

# Comparison of the Anti-Cancer Drugs Camptothecin and 10-Hydroxy-Camptothecin from Different Plant Species by Using ...

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## ABSTRACT

Camptothecin(CPT) is a compound isolated by Wall 's and others in 1966 from *Camptotheca acuminata*. Camptothecin(CPT) and its analogues can inhibit DNA topoisomerase I, which will arrest the DNA of oncogenes from synthesizing and inhibit the growth of tumors. The goals of this research is to find the best analyzing and extraction method for obtaining Camptothecin(CPT) and 10-hydroxy-camptothecin(10-OH-CPT), and compare the CPT and 10-OH-CPT content differences in various Camptothecin(CPT) containing plants. We experimented with leaves of Taiwan 's *Camptotheca acuminata* Decaisne, leaves of Taiwan 's *Nothapodytes foetida* (Wight.) Sleumer, tree branches of Taiwan 's *Nothapodytes foetida* (Wight.) Sleumer, leaves of Hunan Changsha 's *Camptotheca acuminata* Decaisne, and fruits of Hunan Changsha 's *Camptotheca acuminata* Decaisne. This experiment uses the following three extracting systems to conduct research, which are heat recirculation, ultrasound assistant extraction, and microwave assistant extraction. In the area of heating reflux extraction, we used different solvents to find out the best extraction method. Results revealed that the leaves of Taiwan 's *Nothapodytes foetida* (Wight.) Sleumer had the richest CPT content. We repeated three sessions of 3hr heating reflux extraction by using a mixed solvent composed of Methylene dichloride and water (ratio 9:1) under temperatures of 48 . The extraction rate was 0.61 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. Leaves of Taiwan 's *Camptotheca acuminata* Decaisne had the richest 10-OH-CPT content; we repeated three sessions of 3hr heating reflux extraction by using 95% ethanol as a solvent under temperatures of 78.5 . The extraction rate was 0.35 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. Ultrasonic-assisted extraction was used to extract effective elements within the plants, it is faster, easier, does not need heating, achieves higher extraction rates, does not destroy the structure of the extracts, displays good effects, thus proving it to be the better method. The Merck Index indicates that DMSO is a very good solvent, therefore we used various DMSO with different water contents (which are 100%, 80%, 60%, and 40%) to understand the one that would be most effective in extraction, thus establishing a standard for analysis. The results indicated that extraction was at its best when the water content was 80% for duration of 30 minutes. The content for 10-OH-CPT was 5.25g/kg, while the content for CPT was 2.67g/kg. When we compared different plants, we discovered that the leaves of Taiwan 's *Nothapodytes foetida* (Wight.) Sleumer were richest in CPT content. We applied ultrasonic- assisted extraction for 30 minutes, using a mixed solvent composed of Methylene dichloride and water (ratio 9:1) under temperatures of 48 . The extraction rate was 2.89 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. Leaves of Taiwan 's *Camptotheca acuminata* Decaisne had the richest 10-OH-CPT content; we applied ultrasonic-assisted extraction for 30 minutes, using a mixed solvent composed of Methylene dichloride and water (ratio 9:1) under temperatures of 35 . The extraction rate was 0.35 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. The time the ultrasonic took to finish was 18 times shorter than heating reflux extraction. Effects of microwave-assisted extraction. Microwave extraction techniques can shorten experiment and production time, save energy, reduce amounts of solvent used, decrease amounts of waste produced, it can also increase recycle rates and purity of the extraction. When we compared the plants, we discovered that the leaves of Taiwan 's *Camptotheca acuminata* Decaisne had the richest CPT content. We applied microwave-assisted extraction for 1 minute, using a mixed solvent composed of Methylene dichloride and water (ratio 9:1) under temperatures of 48 . The extraction rate was 2.79 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. Leaves of Taiwan 's *Nothapodytes foetida* (Wight.) Sleumer were richest in 10-OH-CPT content. We applied microwave-assisted extraction for 1 minute, using a mixed solvent composed of Methylene dichloride and water (ratio 9:1) under temperatures of 35.1 . The extraction rate was 0.31 times of DMSO analysis [The ultrasonic-assisted extraction took 30 minutes using Dimethyl sulfoxide as a solvent (DMSO), which had a water content of 20%]. Results indicate that although the total extraction amount for ultrasonic-assisted extraction is higher that microwave-assisted extraction, yet the time spent on microwave-assisted extraction is 30 times shorter. Microwave-assisted extraction only needs 1 minute to achieve higher extraction rates and uses fewer solvents. Apparently, microwave-assisted extraction will be the future trend and method adopted by extraction industries.

