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# Pharmaceutical analysis of *Euphorbia cyparissias* included on Beta-cyclodextrin complexes

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**The methods of bio-control have diversified resulting in study of new fighting measures. Studies on *Euphorbia cyparissias* active compounds as well as their *in vivo* and *in vitro* activity revealed a significant pharmacologic activity, encouraging design of a complex original conditioning. Encapsulation techniques can be of considerable help in this endeavour, being a means to protect sensitive active components (such plant's active compounds) from the environment and from other excipients used. The aim of the present study was to evaluate *E. cyparissias* extracts inclusion in β-cyclodextrine complexes and thermo gravimetric (TG-DTG) analysing of *Euphorbia*'s extracts complexes, as a GMP pharmaceutic step, in the study of thermal behaviour of a future finite conditioning destined to demodectic mange treatment, a zoonotic ectoparasitic disease. TG-DTG analysis of pure β-cyclodextrine revealed a mass loss of 11.7%, which actually represent the β-cyclodextrin decomposition. In crude *Euphorbia* extract complexes T5 / β-CD case, the mass loss was corresponding probably to the encapsulated bioactive decomplexing phase of *Euphorbia* extracts' compounds. A similar behaviour to heating was recorded in case of crude *Euphorbia* extract T10 / β-CD complexes. In case of *Euphorbia* concentrated extracts T5 / T10 - β-CD complexes, the loss can be translated as a degradation / loss of complexes' bioactive compounds.**

**Key words:** *Euphorbia cyparissias*, β-cyclodextrine, complexes, TG-DTG analysis.

## INTRODUCTION

In medicine, biotherapy has become a current topic. Methods linked to the parasites' bio-control have diversified; new fighting means being studied (fungus, entomogenous nematodes, vegetal extracts, volatile oils, among others) (Kaaya et al., 2000; Samish and Rehacek, 1999; Sanis et al., 2012; Zahir and Rahuman, 2012). In this respect, plant extracts can be an important alternative control source, being a rich source of efficient bioactive compounds. Researchers try to bring new information regarding the use of spontaneous flora plants from their countries as well as other means to enrich the antparasitary arsenal (Babar et al., 2012; Borges et al., 2011; Chagas de Souza et al., 2012; Reggasa, 2000; Tona et al., 1999).

Numerous components from extracts and latex of *Euphorbiacee* were identified, mostly diterpenes (phorbolester, ingenole, euphorbone, piceatanole, aesculetine, jolkinol, hyperoside, kaempferol, acylphorbol, acylingenol among others) (Appendino et al., 2000; Evanics et al., 2001; Toth-Soma et al., 1993). Previous studies on the active compounds of *Euphorbia cyparissias*, revealed in the plant's inflorescence, thirteen compounds; (sesquiterpenoids being dominant). The *in vivo* and *in vitro* experiments, following this plant's

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**Table 1.** Conditions and results for obtaining the *Euphorbia* / β-CD extract complexes

| No. | Code | Description | m (β-CD) (g) | V (water) (ml) | Temp. (°C) | V (EtOH) (ml) | Cooling Time (h.) | Time perf. (h.) | Yield (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | T5 | *Euforbia* T5% extract | 0.671 | 4.0 | 50 | 4.0 | 4.0 | 12 | 80 |
| 2 | T10 | *Euforbia* T10% extract | | | | | | | 81 |
| 3 | T5c | *Euforbia* T5% conc. 1/5 extract | | | | | | | 79.90 |
| 4 | T10c | *Euforbia* T10% conc. 1/5 extract | | | | | | | 80 |

extracts activity against argasides and demodectic mange, revealed also a significant ectoparasitary activity, encouraging us to design a complex original conditioning, an *Euphorbia* ointment. Encapsulation techniques can be of considerable help in this endeavour, being means to protect sensitive active components (such plant's active compounds) from the environment and from other excipients used, this technique being used in many fields of therapy, from antibiotics, cancer to anti-parasitic domains (Thatiparti et al., 2010; Menuel et al., 2007; Becket et al., 1999).

Cyclodextrin (CD) is the general term of amylose produced by bacillus cyclodextrin glycosyltransferase enzyme generating a series of cyclic oligosaccharides usually containing 6 to 12 D - pyran glucose units. Studied more, and of great practical significance for medicine, molecules containing 6, 7, 8-glucose units are called alpha -, beta - and gamma – cyclodextrin. Cyclodextrins are allowing the encapsulation of active substances, drugs, flavours, enzymes, among others at the lowest possible that is, molecular encapsulation. In this process, each constituent is surrounded by a cyclodextrin ring, which provides almost perfect protection against the harmful effects of the environment (Del Valle, 2004; Biwer et al., 2002). Also cyclodextrins can enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface, the active molecules partition from the cyclodextrin cavity into the lypophilic barrier, thus, drug delivery from aqueous cyclodextrin solutions is both diffusion controlled and membrane controlled. Also cyclodextrins can enhance topical drug delivery in the presence of water (Loftsson and Masson, 2001). So, cyclodextrins are suitable active substances delivery systems, because of their ability to modify the physical, chemical, and biological properties of the guest molecules through labile interactions by formation of inclusion and/or association complexes becoming an important choice for new conceived drugs including therapeutic active substances from plants (Denadai et al., 2006; Karioti et al., 2011). The aim of our study was to accomplish, as novelty, inclusion of *E. cyparissias* extracts in β-cyclodextrine (β-CD) complexes (7-membered sugar ring molecules) and the thermo gravimetric analysis (TG-DTG) of *Euphorbia*'s extracts complexes, as a compulsory GMP pharmaceutic step, in the study of thermal behaviour of a future finite conditioning components destined for treatment of demodectic mange, a zoonotic ectoparasitic disease found in dog and humans.

## MATERIALS AND METHODS

### Obtaining *Euphorbia* / β-cyclodextrine (β-CD) extract complexes

Quantities of β-CD presented in Table 1 (corresponding to 0.5 mmols β-CDs), were weighed and then dissolved in 4 ml distilled water at 50 ± 1°C. After dissolution on this solution, *E. cyparissias* raw or concentrated extract ethanolic solutions (4 ml) were introduced drop wise, corresponding to a 1:1 molar ratio, calculated according to the known major component of the extract (quercitine), within 30 min, under continuous stirring. Resulted solution was slowly stirred for another 15 min, then cooled for 4 h in a water bath and finally stored in refrigerator, at 4°C for 24 h, to complete the crystallization of the newly formed complexes. This suspension was subjected to filtration, being washed with 1 ml of 96% ethanol and dried in desiccators. The obtained samples were subjected then to thermo gravimetric analyze.

### Thermo gravimetric analysis (TG-DTG)

A thermo gravimetric thermo-microbalance TG 209 F3 *Tarsus*® analyzer from Netzsch Instruments apparatus was used. It measures mass change as a function of temperature and has an operating temperature of between 10 and 1100°C. It is also vacuum tight enabling runs to be performed in vacuum, as well as in flowing gases, all measurements being performed in a nitrogen atmosphere to a temperature program: between 20-500°C, to a heating rate of: 10°C /min. Data acquisition was accomplished with the help of Netzsch TG 209-Acquisition program Soft/2000 and them processing with Netzsch Proteus - Thermal Analysis, program ver. 4.0/2000.

