

Purification of Chebulinic acid from the Composition of Medicinal herbs by Column Chromatography

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Abstract: Chebulinic acid is a phenolic compound and found in the medicinal herbs like fruits of Terminalia chebula, Amla and Longan seeds. The objectives of this work were extraction of chebulinic acid from the composition of medicinal herbs by Soxhlet extraction and purify the chebulinic acid by using Column chromatography. From the Soxhlet extraction the chebulinic acid concentration was 8.8 mg/ml and it was increased to 9.2 mg/ml from the Column chromatography. The purity of chebulinic acid was improved by Column chromatography.

Keywords: Chebulinic acid, Soxhlet extraction, Column chromatography, Terminalia chebula, Amla, Longan

I. Introduction

Column chromatography is a technique which is used to purify individual compounds from the mixture of compounds. Silica gel and alumina are commonly used as stationary phase for Column chromatography. In this studies, the purification of chebulinic acid from the composition of medicinal herbs like fruits of Terminalia chebula, Amla and seeds of Longan. Mainly Chebulinic acid is a phenolic compound [1]. It is a Faint yellowish crystalline powder and sparingly soluble in water, soluble in ethanol, methanol and ethyl acetate. Chebulinic acid [2] was helps to remove toxins and unwanted fat from the body, improves skin glow and complexion also. It showed many pharmacological activities [3] including inhibition of cancer cell growth like human colon adenocarcinoma HT-29 cell lines [4] leukemia K562 cells [5], inhibiting the contractile responses of cardiovascular muscles[6], anti-neisseria gonorrhoeae activity, anti-hypertensive [7], anti-oxidant, anti-bacterial activities etc. The dried fruits of Terminalia chebula is used to produced the dye. The appearance of dye powder is brown and the main colouring component is chebulinic acid and this fruit contains an astringent matter. The astringency is because of the characteristic principle of chebulinic acid. Mainly the structural and conformational analysis of chebulinic acid component by using high pressure liquid chromatography [8] and Reverse Phase HPLC [9].

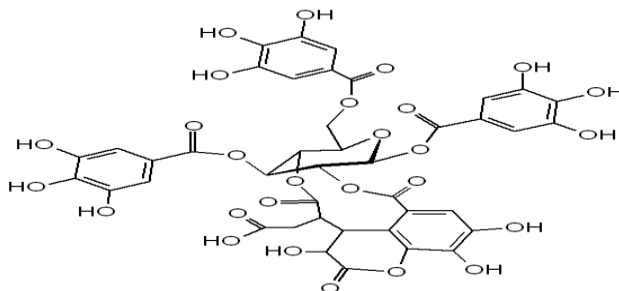


Figure 1: Structure of chebulinic acid

II. Materials And Methods

Chemicals and Reagents

Folin-Denis reagent, sodium carbonate (Na_2CO_3), methanol, silica gel and distilled water.

Materials

The dry fruits of Terminalia chebula, Amla and seeds of longan collected from local market in Visakhapatnam, Andhra Pradesh, India. Clean the fruits and seeds and dried under sunlight for 1 day. These fruits and seeds were cut into small pieces and powdered and stored in the air tight container. It is finely grounded to 120 mesh size.

Solvent extraction using Soxhlet Extractor

8.8 mg/ml of chebulinic acid concentration obtained from the composition of medicinal herbs by using Soxhlet extraction process [10]. So that extract was done in to the column chromatography method [11] for the purification of chebulinic acid.

Column chromatography

In Column Chromatography, 100 micron particle size silica gel was used as stationary phase. Before starting the experiment first insert a piece of cotton into the column towards outlet. Fix the column to the clamp tightly. Pour the sea sand of 1cm bed in the column. Add silica gel powder in the column up to 20cm length from the neck of the column. Run the solvent methanol in the column up to the bed was entirely wet. Add excess solvent on the top of the silica gel bed. Gently tap the column with hand or soft materials. After tapping gentle pressure can be applied. Before loading the sample in the column, little silica gel was added to the extract. Pour the extract and rinse the wall. Add sand on the top of the sample. The entire system was shown in figure 1. After collecting the samples for every 5 minutes from the column, take 1ml of sample from each test tube add 0.5ml FD reagent and 1ml Na₂CO₃. Make up this solution up to 10ml with distilled water. After 30min read the absorbance at 700nm for chebulinic acid concentration.



Figure 2: Column chromatography

III. Results And Discussion

From the Soxhlet extraction studies, ethanol (80% v/v) was the best solvent for chebulinic acid extraction. The highest chebulinic acid concentration was observed to be 8.8 mg/ml and the concentration was increased to 9.2 mg/ml from the Column chromatography. The purity of chebulinic acid was improved by Column chromatography at 15 min. The results of the Column chromatography studies were shown in table 1 and figure 2.