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REFERENCES

- 1.上海藥物研究所植物室喜樹研究組。1975。喜樹果中的抗癌有效成分喜樹鹼與10-羥基喜樹鹼。中草藥通訊 (5):17-18。
- 2.王自芬和劉文哲。2005。不同產地喜樹果實中喜樹鹼及10-羥基喜樹鹼的差異。中草藥 36(5):762-764。
- 3.江蘇植物研究所編。1993。江蘇植誌。
- 4.李立源、張冬?和白鳳武。2001。喜樹鹼及其衍生物的研究進展。大連民族學院學報3(2):02-0017。
- 5.李國雄和王惠康。1995。喜樹鹼的化學及抗癌構效關係研究之最新發展。The Chinese Chemical Society 53:64-75。
- 6.吳立軍。2006。中藥化學。第29頁。科技圖書股份有限公司。台北。台灣。
- 7.邱年永和張光雄。1992a。原色台灣藥用植物圖鑑(3)，南天書局。台北。台灣。
- 8.邱年永和張光雄。1992b。原色台灣藥用植物圖鑑(5)，南天書局。台北。台灣。
- 9.范漢欽、黃士佳和蔡宗賢。2000。田口軟體之應用。義守大學工業工程管理學系。
- 10.徐任生和趙志遠。1977。抗癌植物喜樹化學成分的研究II:喜樹果的化學成分。化學學報。35(3,4):193-199。
- 11.商惠芳、陳作琳、林嘉伯和黃昭蓮。1995。作用於DNA拓樸異構?之抗癌藥物。Journal of Food and Drug Analysis 3(3):145-15
- 12.孫慶磊、梁月榮和陸建良。2006。超聲波在茶葉提取中的應用。Journal of Tea 32(2):79 ~ 82。
- 13.趙東亮。2005。喜樹鹼的萃取、純化工藝研究。貴州大學碩士論文。貴州。
- 14.網路資料來源。2007。山羊百科。喜樹果實網址:  
[http://plant.climb.com.tw/modules/mediawiki/index.php/%E5%9C%96%E5%83%8F:Camptotheca\\_acuminata\\_IMGP4069.JPG](http://plant.climb.com.tw/modules/mediawiki/index.php/%E5%9C%96%E5%83%8F:Camptotheca_acuminata_IMGP4069.JPG)。青脆枝網址:  
[http://plant.climb.com.tw/modules/mediawiki/index.php/%E5%9C%96%E5%83%8F:Camptotheca\\_acuminata\\_IMGP4\\_069.JPG](http://plant.climb.com.tw/modules/mediawiki/index.php/%E5%9C%96%E5%83%8F:Camptotheca_acuminata_IMGP4_069.JPG)。
- 15.劉業經、呂福原和歐辰雄。1994。台灣樹木誌。第925頁中興大學農學院出版委員會。
- 16.劉川生、王平、王立飛、梅成和陳薇薇。2003。微波萃取技術在天然藥物萃取中的研究進展。中國天然藥物 1(3):187-192。
- 17.羅厚蔚。1975。從喜樹果中分離出一種微量生物鹼。中草藥通訊 17 (1): 25-26。
- 18.蘇朝墩。2002。田口式品質工程。國立清華大學。
- 19.Adamovics J. A., China J. A. 1979. Minor alkaloids of *Camptotheca acuminata*. *Phytochemistry* 18:1085-1086。
- 20.Balandrin M. F., Kinghorn A. D. and Farnsworth N. R. 1993. Plant-derived natural products in drug discovery and development: an overview. *Am Chem Soc Symp Ser.* p2-12. Washington DC. USA.
- 21.Champoax, J. J. 1981. DNA is linked to the rat liver nicking-closing enzyme by a phosphodiester bound to tyrosine. *Journal of Biological Chemistry* 256:4805-4809。
- 22.Devanand, P. F. and Ramesh, K. S. 2005. Distribution of anticancer drug camptothecin in *Nothapodytes foetida*. *Fitoterapia*, 76:643-648。
- 23.Devanand, P. F. and Ramesh, K. S. 2005. Comparison of techniques for the extraction of the anti-cancer drug camptothecin from *Nothapodytes foetida*. *Journal of chromatography A*, 1063:9-13。
- 24.Fulzele, D. P., Satdive, R. K. and Pol, B. B. 2001. Growth and production of camptothecin by cell suspension cultures of *Nothapodytes foetida*. *Plant Med.* 67:150-152。