Thermo gravimetric analysis (TGA) is commonly used to determine selected characteristics of materials that exhibit either mass loss or gain due to decomposition, oxidation, or loss of volatiles (such as moisture). The principle is to measure the mass change of a sample as a function of temperature or time, under a defined and controlled environment with respect to heating rate, gas atmosphere, flow rate, crucible type, among others. Common applications of TGA are materials characterization through analysis of characteristic decomposition patterns, studies of degradation mechanisms and reaction kinetics, determination of organic content in a sample, and respectively, determination of inorganic (for example, ash) content in a sample (Brown, 2001).

To establish aspects of thermal behaviour of the involved components, plants extract different concentrations and the associations and influence of rough β-cyclodextrine, different TG-

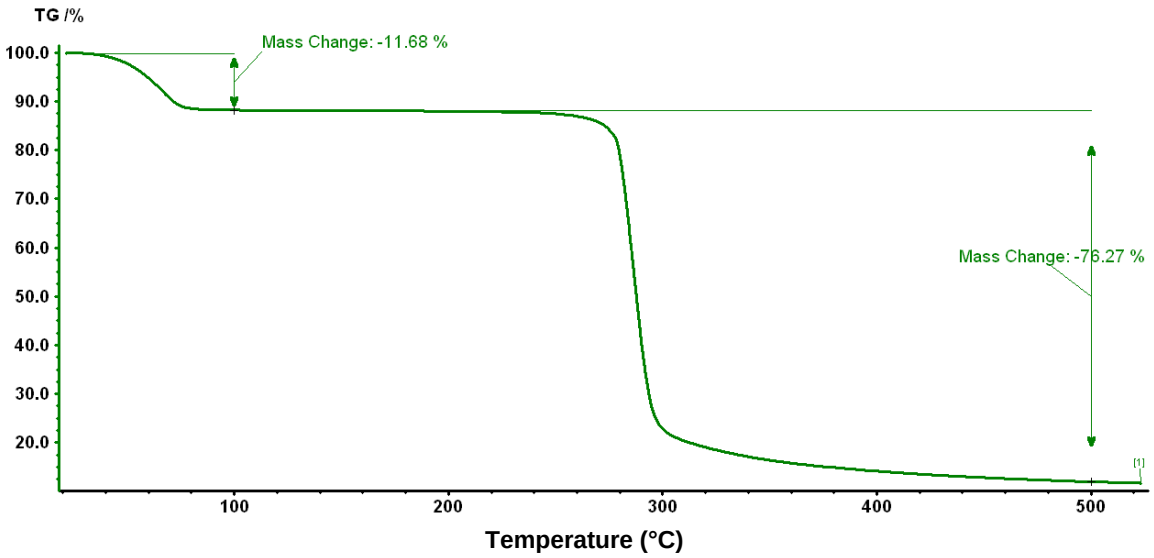


**Figure 1.** Pure β-cyclodextrine TG-DTG analysis.

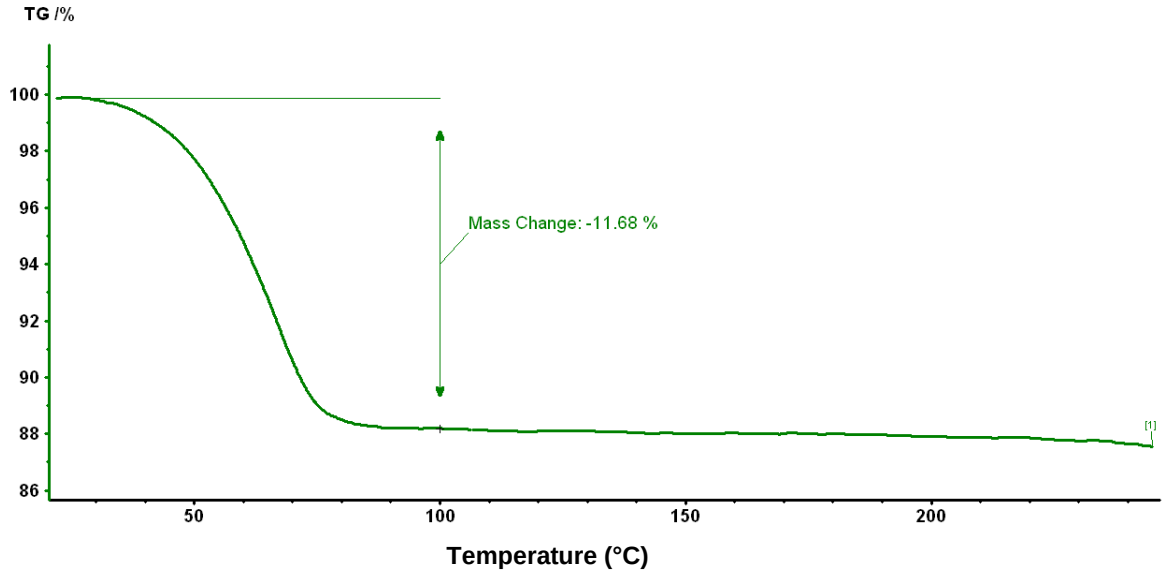


**Figure 2.** Pure β-cyclodextrine (low-temperature domain) TG-DTG analysis.

DTG tests were done after thermal analysis procedure known in pharmaceutical sciences, with the aim of mass degradation / loss of complexes bioactive compounds behaviour establishing of the conceived associations.

## RESULTS

### Pure β-cyclodextrin TG-DTG analysis

In Figures 1 and 2, pure β-cyclodextrine TG-DTG analysis is presented. TG-DTG analysis of pure β-cyclodextrine showed a mass loss of 11.68% up to 100°C, this corresponds to the release of crystalline water, and a 76.27% loss, in the 100 to 500°C temperature range, which represents the decomposition of β-cyclodextrin.

