additionally applied to all four SS extract types under oxidative conditions in order to study the thermo-oxidation stability of guest molecules of SS extract, encapsulated or not (Figure 4). The entrapment of guest molecule inside the cavity provides prolonged protection against oxidation and this phenomenon can be considered as an indirect indication of microencapsulation accomplishment (Karathanos *et al.*, 2007).

The initiation of oxidation can be exported relying on DSC Pyris software by extrapolating the tangent drawn on the steepest slope of the exothermic curve. The onset of oxidation of SS aqueous extract was found at 189.58°C. On the other hand, the SS extracts with β-CD solutions of 8 mM and 16 mM oxidized at 236.63°C and 229.63°C respectively, showing a clear protection against oxidation provided by β-CD solutions. Even bigger was the shift observed in the beginning of oxidation in the case of SS extract with HP-β-CD solution (27 mM). In particular, the oxidation started at 270.89°C, indicating an explicit protection, which is the highest amongst the SS extracts with CDs solutions. In accordance with the results from DSC scanning under inert conditions, DSC analysis under oxidative environment suggests that the extraction with aqueous solution of HP-β-CD (27 mM) seemed to be the most effective concerning the prospect of microencapsulation.

Conclusion

Cyclodextrin solutions were efficiently applied as extraction media for the extraction of SS leaves. The presence of β-CD and HP-β-CD during extraction enhanced the total phenol content and the radical scavenging activity of Sideritis scardica extracts. Furthermore, greater thermal stability and stability over oxidation were observed. HP-β-CD solution (27 mM) acted, comparatively, as the most advantageous extractant. All extracts could be used as raw materials for the development of functional foods or could be components of nutritional supplements, presenting stability and enhanced specifications.

Acknowledgement

The authors express their gratitude to the cosmetics company APIVITA S.A. for kindly providing the Sideritis aerial parts.

References

Alexandru, L., Binello, A., Mantegna, S., Boffa, L., Chemat, F. and Cravotto, G. 2014. Efficient green extraction of polyphenols from post-harvested agro-industry vegetal sources in Piedmont. Comptes Rendus Chimie 17: 212-217.

Alipieva, K., Petreska, J., Gil-Izquierdo, A., Stefova, M., Evstatieva, L. and Bankova, V. 2010. Influence of the extraction method on the yield of flavonoids and phenolics from *Sideritis* spp. (Pirin mountain tea). Natural Product Communications 5(1): 51-54.

Arnous, A., Makris, D. P. and Kefalas P. 2002. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. Journal of Food Composition and Analysis 15: 655-665.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R. and Simal-Gándara, J. 2009. A review on the use of cyclodextrins in foods. Food Hydrocolloids 23: 1631-1640.

Chemat, F., Vian, M. A. and Cravotto, G. 2012. Green extraction of natural products: concept and principles. International Journal of Molecular Sciences 13: 8615-8627.

Danesi, F., Saha, S., Kroon, P. A., Glibetic, M., Konic-Ristic, A., D'Antuono, L. F. and Bordoni, A. 2013. Bioactive-rich *Sideritis scardica* tea (mountain tea) is as potent as *Camellia sinensis* tea at inducing cellular antioxidant defences and preventing oxidative stress. Journal of the Science of Food and Agriculture 93: 3558-3564.

Hădărugă, D. I., Hădărugă, N. G., Bandur, G. N. and Isengard, H.-D. 2012. Water content of flavonoid/cyclodextrin nanoparticles: Relationship with the structural descriptors of biologically active compounds. Food Chemistry 132: 1651-1659.

Hsu, C. M., Yu, S. C., Tsai, F. J. and Tsai, Y. 2013. Enhancement of rhubarb extract solubility and bioactivity by 2-hydroxypropyl-beta-cyclodextrin. Carbohydrate Polymers 98: 1422-1429.

Ishizu, T., Hirata, C., Yamamoto, H. and Harano, K. 2006. Structure and intramolecular flexibility of β-cyclodextrin complex with epigallocatechin gallate in aqueous solvent. Magnetic Resonance in Chemistry 44: 776-783.

Kalogeropoulos, N., Konteles, S., Mourtzinos, I., Troullidou, E., Chiou, A. and Karathanos, V. T. 2009. Encapsulation of complex extracts in beta-cyclodextrin: an application to propolis ethanolic extract. Journal of Microencapsulation 26: 603-613.

Karathanos, V. T., Mourtzinos, I., Yannakopoulou, K. and Andrikopoulos, N. K. 2007. Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with β-cyclodextrin. Food Chemistry 101: 652-658.

Koutsaviti, A., Bazos, I., Milenković, M., Pavlović-Drobac, M. and Tzakou, O. 2013. Antimicrobial activity and essential oil composition of five *Sideritis taxa* of Empedoclia and Hesiodia Sect. from Greece. Records of Natural Products 7: 6-14.

