



A Novel Green Extraction and Molecular Docking Study: Effect of Native Cyclodextrins and Derivatives on the Extraction of Andrographolide from *Andrographis paniculata* (Burm.f.) Nees

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ABSTRACT

Andrographolide (AP) is a sparingly water solubility bioactive compound from *Andrographis paniculata*. It exhibits several pharmacological activities. With respect to its hydrophobicity, the high-yield extract of AP is primarily obtained from organic solvents, which are considered as environmentally harmful substances. Therefore, the green extraction using an alternative solvent is eventually performed to obtain the highest AP extract. The objectives of this study are to investigate the extraction efficiency of AP using different types of cyclodextrins (CDs) in aqueous media and *in silico* molecular docking study of AP with the corresponding CDs. The cyclodextrin-assisted extraction of AP using alpha cyclodextrin (α CD), beta cyclodextrin (β CD), gamma cyclodextrin (γ CD), methyl beta cyclodextrin (M β CD), trimethyl beta cyclodextrin (triM β CD) and hydroxypropyl beta cyclodextrin (HP β CD) at a concentration of 1 mM was investigated in aqueous media comparing with water and methanol by ultrasonication. The amount of AP in each extract was determined by an HPLC method. In screening process, HP β CD exhibited the highest extraction yield of AP compared with the other CDs. However, it was significantly lower than that in the methanolic extract ($P < 0.05$). Subsequently, the extraction of AP was performed as a function of HP β CD concentration. Increasing the concentration of HP β CD resulted to obtain the better extraction yield of AP comparing with a positive control of methanol ($P < 0.05$). In conclusion, the green extraction using HP β CD could be an alternative method to achieve a higher AP in the extract than that in the methanolic extract.

Keywords: cyclodextrin-assisted extraction, host-guest interaction, inclusion complex, labdane diterpenoid, water extraction

Introduction

“King of bitter” or *Andrographis paniculata* (Burm.f.) Nees, a plant in Acanthaceae family, has been included in the National List of Essential Medicine, Herbal Medicinal Product chapter. The plant has been found in tropical countries including India, Sri Lanka, China, Indonesia, Malaysia and Thailand. In traditional medicine, the whole plant and its leaves are commonly used for the treatment of upper respiratory tract infection, antipyretic, skin rashes, malaria and peptic ulcer.¹ Labdane diterpenoids, specifically andrographolide, neoandrographolide, and isoandrographolide, are the primary bioactive components of *A. paniculata*.²⁻³ These compounds possess a variety of pharmacological properties, including antibacterial, anti-biofilm formation, anti-HIV, anti-flu, antimalarial, anti-leishmaniasis, and antiviral properties.⁴⁻⁶

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Extraction of bioactive compounds from medicinal plants is aimed to obtain high yield of active phytochemicals in the extract or to remove fiber and undesired plant materials. The selection of extraction technique is a crucial step, which has to be considered depending on the physicochemical characteristics of the phytochemicals as well as the advantages or disadvantages of that technique. Green extraction is relied on the design of extraction processes such as reductions of energy consumption and organic solvents or using alternative solvents to ensure a safety and environmentally friendly concerns.⁷⁻⁸ Water is considered as harmless solvent for extraction. However, the extraction efficiency of phytochemicals using water is relatively low due to its high polarity.⁹ The use of cyclic oligosaccharides-cyclodextrins in aqueous medium probably improves the extraction efficiency of bioactive compounds such as phenolic compounds, flavonoids and terpenoids.¹⁰ The cyclic oligosaccharides known as cyclodextrins (CDs) are linked by α -1,4 glycosidic bonds and contain D-(+)-glucopyranose subunits. Due to the hydroxyl groups of the sugars, the cyclic structure of CDs exhibits external hydrophilic properties, whilst the internal cavity exhibits lipophilic properties.¹¹ CDs lipophilic internal cavities can encapsulate low- or medium-polar molecules by non-covalent interactions, van der Waal forces, hydrophobic interactions, and hydrogen bonds to generate host-guest inclusion complexes.¹² Native CDs consist of 3 different sizes of macrocyclic oligosaccharides, namely, α CD (6 glucose subunits), β CD (7 glucose subunits) and γ CD (8 glucose subunits), which provide different interaction pocket sizes for phytochemicals.¹³ CDs are widely used in pharmaceutical-, food-,

cosmetics- and chemical industries to improve stability and increase water solubility or mask undesired taste.¹⁴