Table 1: Effect of Extraction yield with Extraction time for Chebulinic acid by Column chromatography

Time (min)	Concentration of Chebulinic acid (mg/ml)
5	5.2
10	7.8
15	9.2
20	4.0
25	1.6
30	0.05

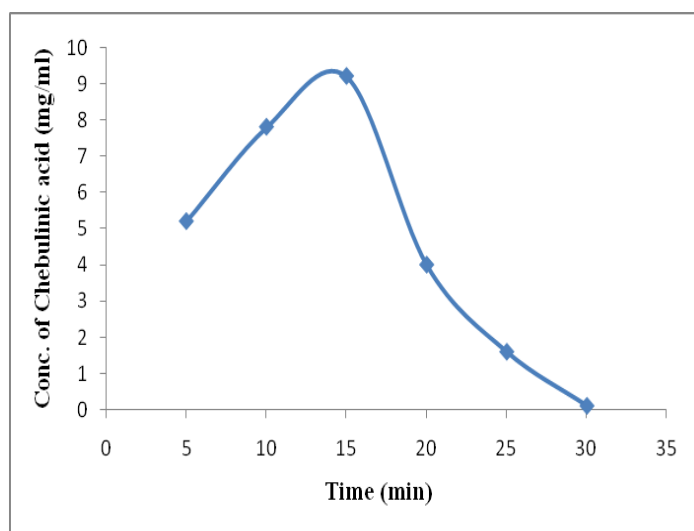


Figure 3: Effect of Extraction yield with Extraction time for Chebulinic acid by Column chromatography

IV. Conclusion

Chebulinic acid is a main component in this dry fruits of *Terminalia chebula*, Amla fruit and longan seeds. In this present work the extraction of chebulinic acid from the composition of medicinal herbs by Soxhlet extraction studies and purification by Column chromatography were performed. From the Soxhlet extraction the chebulinic acid concentration was 8.8 mg/ml and it was increased to 9.2 mg/ml from the Column chromatography. The purity of chebulinic acid was improved by Column chromatography.

References

- [1]. T Yoshida, R Fujii and T Okuda. Revised structures of chebulinic acid and chebulagic acid, *Journal of Chemical and Pharmaceutical Bulletin* Yoshida, 28(12), 1980, 3713-3715.
- [2]. T. N. Ranjini, M. A. Suryanarayana, K. Bhanuprakash and Umesha. Estimation of Chebulagic and Chebulinic acid in *Terminalia chebula*, *Journal of the Bioscan*, 10(2), 2015, 549-551.
- [3]. Di Bei, Leon Xie, Kai Lu, Kenneth Chan, Sujit Basu and Zhongfa Liu. Determination of chebulagic acid and chebulinic acid and their pharmacokinetics in mouse, *Journal of Cancer Research*, 73, 2013, 4879-4882.
- [4]. A Saleem, M Husheem, P Harkonen and K Pihlaja. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz fruit. *Journal of Ethnopharmacology*, 81(3), 2002, 327-336.
- [5]. Yi ZC, Z. Wang, Li HX, MJ. Liu, Wu RC and XH.Wang, Effects of chebulinic acid on differentiation of human leukemia K562 cells, *Journal of Acta Pharmacological Sinica*, 25(2), 2004, 231-238.
- [6]. YY. Guan, CY. Kwan, FL. Hsu and JT. Cheng, In vitro Inhibitory effects of chebulinic acid on the contractile responses of cardiovascular muscles, *Journal of Clinical and Experimental Pharmacology and Physiology*, 23, 1996, 745-50.
- [7]. Ta-Chen Lin, Fengun Hsu and Juei-Tang Cheng, Antihypertensive activity of corilagin and chebulinic acid, tannins from *lumnitzera racemosa*, *Journal of Natural Products*, 56(40), 1993, 629-632.
- [8]. Anil D. Mahajan and Nandini R. Pai. Simultaneous Determination of Eight Phytoconstituents in Triphala churna by HPLC–DAD. *Research J. Pharmacognosy and Phytochemistry*, 3(2), 2011, 62-66.
- [9]. V. Pawar, P. Lahorkar and D. B. Anantha Narayana. Development of a RP-HPLC Method for Analysis of Triphala Churna and its Applicability to Test Variations in Triphala Churna Preparations. *Indian J Pharm Sci.*, 71(4), 2009, 382–386.

- [10]. D. V. Surya Prakash and Meena Vangalapati. Experimental and Modelling Equation of Chebulinic acid from the composition of medicinal herbs by using soxhlet extractor, International Journal of Innovative Research in Science, Engineering and Technology, 4(7), 2015, 5217-5220.
- [11]. D.V Surya Prakash, N SreeSatya and V Meena. Purification of chebulinic acid from Terminalia chebula species by Column chromatography. Journal of chemical, biological and physical sciences, 2(4), 2012, 1753-1758.