### Crude *Euphorbia* T5 / β-cyclodextrine extract complex TG-DTG analysis

In the case of *Euphorbia* complex T5 / β-CD crude extract, mass loss up to 100°C was only of 8.9%, but up

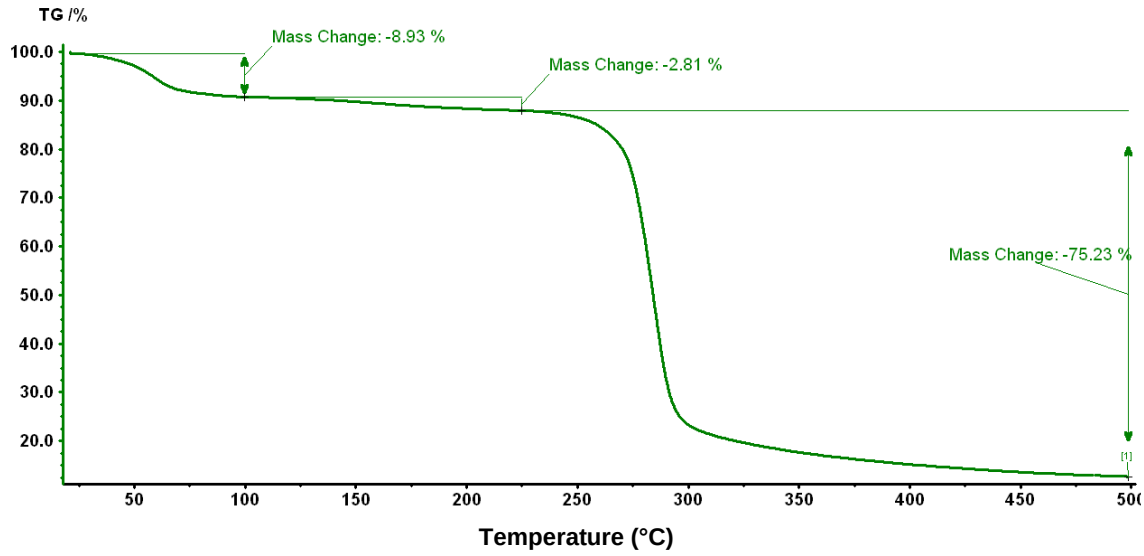


**Figure 3.** Crude *Euphorbia* T5 / β-CD extract complex TD-DTG analysis.

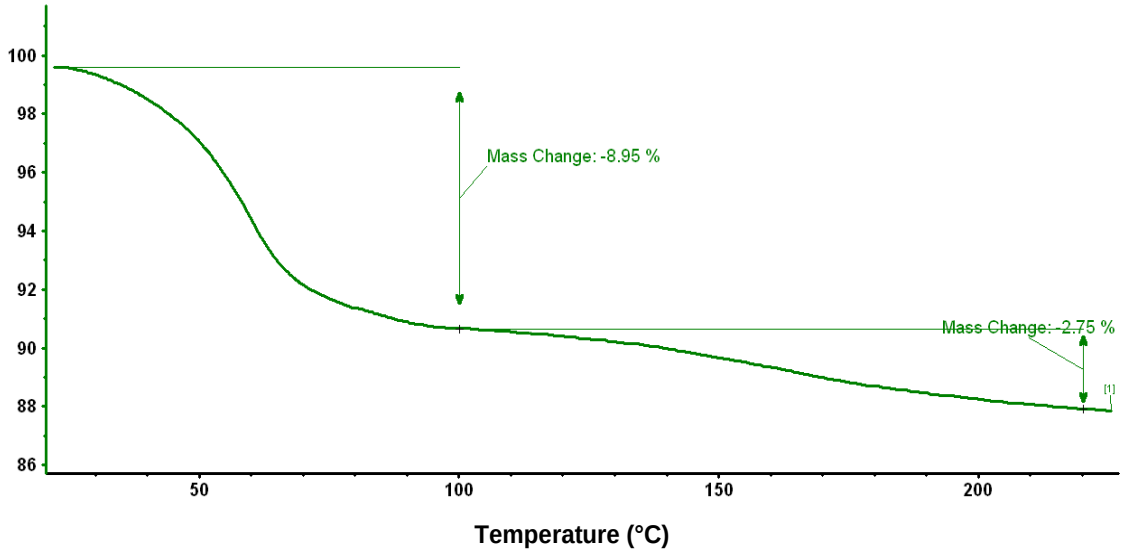


**Figure 4.** Crude *Euphorbia* T5 / β-CD extract complex (low-temperature field) TG-DTG analysis.

to a temperature of 225°C, there was an additional mass loss of 2.8%, mass loss decomposition being approximately equal (Figures 3 and 4).

## Crude *Euphorbia* T10 / β-cyclodextrine complex extract TG-DTG analysis

A similar behaviour to heating had also the crude *Euphorbia* T10/β-CD extract complex, so that up to 100°C, mass loss was 8% and between 100 and 225°C range of 2.9%, the decomposition determining a mass loss of ~ 78% (Figures 5 and 6).

## T5 and T10 *Euphorbia* / β-cyclodextrine concentrated complex TG-DTG analysis

In the case of *Euphorbia* complexes T5/T10 - β-CD concentrated extracts, mass loss up to 100°C, was more than 11.91% and from 100 to 225°C range, below to 1%. This probably means the initial extracts complexes' bioactive compounds loss / degradation (Figures 7, 8 9 and 10). Differences between thermal behaviour of crude