Cyclodextrin-assisted extraction (CAE) is one of the green extraction methods. The trend of CAE of plant secondary metabolites has been increasing due to its extraction efficiency and primarily using aqueous medium.¹⁰ Previous researches on CAE of phytochemicals were mostly focused on the extraction of phenolic compounds *e.g.* phenolic acid in Chinese date,¹⁵ anthocyanin in mulberry fruits¹⁶ and mangiferin in mango stem barks.¹⁷ There are various factors influencing the CAE efficiency such as CD types, CD concentration, temperature, extraction time and extraction technique.¹⁸ Additionally, molecular docking of phytochemicals and CDs may be performed to investigate host-guest binding energy and functional group interaction such as CDs and scandenin, a bioactive compound of *Deris scandens*.¹⁹ The objectives of this study were to investigate the extraction efficiency of andrographolide from *A. paniculata* using various types of CDs and binding free energy of andrographolide-CDs complexes using *in silico* molecular docking technique. The results of CAE of andrographolide and molecular docking study were also compared.

Materials and Methods

Chemicals and reagents

Alpha cyclodextrin (α CD) and gamma cyclodextrin (γ CD) were purchased from Wako chemicals (Osaka, Japan). Beta cyclodextrin (β CD), methyl beta cyclodextrin (M β CD) and trimethyl beta cyclodextrin (triM β CD) were obtained from Acros Organics (Geel, Belgium). Hydroxypropyl beta cyclodextrin (HP β CD) was from CycloLab (Budapest, Hungary). A standard andrographolide (AP, 98%) was from Sigma Aldrich (Saint Louis, MO, USA). Ultra-purified water was generated by Milli-Q academic A10 (Millipore, Darmstadt, Germany). Methanol AR grade was obtained from Labscan (Bangkok, Thailand). All structures of CDs and andrographolide were shown in Figure 1.

Plant materials and HPLC analysis of andrographolide

Andrographis paniculata was harvested in Sakon Nakhon Province (GPS position: 17.537168°N, 103.729399°E) in August 2021. The voucher specimen was collected at the herbarium of Faculty of Pharmacy, Mahasarakham University, Thailand (Herbarium Specimen Number: MSU.PH-ACA-AP-1). The whole plant was cleaned with deionized water and dried under 50°C hot air oven (Model 2000, Contherm Scientific, New Zealand). The dried material was further pulverized (Sunsolid, Samut Prakan, Thailand) and sieved (Retsch GmbH, Haan, Germany) with 60 mesh to obtain fine powder. The powder was then kept under cool condition and protected from light prior to experiment. The analysis of andrographolide was performed under the Thai Herbal pharmacopoeia (THP) monograph.²⁰ Briefly, the HPLC analytical method of AP was performed on a Primaide 1000 series (Hitachi, Tokyo, Japan). Mobile phase was a mixture of 52 volumes of ultrapure water and 48 volumes of methanol. The separation of andrographolide was carried out using Dr.Maisch Reprosil-Pur C₁₈ 5- μ m, 150 x 4.6 mm (Ammerbuch-Entringen, Germany) with Luna C₁₈ 5- μ m guard column, 20 x 3.9 mm (Phenomenex, Torrance, CA, USA) at a flow rate of 1 mL per minute. The analysis of andrographolide was monitored at 224 nm with Primaide 1430 Diode Array Detector (Hitachi, Tokyo, Japan).

Effect of CD and its derivatives on the extraction of andrographolide

The extraction of andrographolide (AP) was performed at 10 minutes using 30% amplitude of 296 W, 40 kHz ultrasonic probe (Sonics & Materials Inc, Newtown, CT, USA). *A. paniculata* was weighed at 300 mg (Sartorius, Goettingen, Germany) in the extraction media of 10 mL. The extraction temperature was set at 25°C. All CDs were prepared at a concentration of 1 mM to evaluate the extraction efficiency in comparison with water and methanol extracts. After extraction, the samples were centrifuged at 5,000 rpm for 10 minutes (Hettich GmbH, Tuttlingen, Germany). Subsequently, the supernatants were filtered with 0.45 μ m PTFE syringe filter prior to injection to the HPLC system. Finally, the CD with highest extraction efficiency was further studied as a function of CD concentration. The extraction yield of AP was

calculated using a standard curve of peak areas versus concentrations ($y = 89750x - 19007$, $R^2 = 0.9996$).

