



OPTIMIZATION OF PHYSICO-CHEMICAL PARAMETERS FOR THE EXTRACTION OF CHEBULINIC ACID FROM MEDICINAL HERBS

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Abstract. Chebulinic acid is a phenolic compound found in the fruits of *Terminalia chebula* (Haritaki), *Phyllanthus emblica* (Amla) and seeds of *Dimocarpus longan* (Longan) species etc. It showed many pharmacological activities including inhibition of cancer cell growth like human leukemia K562 cells, colon adenocarcinoma HT-29 cell lines, anti-neisseria gonorrhoeae activity etc. The present studies on optimization of physico-chemical parameters like effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol and methanol as solvents, pH and different weight dosages for the extraction of Chebulinic acid from Medicinal herbs. The highest chebulinic acid concentration for optimized conditions of *Terminalia chebula* fruit, Amla fruit and Longan seeds were 6.2 mg/mL, 4.8 mg/mL and 5.7 mg/mL respectively.

Key words: Chebulinic acid, *Terminalia chebula* fruit, Amla fruit, Longan seed, Folin-Denis reagent.

Introduction

Terminalia chebula species commonly called as *Black myrobalan*, Ink tree or *Chebolic myrobalan*.

It belongs to the family *combretaceae*. It is used for the treatment of number of diseases like cardio vascular diseases, arthritis, paralysis, cancer, gout, ulcers, epilepsy etc. contains tannins up to 30%, chebulic acid 3-5%, chebulinic acid 30% [KAREL D. KLIKA *et al.*, 2004], tannic acid 20-40%, ellagic acid, 2,4-chebulyi- β -D-gluco pyranose, gallic acid, ethyl gallate, flavonoids like luteolin, rutins, and quercetin etc.

It shows several pharmacological activities [SURYA PRAKASH and MEENA, 2014] like anti-oxidant, anti-cancer [SALEEM *et al.*, 2002], anti-diabetic, anti-microbial etc.

Phyllanthus emblica, also known as Emblic, *Emblic myrobalan* or *Indian gooseberry* (Amla).

It belongs to the family *Phyllanthaceae*. These fruits are reputed to contain high amounts of ascorbic acid (vitamin C), ellagitannins such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%).

It also contains punicafolin, phyllanemblinin, gallic acid, ellagic acid, chebulinic acid [EKTA SINGH *et al.*, 2011], flavonoids like quercetin and kaempferol etc.

It used as a rasayana (rejuvenative) to promote longevity, and traditionally to enhance digestion, treat constipation, reduce fever, purify blood, reduce cough, alleviate asthma, strengthen the heart, benefit the eyes, stimulate hair growth, enliven the body, and enhance intellect.

It shows antitumor activity, anti-inflammatory activity [ASMAWI *et al.*, 1993], antimicrobial activity, cytoprotective activity, antioxidant activity [JUREE CHAROENTEERABOON *et al.*, 2010] etc.

Dimocarpus longan, commonly known as the Longan and belongs to the family Sapindaceae.

It is a tropical tree that produces edible fruit.

This fruit used as a traditional Chinese medicine for different treatments, such as soothing nerves, relieving insomnia and promoting blood metabolism.

Longan pulps are tasty and rich in nutritious ingredients.



The dried longan pulps are used as a tonic in traditional Chinese medicine to nurture heart and spleen, nourish blood, calm mind, add luster and beauty to the skin, and have therapeutic effects on heart palpitation, insomnia, amnesia, and anxiety.

Longan seeds have long been used as a folk medicine in China for treatment of pains, hernia, and skin diseases, acariasis, hernia, wound hemorrhages, eczema and scrofula *etc.*

This seeds have been found to be a rich source of poly phenolic compounds [SOONG and BARLOW, 2005] such as gallic acid, corilagin, chebulinic acid, chebulagic acid, ellagic acid flavonoids like quercetin *etc.*

Longan seeds [ATITA PANYATHEPA *et al.*, 2013] extract showed strong scavenging activities of free radicals, inhibition on the proliferation of human colorectal carcinoma cells, antifatigue effect, hypoglycemic effects *etc.*

Chebulinic acid ((2R)-2-[(3S)-3-carboxy-5,6,7-trihydroxy-1-oxo-3,4-dihydroisochromen-4-yl]butanedioic acid) is a phenolic compound [MANOSROI *et al.*, 2010] found in the fruits of *Terminalia chebula*, *Phyllanthus emblica* and seeds of *Dimocarpus longan* species *etc.*

It helps to remove toxins and unwanted fat from the body. It showed many pharmacological activities including inhibition of cancer cell growth like human leukemia K562 cells [Yi *et al.*, 2004], colon adenocarcinoma HT-29 cell lines, anti-neisseria gonorrhoeae activity, inhibiting the contractile responses of cardiovascular muscles activities *etc.*

The present paper Optimization of Physico-Chemical Parameters for the Extraction of Chebulinic acid (C₁₄H₁₂O₁₁) from fruits of *Terminalia chebula*, *Phyllanthus emblica* and seeds of *Dimocarpus longan* species.