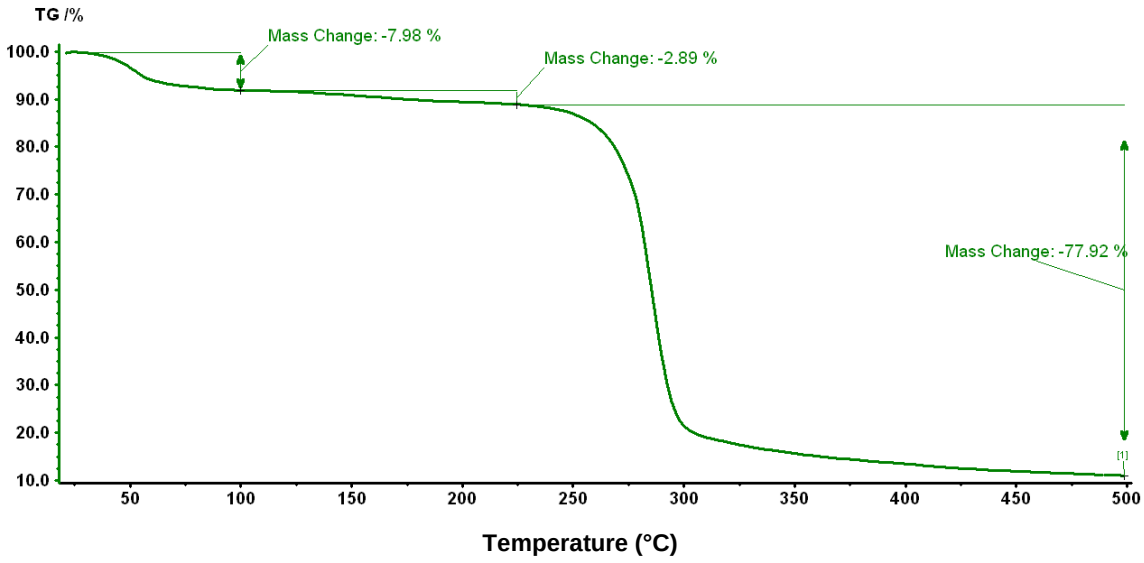


**Figure 5.** Crude *Euphorbia* T10 / β-CD extract complex TG-DTG analysis.

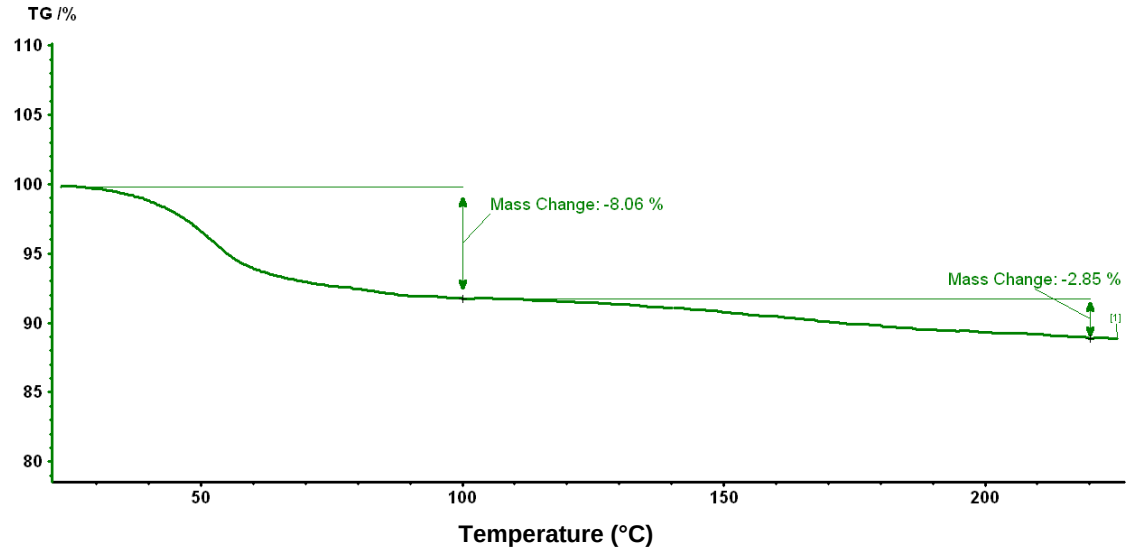


**Figure 6.** Crude *Euphorbia* T10/β-CD extract complex (low-temperature domain) TG-DTG analysis.

or concentrated T5 and T10 / β-CD *Euphorbia* extract complexes can be seen in Figures from 11, 12, 13, 14, 15 and 16.

## DISCUSSION

Studies of numerous macromolecule vehicles on topical delivery and understanding the basic relationship between solvent and solute penetration is an important issue to the researchers focussed on finding more effective pharmaceutical conditionings (Karande and Mitragotri, 2004; Magnusson et al., 2001).

Cyclodextrins can effectively increase the water-soluble adverse drug solubility in water and dissolution rate. They can improve the drugs stability and bioavailability of intestinal granules of volatile oil, reduce irritation and toxicity of drugs and drugs releasing and improving formulations (Cross et al., 2001; Inamori et al., 1994). In the recent years many researchers have reported on the topic of active substances' delivery potential of cyclodextrins and their possible applications in medicine. For example Liu et al. (2012) explored the formation of inclusion complex between puerarin and glucosyl-β-