Molecular docking study

The optimized structures of β CD, M β CD and HP β CD from our prior research was used.²¹⁻²³ The triM β CD was created through methyl substitutions at the R1, R2, and R3 positions of the natural β CD. The structural configurations of α CD (PDB: 4FEM) and γ CD (PDB: 5E70) were sourced from the Protein Data Bank (<https://www.rcsb.org>). The two-dimensional (2D) configurations of AP were acquired in SDF format from PubChem Compound (<http://pubchem.ncbi.nlm.nih.gov/>) using the compound CIDs of 5318517. The preparation of all docking input files was carried out using AutoDockTools (ADT).²⁴ For the CDs, the Kollman united atom charges were applied²⁵, while the Gasteiger-Marsili charges²⁶ were assigned to the partial atomic charges of the AP molecule. The dimensions of 18 x 18 x 18 Å³ was established and centered on the grid box of each CDs molecule. The grid spacing was set to 1.0 Å. Host-guest inclusion structures were generated through molecular docking calculations utilizing AutoDock Vina. Default values were utilized for the other parameters. In the search protocol, the guest molecule had the freedom of unrestricted movement within the CD cavity, while the host molecule remained fixed in a truncated-cone structure. The analysis and visualization of the results of inclusion complexation between AP and various CDs were conducted using Biovia Discovery Studio and ChimeraX software.²⁷

Data analysis

All collected data were presented in mean \pm SD (n=3) using Microsoft Excel. IBM SPSS Statistics Version 29 was used to analyze the statistical difference of the extracted AP among the experimental groups using One-way ANOVA. A Tukey post hoc test was applied to compare the amount of AP in various extraction media with control groups, water- and methanolic extract. Statistically significant difference was considered at p value < 0.05.

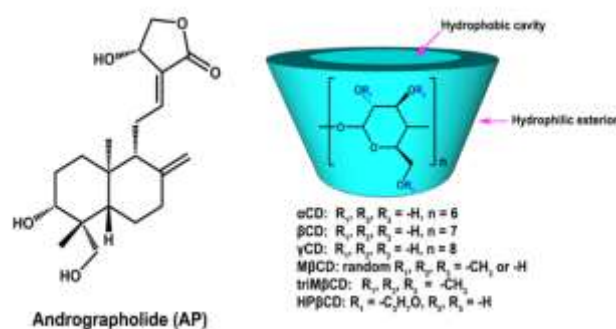


Figure 1: Structures of andrographolide and native CDs and derivatives

Results and Discussion

Cyclodextrin-assisted extraction (CAE) of andrographolide from *A. paniculata*

AP exhibits a very low solubility in aqueous medium with log P at 2.2.²⁸ Due to low water solubility, CDs were considered to form host-guest inclusion complexes, which resulted in enhanced water solubility of AP in aqueous media. The amounts of AP extracted from *A. paniculata* using various types of CDs at a concentration of 1 mM in aqueous media were substantially compared with water and methanol as shown in Table 1. The results revealed that γ -, M β - and HP β -CDs exhibited the highest yield at 5.69 \pm 0.04, 5.78 \pm 0.14 and 5.98 \pm 0.17 mg AP/g dried weight, respectively comparing with other CDs and water extract ($P < 0.05$). However, the highest extraction efficiency at 6.45 \pm 0.16 mg AP/g dried weight was observed in methanolic extract. The extraction efficiency is derived from the hydrophobic internal cavity size and molecular interaction of host-guest compounds. In the case of AP containing cyclic moieties, the compound could not form a host-guest inclusion complex with α CD due to its small internal cavity.¹⁰ The low

extraction efficiency of AP was also observed in β CD-assisted extraction. It is probably from a low water solubility of β CD at 18.5 mg/mL which could compete in water solubility with AP. On the other hand, the water solubilities of other CD derivatives range between 145 to more than 2,000 mg/mL, at 25°C. The substitution of native β CD with various functional groups may lead to improve the interaction of the guest compounds as well as the increment of water solubility of CD derivatives.²⁹ In comparison to other CDs, HP β CD demonstrated the highest extraction efficiency (Table 1).