Material and methods

Chemicals and reagents

Folin Denis reagent, Sodium carbonate (Na₂CO₃), Methanol, Ethanol, Ethyl acetate, Hexane, Distilled water.

Collection of Plant material

The dry fruits of *Terminalia chebula*, Amla and seeds of Longan collected from

local market in Visakhapatnam, Andhra Pradesh, India.

Processing of the Plant material

These fruits and seeds were cut into small pieces and powdered.

The total powder done in to different mesh sizes from 44 to 120.

The different size powders were stored in the air tight small covers.

Extract preparation

Weigh the different amounts of 7.2 gm of *Terminalia chebula* fruit, 8.0 gm of Amla fruit and 7.4 gm of Longan seeds of powders and add ethanol (50%) and methanol (80%), in different flasks and makeup this solution up to 50 mL.

Soak the solution for 1 day and 2 days respectively.

After the soaking time filtrate the solution by using Whatman No. 1 filter paper and heat the filtrate solution at 78°C and 65°C respectively.

So that the solvent which is taken in the glass wear is evaporated and make up this solution up to 25 mL with distilled water to this solution add 25 mL of hexane solvent [HARPREET WALIA *et al.*, 2011] mix the solution thoroughly.

Pour the entire mixture in the separating funnel by using glass funnel.

Incubate the solutions of ethanolic and methanolic extract for 1hr.

Determination of Chebulinic acid (CBA) by Colorimeter

By Folin-Denis Method: 1mL of ethanolic and methanolic extracts was withdrawn in a 10 mL volumetric flask separately.

To each flask 0.5mL of Folin Denis reagent [AVANI PATEL *et al.*, 2010; BUTNRIU *et al.*, 2011] and 1mL of Sodium carbonate were added and volume is made up to 10 mL with distilled water.

The mixtute was allowed to stand for 30 min at room temperature.

The absorbance of the reaction mixture was measured at 700 nm using colorimeter.

The Chebulinic acid was determined by using calibration curve.



Results and discussion

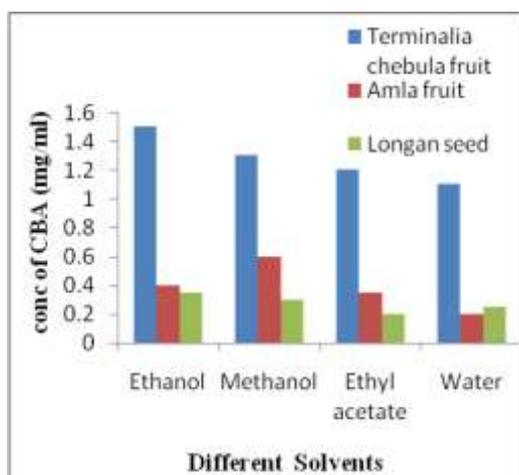
a) Effect of Different Solvents for Extraction of Chebulinic acid (CBA):

Different organic solvents such as methanol, ethanol, ethyl acetate and water were used to extract the optimum yield of chebulinic acid from these plants.

The solvent, ethanol of *Terminalia chebula* fruit and Longan seeds were shows best results of chebulinic acid and its concentrations were 1.5 mg/mL and 0.35 mg/mL respectively.

The solvent, methanol of Amla fruit was shows best results of chebulinic acid and it concentration was 0.6 mg/mL.

The results were shown in [figure 1](#).



[Figure 1](#). Effect of Different Solvents for Extraction of Chebulinic acid

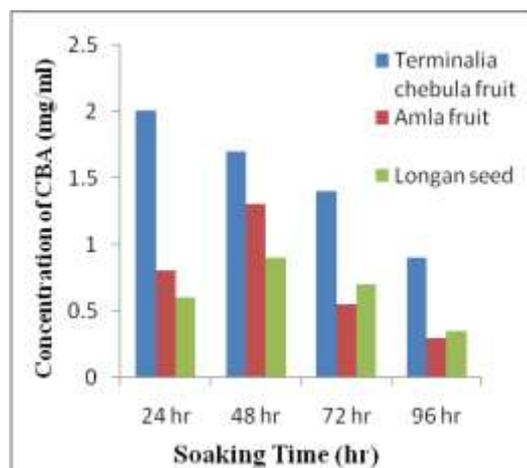
b) Effect of Soaking Time for Extraction of Chebulinic acid (CBA):

The samples were incubated under proper conditions at different time intervals viz., 1, 2, 3 and 4 days to investigate the influence on extraction of Chebulinic acid.