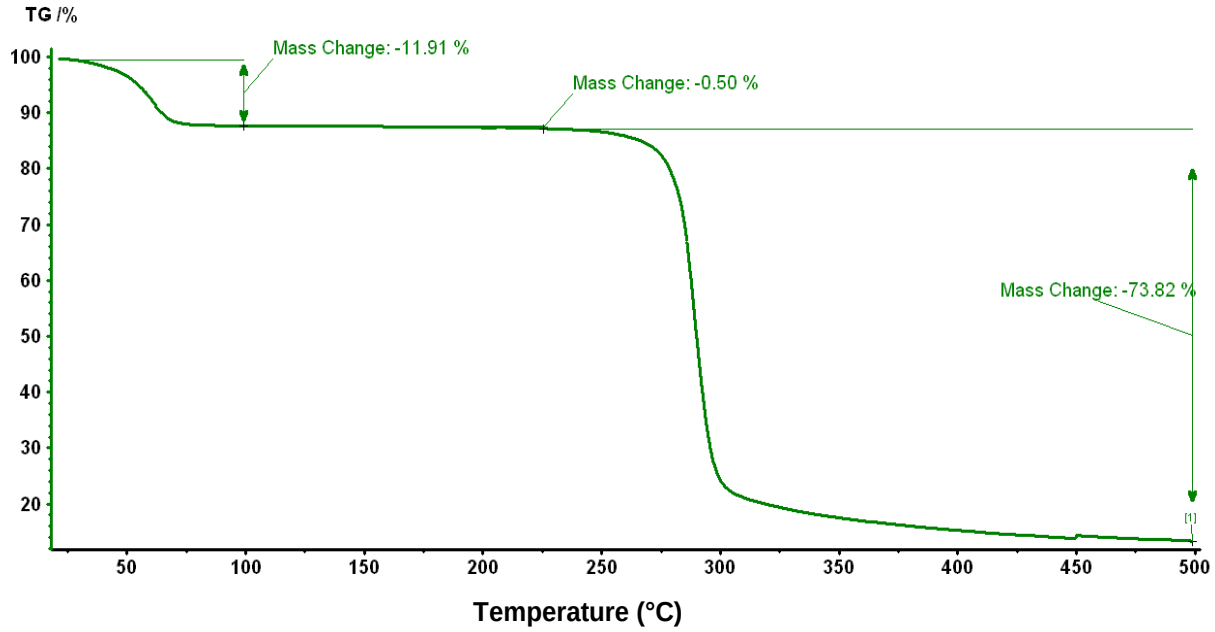


**Figure 7.** The *Euphorbia* T5 / β-CD concentrated complex TG-DTG analysis.

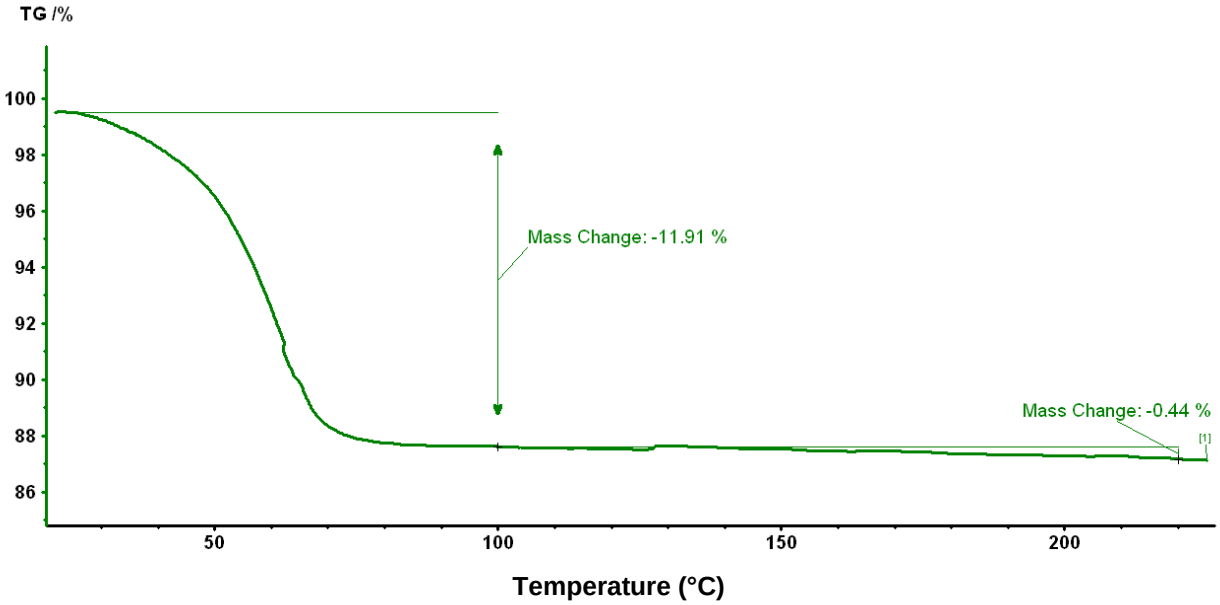


**Figure 8.** The *Euphorbia* T5 / β-CD concentrated complex (low-temperature domain) TG-DTG analysis.

cyclodextrin (G-β-CD) to improve the aqueous solubility of puerarin. Results showed clearly that the process led to the formation of a supramolecular complex in which the guest molecule, puerarin, was entrapped inside the cavity of the host, G -β-CD enhancing its therapeutic potential.

Sudha and Enoch (2011) studied interaction of curculigosides, phyto-constituents of plant *Curculigo orchioides*, and their β-cyclodextrin complexes with bovine serum albumin. As results, curculigoside–cyclodextrin complexes were found to bind more weakly to the bovine serum albumin molecule than their free forms. Petrović et al. (2010) studied the inclusion complexes between the *Cinnamomum verum* essential oil and β-cyclodextrin, prepared by co-precipitation method, in order to determine the effect of the ratio on the inclusion efficiency for encapsulating oil volatiles. Results revealed that the chromatographic profile of the