As shown in Table 1, the extraction using HP β CD exhibited the highest extraction yield. Therefore, HP β CD was considered to perform a further experiment on CAE of AP as a function of the CD concentration. The HP β CD was prepared in different concentrations, *i.e.*, 1%, 2% and 3% w/v in water as extraction media. Our findings revealed that increasing HP β CD concentration tend to enhance the solubility of AP by increasing the chance to encapsulate the compound into the interior cavity. The strong binding affinity of AP and HP β CD could be predominantly from hydrogen bonding interaction.^{11,29-30} The solubility enhancement of HP β CD in various concentrations showed a better extraction efficiency than those in water and methanol (Figure 2). The AP extraction yields using 1%, 2% and 3% w/v HP β CD substantially increased from 7.90 \pm 0.11 to 9.06 \pm 0.01 and 10.27 \pm 0.17 mg AP/g dried weight, respectively. The AP extraction yield had linearly complied with the CD concentrations with a correlation coefficient of 0.999, $y = 1.181x + 6.7154$. As a result of its water solubility at more than 1,200 mg/mL as well as hydroxypropyl groups, increasing the concentration of HP β CD may probably tend to improve the extraction yield of AP.²⁹ This phenomenon had been observed in CAE using β CD and HP β CD for the extraction of *Hibiscus sabdariffa* anthocyanins.³¹ The extraction yield of anthocyanins was comparable to a positive control of 40% ethanol solution ($P > 0.05$), when the concentration of HP β CD had been increased from 1% to 5% w/v. However, increasing the concentration of β CD could not demonstrate the same effect as HP β CD. It is due to the low water solubility of β CD as well as the saturation of internal cavity of β CD at the higher concentration. These could limit further interactions of the compound and β CD.³² The formation of host-guest inclusion complex is a major role of the extraction which related to the encapsulation of phytochemicals in hydrophobic cavity.¹⁰ The inclusion complexes of CDs and phytochemicals depend on 2 factors *i.e.* (1) size and structure of guest molecules and internal cavity of host CDs, and (2) thermodynamics interaction of CD, guest molecule and solvent.^{29,33}

Molecular docking

The modeling results of AP with CDs in all inclusion complexes are shown in Figure 3. The AP molecule was configured to move freely, resulting in the binding of the curved structure between AP and α CD.

Table 1: The CAE of andrographolide (AP) from *A. paniculata* using various CDs at a concentration of 1 mM, water and methanol

Extraction media	Extraction yield of AP (mg/g dried weight)*
α CD	4.32 \pm 0.04 ^a
β CD	5.25 \pm 0.11 ^c
γ CD	5.69 \pm 0.04 ^d
M β CD	5.78 \pm 0.14 ^d
triM β CD	5.16 \pm 0.07 ^c
HP β CD	5.98 \pm 0.17 ^d
Water	4.85 \pm 0.04 ^b
MeOH	6.45 \pm 0.16 ^c

Different alphabets (a – e) indicate statistically significant differences ($P < 0.05$)

*The result shows in mean \pm SD (n=3).

Table 2: The binding free energy (ΔG_{bind}) of andrographolide (AP) encapsulated to different types of CDs

Host-guest inclusion complexes	ΔG_{bind} (kcal/mol)
AP- α CD	-4.3
AP- β CD	-5.5
AP- γ CD	-5.5
AP-M β CD	-5.5
AP-triM β CD	-3.6
AP-HP β CD	-6.1

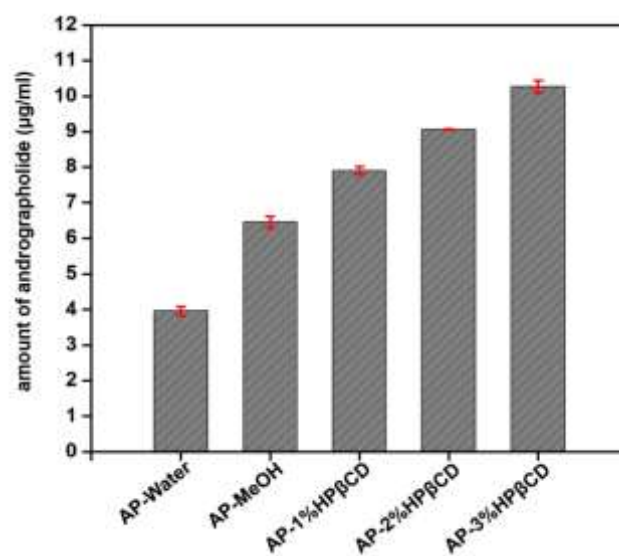


Figure 2: The CAE of andrographolide (AP) from *A. paniculata* as a function of CD concentration comparing with water and methanol, the result shows mean \pm SD (n=3)

The decalin rim of the AP molecule was partially enclosed in the fairly hydrophobic cavity of the structure of AP- α CD (Figure 3a), while the lactone group extends beyond the surrounding of α CD. Particularly, AP moving its entire structure into the β CD, γ CD, M β CD or HP β CD hydrophobic interior (Figure 3b-d and f) appeared on the CD primary rim. Furthermore, the interaction of AP and hydroxyl groups of native CDs or substituted functional groups was apparently a consequence of either a steric hindrance or inclusion complex forming by non-covalent interaction. In this context, the guest compound could be completely encapsulated into the β CD, γ CD, M β CD and HP β CD internal cavity resulting to a low binding free energy. Nevertheless, the methyl substituents on 2,3,6-position of the glucose subunits, triM β CD exhibited more pronounced steric hindrance. Although the hindered AP molecule probably employed a superficial interaction with the CD, it was unable to fully occupy this particular lipophobic cavity of the CD (Figure 3e).