It was observed that *Terminalia chebula* fruit was shows first day was the best soaking time for the extraction of chebulinic acid and the concentration was 2.0 mg/mL.

Amla fruit and Longan seeds were shows second day was the best soaking time and its concentrations were 1.3 mg/mL and 0.9 mg/mL respectively.

The results were shown in [figure 2](#).



[Figure 2](#). Effect of Soaking Time for Extraction of Chebulinic acid

c) Effect of Extraction Time with hexane for extraction of Chebulinic acid (CBA):

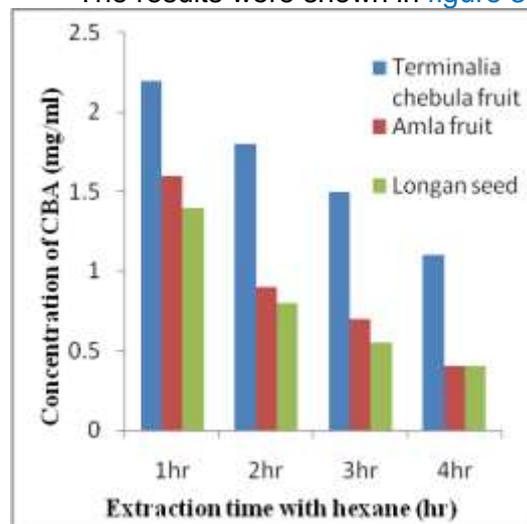
To investigate the influence of hexane on extraction of Chebulinic acid different time intervals were taken viz., 1, 2, 3 and 4 hr.

Solvent-Solvent extraction was done with hexane as one of the solvent.

The fruits of *Terminalia chebula*, Amla and Longan seeds were shows that optimum concentrations were observed at first hour extraction time with hexane for extraction of chebulinic acid.

The concentrations were 2.2 mg/mL, 1.6 mg/mL and 1.4 mg/mL respectively.

The results were shown in [figure 3](#).



[Figure 3](#). Effect of Extraction time with hexane for Extraction of Chebulinic acid



d) Effect of Different Particle size for the Extraction of Chebulinic acid (CBA):

Different particle size viz., 354, 328, 250, 205, 149 and 125 microns were used to find out the optimum concentrations of Chebulinic acid from these plants.

The present investigation suggests that the extraction of Chebulinic acid at different particle sizes indicates that the optimum particle size [ANIL D. MAHAJAN *et al.*, 2011; BUTNRIU and BOSTAN, 2011] was 125 microns of *Terminalia chebula*, Amla fruits and Longan seeds for extraction of Chebulinic acid. The optimum concentrations were 2.6 mg/mL, 2.0 mg/mL and 2.2 mg/mL.

The results were shown in figure 4.

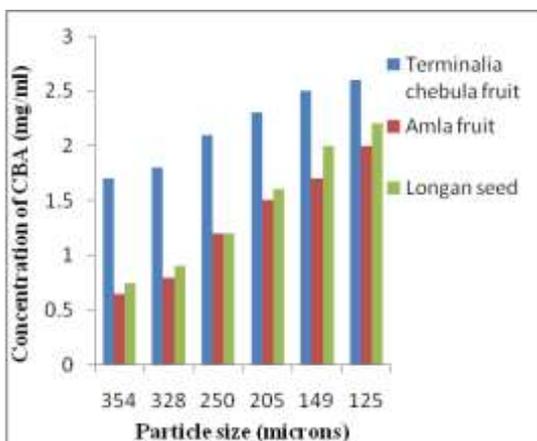


Figure 4. Effect of Different Particle size for the Extraction of Chebulinic acid

e) Effect of Different Solvent percentages for the Extraction of Chebulinic acid (CBA):

Percentage of the solvent is also plays a vital role for the extraction of components.

The study on different solvent (ethanol, methanol) percentages like 0%, 20%, 40%, 50%, 60%, 80% and 100% shows significant variations.

Figure 5 shows that the optimum solvent percentages were found to be at 50% ethanol of both *Terminalia chebula* fruit & Longan seeds and 80% methanol of Amla fruit for the extraction of chebulinic acid.

The optimum concentrations of 50% ethanol were 3.0 mg/mL & 2.6 mg/mL and 80% methanol was 2.3 mg/mL.

The results were shown in figure 5.