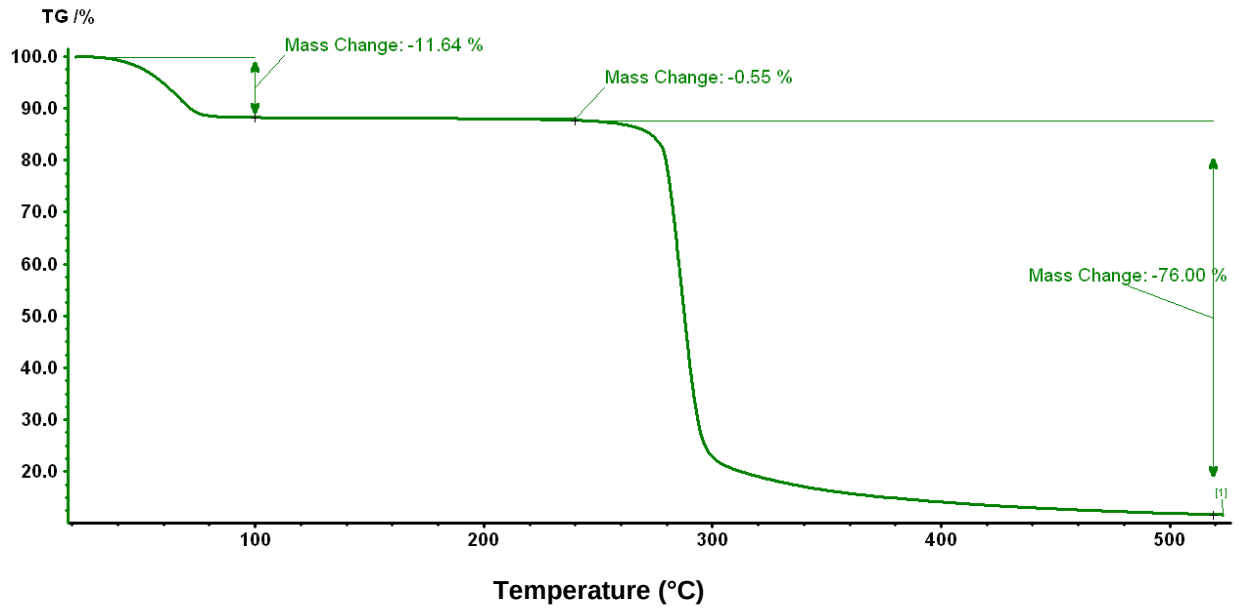


**Figure 9.** The *Euphorbia* T10 / β-CD concentrated complex TG-DTG analysis.

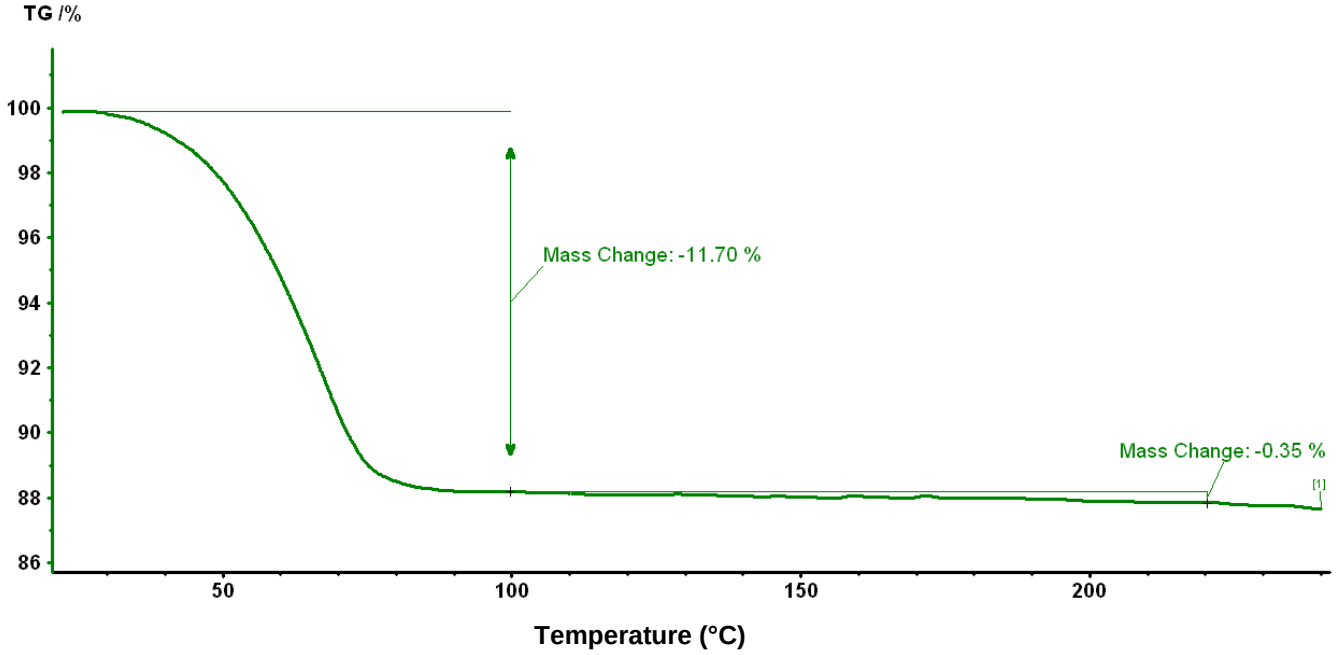


**Figure 10.** The *Euphorbia* T10 / β-CD concentrated complex (low-temperature domain). TG-DTG analysis.

surface adsorbed oil was different. The thirteen major compounds, found in the commercial *C. verum* essential oil, were present in all of the extracts, but in the different proportions between the total and surface oil extracts indicating the best therapeutic choice to choose. Yadav et al. (2009) prepared and evaluated the anti-inflammatory activity of cyclodextrin complex of curcumin for the treatment of inflammatory bowel disease (IBD) in a colitis-induced rat model. Results revealed that curcumin β-CD complexes proved to be potent angioinhibitory agents. It was concluded that the degree of colitis caused by administration of dextran sulphate solution (DSS) was significantly attenuated by CD of curcumin, recommending this nontoxic natural dietary complex as useful means in the therapeutic strategy for IBD patients. Finally, Panichpakdee and Supaphol (2011) studied the

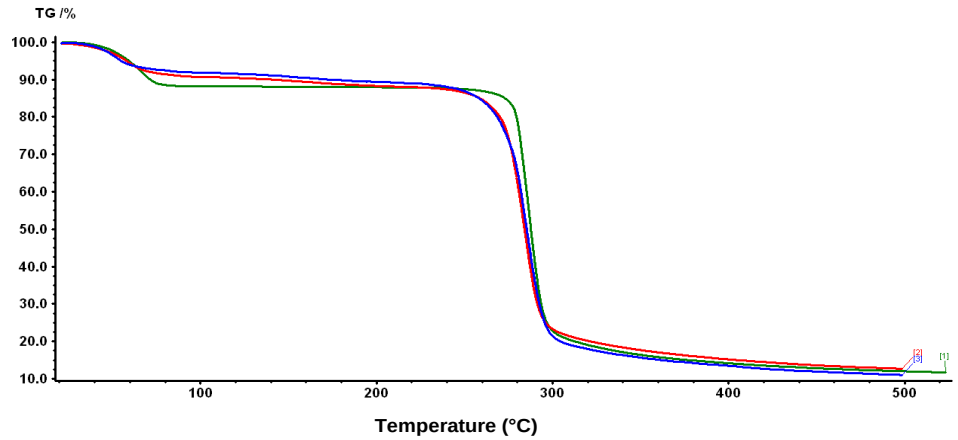


**Figure 11.** Pure β-CD thermograms overlap, pure (green) and Euphorbia T5 / β-CD (red) and T10 / β-CD (blue) crude extract complexes

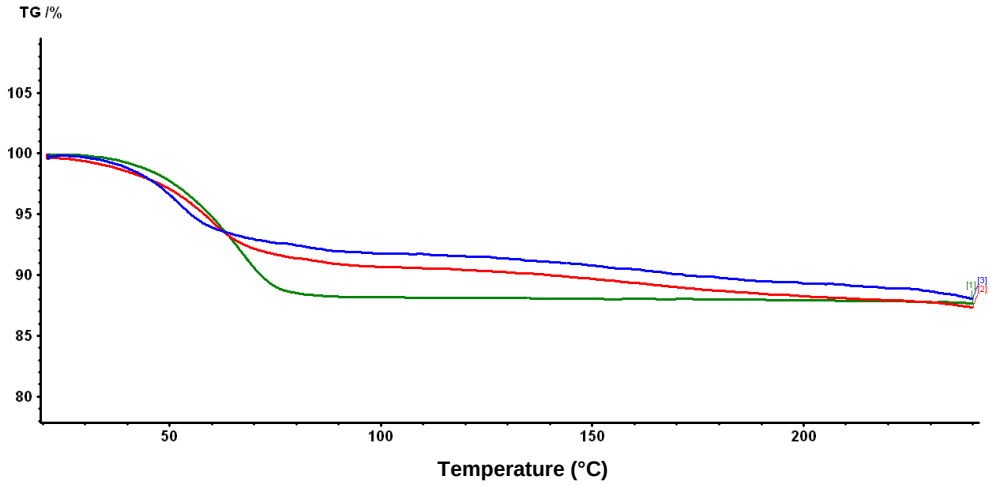


**Figure 12.** Pure β-CD thermograms overlap (green) and *Euphorbia* T5/β-CD (red) and T10/β-CD (blue) (low-temperature domain) crude extract complexes.

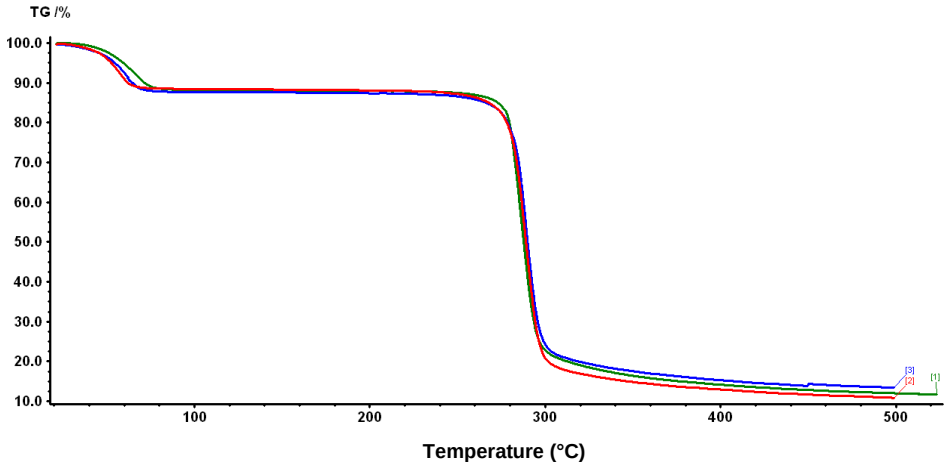


**Figure 13.** Pure β-CD thermograms overlap (green) and *Euphorbia* T5/β-CD (red) and T10/β-CD (blue) concentrated complexes