The overall host-guest binding free energy (ΔG_{bind}) of AP- α CD, AP- β CD, AP- γ CD, AP-M β CD, AP-triM β CD, and AP-HP β CD are shown in Table 2. The binding energies are found in the range of -3.6 to -6.1 kcal/mol. The binding strengths relative to binding energies of AP encapsulated into different types of CDs are presented in the order; AP-HP β CD (-6.1 kcal/mol) > AP- β CD (-5.5 kcal/mol) = AP- γ CD (-5.5 kcal/mol) = AP-M β CD (-5.5 kcal/mol) > AP- α CD (-4.3 kcal/mol) > AP-triM β CD (-3.6 kcal/mol). The data shows that the AP-HP β CD complex demonstrates the strongest binding interaction between the HP β CD and AP molecules. This ability arises from the capability of the AP structure to completely enter the cavity, preferably by the formation of hydrogen bonds. These binding energies are consistent with previous studies, indicating their favorable energetic agreement.³⁴ The weakest binding capability was attributed to AP-triM β CD due to the positioning of the

methyl group in triM β CD hindering the AP molecule at the terminal outside the CD hydrophobic cavity, as previously mentioned. Increasing the degree of substitution at a lower range could improve solubility of guest compounds, whereas the very high degree of substitution, as the case of triM β CD, could result to steric hindrance of the guest compounds incorporated to the host molecule.³⁵

Conclusion

Among other CDs, the CAE of AP employing HP β CD possessed the highest extraction efficiency. The results from *in silico* molecular docking study mostly complied with the CAE experiment. Therefore, molecular docking can be used for the screening of potential CDs in CAE. The influence of extraction factors *e.g.* liquid-solid ratio, extraction technique, temperature and time should be performed to evaluate the extraction efficiency of AP. Additionally, a study on the optimization of CAE of AP using response surface methodology (RSM) of particular factors would be further investigated.

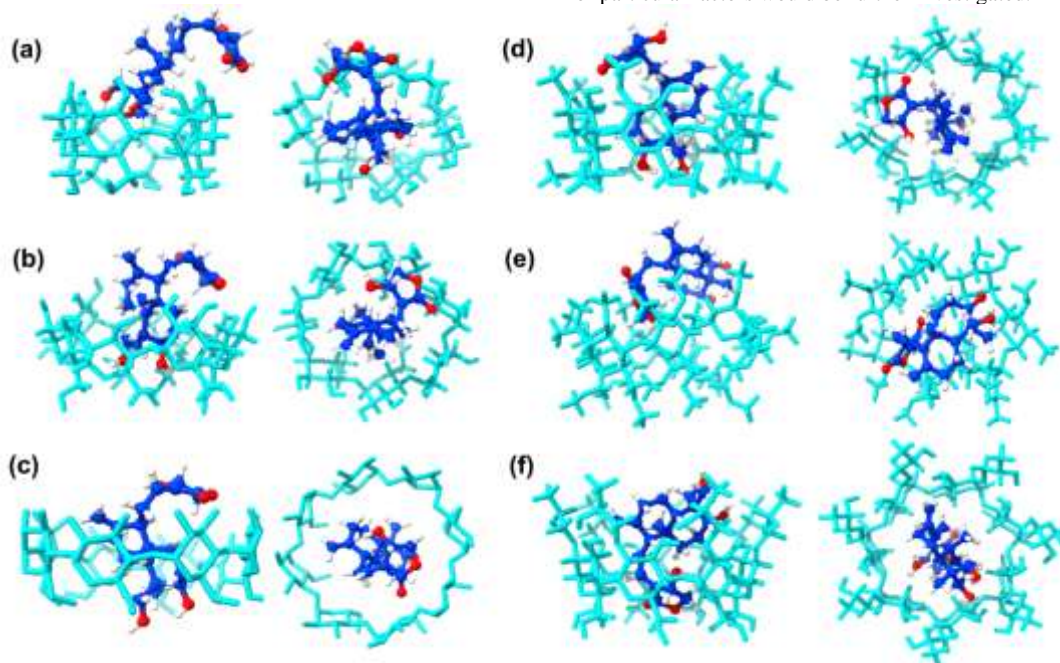


Figure 3: Both front and side view simulations of host-guest inclusion complexes of andrographolide (AP) and CD structures; (a) AP- α CD, (b) AP- β CD, (c) AP- γ CD, (d) AP-M β CD, (e) AP-triM β CD and (f) AP-HP β CD.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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