Kratchanova, M., Denev, P., Ciz, M., Lojek, A. and Mihailov, A. 2010. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta Biochimica Polonica 57: 229-234.

Krishnaswamy, K., Orsat, V. and Thangavel, K. 2012. Synthesis and characterization of nano-encapsulated catechin by molecular inclusion with beta-cyclodextrin. Journal of Food Engineering 111: 255-264.

Kurkov, S. V. and Loftsson, T. 2013. Cyclodextrins. International Journal of Pharmaceutics 453: 167-80.

Kyriakidou, K., Mourtzinos, I., Biliaderis, C.G. and Makris, D.P. 2016. Optimization of a Green Extraction/Inclusion Complex Formation Process to Recover Antioxidant Polyphenols from Oak Acorn Husks (Quercus Robur) Using Aqueous 2-Hydroxypropyl-b-Cyclodextrin/Glycerol Mixtures. Environments 3: 1-11.

Liu, B., Li, W., Nguyen, T. A. and Zhao, J. 2012. Empirical, thermodynamic and quantum-chemical investigations of inclusion complexation between flavanones and (2-hydroxypropyl)-cyclodextrins. Food Chemistry 134: 926-932.

Lucas-Abellan, C., Fortea, I., Gabaldon, J. A. and Nunez-Delicado, E. 2008. Encapsulation of quercetin and myricetin in cyclodextrins at acidic pH. Journal of Agriculture and Food Chemistry 56: 255-259.

Mantegna, S., Binello, A., Boffa, L., Giorgis, M., Cena, C. and Cravotto, G. 2012. A one-pot ultrasound-assisted water extraction/cyclodextrin encapsulation of resveratrol from Polygonum cuspidatum. Food Chemistry 130: 746-750.

Marques, H. M. C., Hadgraft, J. and Kellaway, I. W. 1990. Studies of cyclodextrin inclusion complexes. The salbutamol-cyclodextrin complex as studied by phase solubility and DSC. International Journal of Pharmaceutics 63: 259-266.

Mourtzinos, I., Salta, F., Yannakopoulou, K., Chiou, A. and Karathanos, V. T. 2007. Encapsulation of olive leaf extract in beta-cyclodextrin. Journal of Agricultural and Food Chemistry 55: 8088-94.

Mura, P. 2014. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: a review. Journal of Pharmaceutical and Biomedical Analysis 101: 238-250.

Nguyen, T. A., Liu, B., Zhao, J., Thomas, D. S. and Hook, J. M. 2013. An investigation into the supramolecular structure, solubility, stability and antioxidant activity of rutin/cyclodextrin inclusion complex. Food Chemistry 136: 186-192.

Pinho, E., Grootveld, M., Soares, G. and Henriques, M. 2014. Cyclodextrins as encapsulation agents for plant bioactive compounds. Carbohydrate Polymers 101: 121-135.

Ratnasooriya, C. C. and Rupasinghe, H. P. V. 2012. Extraction of phenolic compounds from grapes and their pomace using β-cyclodextrin. Food Chemistry 134: 625-631.

Samanidou, V., Tsagiannidis, A. and Sarakatsianos, I. 2012. Simultaneous determination of polyphenols and major purine alkaloids in Greek *Sideritis* species, herbal extracts, green tea, black tea and coffee by high-performance liquid chromatography - diode array detection. Journal of Seperation Sciences 35: 608-615.

Shan, B., Cai, Y. Z., Sun, M. and Corke, H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry 53: 7749-7759.

Tadic, V. M., Jeremic, I., Dobric, S., Isakovic, A., Markovic, I., Trajkovic, V., Bojovic, D. and Arsic, I. 2012. Anti-inflammatory, gastroprotective, and cytotoxic effects of *Sideritis scardica* extracts. Planta Medica 78: 415-27.

Tadić, V., Bojović, D., Arsić, I., Đorđević, S., Aksentijevic, K., Stamenić, M., Janković, S. 2012. Chemical and Antimicrobial Evaluation of Supercritical and Conventional Sideritis scardica Griseb., Lamiaceae. Extracts Molecules 17: 2683-2703.

Todorova, M. and Trendafilova, A. 2014. *Sideritis scardica* Griseb., an endemic species of Balkan peninsula: traditional uses, cultivation, chemical composition, biological activity. Journal of Ethnopharmacology 152: 256-65.

Wang, J., Cao, Y., Sun, B. and Wang, C. 2011. Physicochemical and release characterisation of garlic oil-β-cyclodextrin inclusion complexes. Food Chemistry 127: 1680-1685.

Zhao, M., Wang, H., Yang, B. and Tao, H. 2010. Identification of cyclodextrin inclusion complex of chlorogenic acid and its antimicrobial activity. Food Chemistry 120: 1138-1142.