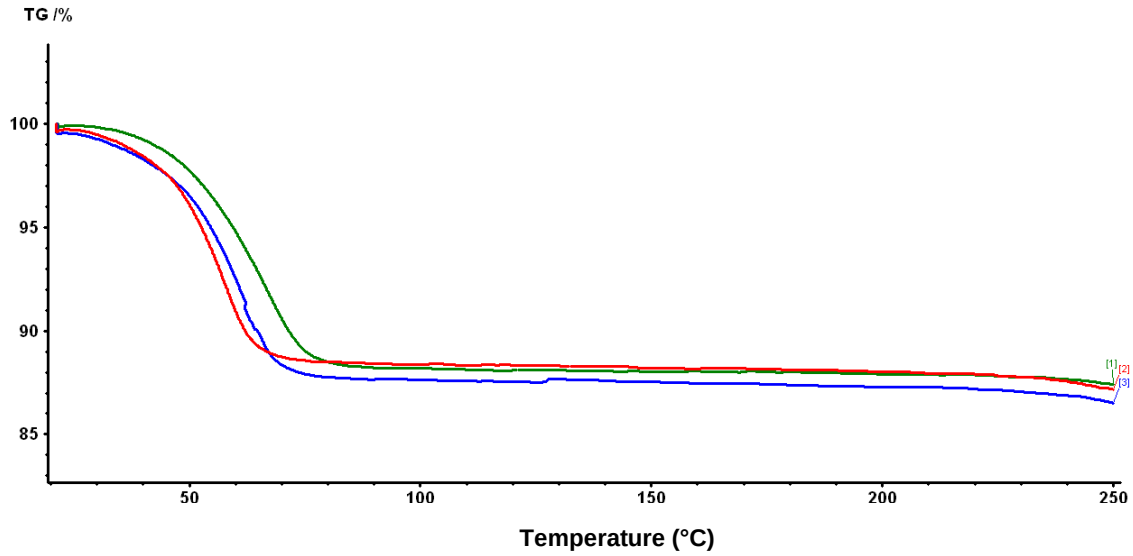


**Figure 14.** Pure β-CD thermograms overlap (green) and *Euphorbia* T5/β-CD (red) and T10/β-CD (blue) concentrated complexes (low-temperature field)

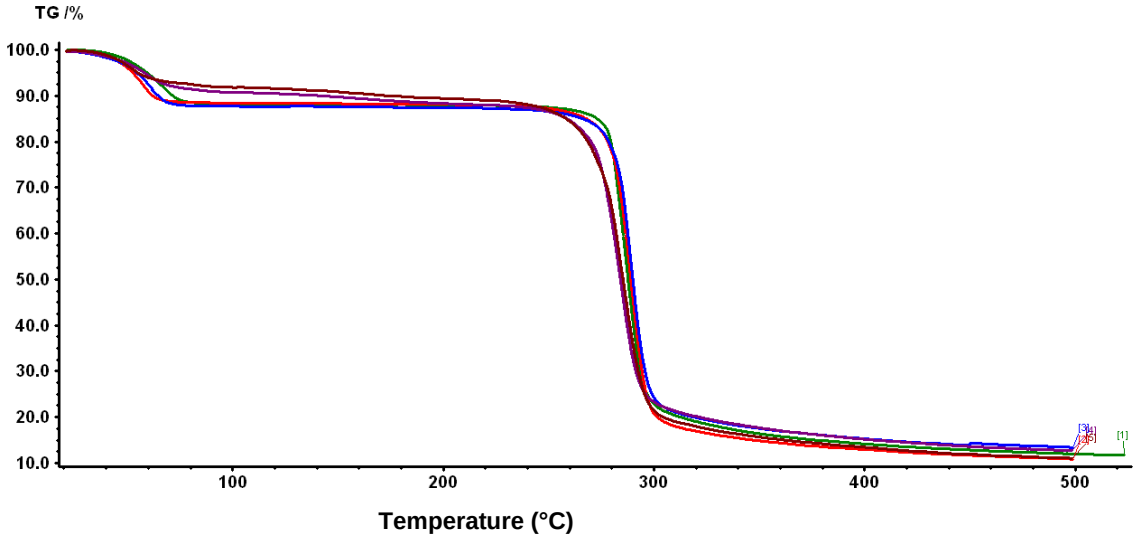


**Figure 15.** Pure β-CD thermograms overlap (green) and crude *Euphorbia* T5 / β-CD (violet) and T10/β-CD **(tan),** extract complexes and *Euphorbia* T5/β-CD (red) and T10/β-CD concentrated complexes (blue)

inclusion complex between an active substance from the medicinal plant *Centella asiatica L*. asiaticoside and 2-hydroxypropyl-cyclodextrin, respectively, demonstrating the practical and therapeutic advantages of the analyzed complexes. The thermo gravimetric analysis (TG-DTG) of *Euphorbia* extracts / β-cyclodextrine complexes was important to establish that the actives' compounds are complexed on cyclodextrin nuclei and different aspects of its behaviour. This has added further to the utility of *in vitro* studies on the lines of modern pharmaco-phytotherapy.

## Conclusions

TG analysis of pure β-cyclodextrine showed a mass loss of 11.7%, which actually represent the β-cyclodextrin decomposition. In crude *Euphorbia* extract complexes T5 / β-CD case, the mass loss was corresponding probably to the encapsulated bioactive decomplexing phase of *Euphorbia* extracts' compounds. A similar behaviour to heating was recorded in case of crude *Euphorbia* extract T10 / β-CD complexes, demonstating a normal decomposition and loss of water of crystallization and

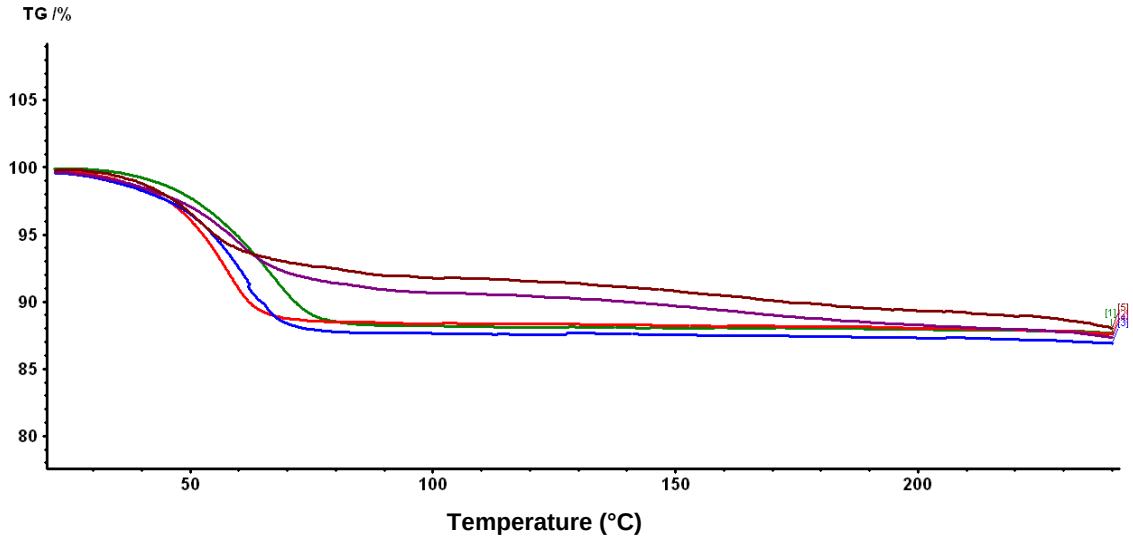


**Figure 16.** Pure β-CD thermograms overlap (green) and crude *Euphorbia* T5 / β-CD (violet) and T10/β-CD **(tan),** extract complexes and *Euphorbia* T5/β-CD (red) and T10/β-CD concentrated complexes (blue) (low-temperature domain).

also expected normal physical transitions: initial vaporization, evaporation, sublimation, desorbtion and finally drying. In case of *Euphorbia* concentrated extracts T5 / T10 - β-CD complexes, the loss can be translated as a degradation / loss of complexes' bioactive compounds weight being an expected and true mass change.