- 25.Giovanella, B. C. 1997. Topoisomerase I inhibitors. *Cancer therapeutics:Experimental and clinical agents* p137-152。
- 26.Giovanella, B. C., Stehlin J. S., Wall, M. E., Wani M. C. Allan W., Nicholas, Liu, L. f., Robert Silber and Milan Potmesil. 1989. DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 246:1046-1048。
- 27.Hertzberg, R., Busby, R. W., Caranfa, M. J., Holden, K. G., Johnson, R. K., Hecht, S. and Kingsburg, W. D. 1990. Irreversible trapping of the DNA- topoisomerase I covalent complex. *Journal of Biological Chemistry* 265:19287-19295。
- 28.Hsiang, Y. H., Herizberg, R. and Hecht, S. 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *The Journal of Biological Chemistry* 260:14873-14878。
- 29.Hsiang, Y. H., Liu, L. F., Wall, M. E., Wani M. C. and Nicholas, A. W. 1989. DNA Topoisomerase I Mediated DNA Cleavage and Cytotoxicity of Camptothecin Analog. *Cancer Research* 49:4385-4389。
- 30.Kjeldsen E., Svejstrup J. Q., Gromova II, Alsner J. and Westergard O. 1992. Camptothecin inhibits both the cleavage and relegation reaction of eukaryotic DNA topoisomerase I. *Journal of Molecular Biology* 228:1025-1030。
- 31.Li, Z. and Z. Liu. 2003. Effects of benzyladenine and Naphthalene acetic acid on growth and camptothecin accumulation in *Camptotheca* seedlings. *Journal of Plant Growth Regulation* 22(3):205-216。
- 32.Muggia, F. M., Creaven, P. J., Hansen, H. H.,

Cohen, M. H. and Selawry, O. S. 1972. Phase I Clinical Trial of Weekly and Daily Treatment with Camptothecin(NSC-100880); Correlation with Preclinical Studies. *Cancer Chemother. Rep. Part I.* 56:515-521. 33. Tsao, Y. P., Russo A., Nyamuswa, G., Silber, R. and Liu, L. F. 1993. Interaction between Replication Forks and Topoisomerase I- DNA Cleavable Complexes: Studies in a Cell- free SV40 DNA Replication System. *Cancer Research* 53:1-8. 34. Wall M. E., Wani M. C. and Cook C. E. 1986. Plant antitumor agents I. The isolation and structure of camptothecin, a novel alkaloidal leukaemia and tumor inhibitor from *Camptotheca acuminata* Dence. *Journal of medicinal chemistry* 29(8):1553-1555. 35. Wall M. E. and Wani M.C. 1991. Antitumor and topoisomerase I inhibition activity of camptothecin and its analogues. *Economic and medicinal plant research, Vol. 5. Plants and traditional medicine.* p111-127. London: Academic Press. 36. Wall M. E. and Wani M. C. 1996. Camptothecin and taxol: from discovery to clinic. *Journal of Ethnopharmacology* 51:239-254.