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## ABBREVIATIONS

**CD,** Cyclodextrin; **TGA,** thermo gravimetric analysis; **IBD,** inflammatory bowel disease; **DSS,** dextran sulfate solution.

### REFERENCES

Appendino G, Belloro E, Tron GC, Jakupovic J, Ballero M (2000). Polycyclic diterpenoids from *Euphorbia characias*. Fitoterapia 71(2):134-142.

Babar W, Iqbal Z, Khan MN, Muhammad G (2012). An inventory of the plants used for parasitic ailments of animals. Pak. Vet. J. 32(2):183-187.

Becket G, Schep LJ, Tan MY (1999). Improvement of the *in vitro* dissolution of praziquantel by complexation with α-, β- and γ-cyclodextrins". Int. J. Pharm. 179(1):65–71.

Biwer A, Antranikian G, Heinzle E (2002). Enzymatic production of cyclodextrins. Appl. Microbiol. Biotechnol. 59(6):609–617.

Borges F, Miranda L, Sousa D, Barbosa, AL, Silva C (2011). Perspectivas para o uso de extratos de plantas para o controle do carrapato de bovinos *Rhipicephalus (Boophilus) microplus*. Rev. Bras. Parasitol. Vet. 20(2):89-96.

Brown ME (2001). Introduction to Thermal Analysis, Kluwer Academic Publisher, London, UK.

Chagas de Souza AC, de Barros LD, Cotinguiba F, Furlan M, Giglioti R, Oliveira de Sena MC, Bizzo HR (2012). *In vitro* efficacy of plant extracts and synthesized substances on *Rhipicephalus* (*Boophilus*) *microplus* (Acari: *Ixodidae*). Parasitol. Res. 110(1):295-303.

Cross SE, Pugh WJ, Hadgraft J, Roberts MS (2001). Probing the effect of vehicles on topical delivery: understanding the basic relationship between solvent and solute penetration using silicone membranes. Pharm. Res. 18:999-1005.

Del Valle EMM (2004). Cyclodextrins and their uses: a review. Process Biochem. 39(9):1033–1046.

Denadai AM, Santoro MM, Lopes MT, Chenna A, de Sousa FB, Avelar GM, Gomes MR, Guzman F, Salas CE, Sinisterra RDA (2006). Supramolecular complex between proteinases and beta-cyclodextrin that preserves enzymatic activity: physicochemical characterization. BioDrugs 20(5):283-291.

Evanics F, Hohmann J, Redei D, Vasas A, Gunther G, Dombi G (2001). New diterpene polyesters isolated from Hungarian *Euphorbia* species. Acta Pharm. Hung. 71(3):289-292

Inamori T, Ghanem AH, Higuchi WI, Srinivasan V (1994). Macromolecule transport in and effective pore size of ethanol pre-treated human epidermal membrane. Int. J. Pharm. 105:113-123.

Kaaya GP, Samish M, Itamar G (2000). Laboratory evaluation of pathogenicity of entomogenous nematodes to African tick species. Ann. N Y Acad. Sci. 916:303-308.

Karande PA, Mitragotri S (2004). Discovery of transdermal penetration enhancers by high throughput screening. Nat. Biotechnol. 22:192-197.

Karioti A, Leonti M, Bergonzi M, Bilia A (2011). Inclusion studies of falcarinol in β-cyclodextrin. Planta Med, 77 - PK7.

Liu B, Zhao J, Liu Y, Zhu X, Jie Z (2012). Physiochemical Properties of the Inclusion Complex of Puerarin and Glucosyl-β-Cyclodextrin. J. Agric. Food Chem. 60(51):1250-1257.

Loftsson T, Masson M (2001). Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. 225:15–30

Magnusson BM, Walters KA, Roberts MS (2001). Veterinary drug delivery: potential for skin penetration enhancement. Adv. Drug Del. Rev. 50:205-227.

Menuel S, Joly JP, Courcot B, Elysée J, Ghermani NE, Marsura A (2007). Synthesis and inclusion ability of a bis-β-cyclodextrin pseudo-cryptand towards Busulfan anticancer agent. Tetrahedron 63(7):1706.

Panichpakdee J, Supaphol P (2011). Use of 2-hydroxypropyl-cyclodextrin as adjuvant for enhancing encapsulation and release characteristics of asiaticoside within and from cellulose acetate films. Carbohydr. Polym. 85:251–260.

Petrović GM, Stojanović GS, Radulović NS (2010). Encapsulation of cinnamon oil in β-cyclodextrin. J. Med. Plants Res. 4(14):1382-1390.

Regassa AJ (2000). The use of herbal preparations for tick control in western Ethiopia. J. South Afr. Vet. Assoc. 71(4):240–243.

Samish M, Rehacek J (1999). Pathogens and predators of ticks and their potential in biological control. Ann. Rev. Entomol. 44:159-182.

Sanis J, Ravindran R, Ramankutty SA, Gopalan AK, Nair SN, Kavillimakkil AK, Bandyopadhyay A, Rawat AK, Ghosh S (2012). *Jatropha curcas (Linne)* leaf extract - a possible alternative for population control of *Rhipicephalus (Boophilus) annulatus*. Asian Pac. J. Trop. Dis. 225-229.

Thatiparti TR, Shoff AJ, Von Recum HA (2010). Cyclodextrin-based device coatings for affinity-based release of antibiotics. Biomaterials 31(8):2335–2347.

Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totte J, Vlietinck AJ (1999). Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J. Ethnopharmacol. 15; 68(1-3):193-203.

Sudha NI, Enoch MV (2011). Interaction of curculigosides and their β-cyclodextrin complexes with bovine serum albumin: a fluorescence spectroscopic study. J. Solution Chem. 40(10):1755-1768.

Toth-Soma LT, Gulyas S, Szegletes Z (1993). Functional connection between intracellular and extracellular secretion in species of *Euphorbia* genus. Acta Biol. Hung. 44(4):433-443.

Yadav VR, Suresh S, Devi K, Yadav S (2009). Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat. AAPS Pharm. Sci. Tech. 10(3):752-762.

Zahir AA, Rahuman AA (2012). Evaluation of different extracts and synthesised silver nanoparticles from leaves of *Euphorbia prostrata* against *Haemaphysalis bispinosa* and *Hippobosca maculata.* Vet. Parasitol. 187(3-4):511-520.