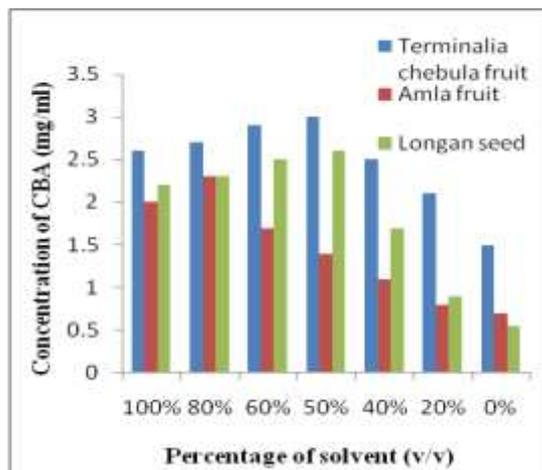


Figure 5. Effect of Different Solvent percentages for the Extraction of Chebulinic acid

f) Effect of Different volumes of Hexane for the Extraction of Chebulinic acid (CBA):

To determine the volume of hexane for the extraction of Chebulinic acid at the different volumes of hexane with solvent (ethanol, methanol) were considered such as 1:1, 1:2 and 2:1.

The optimum extraction of Chebulinic acid was achieved at 1:1 with ethanol as a solvent of *Terminalia chebula* fruit & Longan seeds and the optimum concentrations were 3.2 mg/mL and 2.8 mg/mL. The optimum extraction was also recorded at 1:1 with methanol of Amla and the concentration was 2.5 mg/mL. The observed results were shown figure 6.

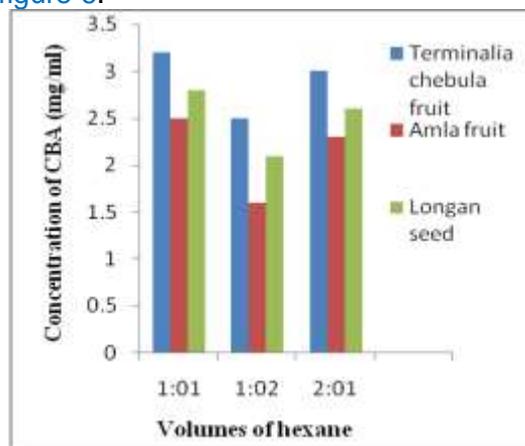


Figure 6. Effect of Different Volumes of Hexane for the Extraction of Chebulinic acid

g) Effect of pH for the Extraction of Chebulinic acid (CBA):



pH places a major role for the extraction of Chebulinic acid.

To optimize the pH for this process different pH extract samples viz., 4, 5, 6, 7 and 8. It was observed that the extraction of Chebulinic acid of *Terminalia chebula* was found to be optimum pH at 6.0 [LOKESWARI *et al.*, 2006; BOSTN *et al.*; 2013] and optimum concentration was 3.4 mg/mL.

The Amla fruit and Longan seeds shows optimum pH at 5.0 and optimum concentrations were found to be 2.7 mg/mL and 3.0 mg/mL.

The results were shown in figure 7.

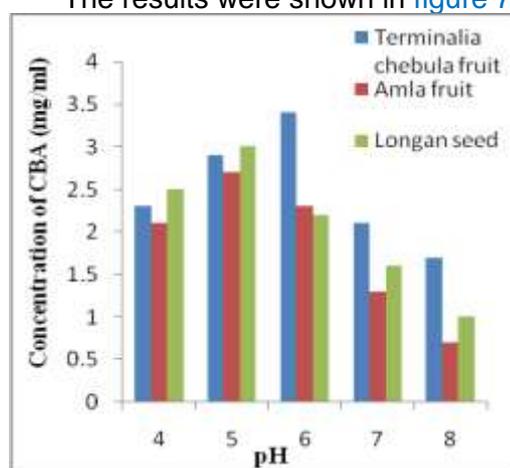


Figure 7. Effect of pH for the Extraction of Chebulinic acid (CBA)

h) Effect of Weight Dosages for the Extraction of Chebulinic acid (CBA):

The results were shown in figure 8.

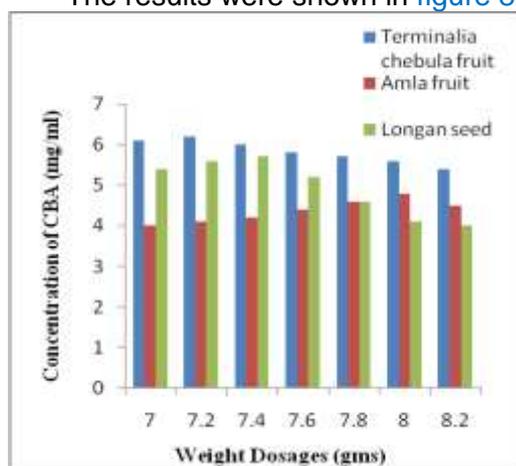


Figure 8. Effect of Weight Dosages for the Extraction of Chebulinic acid

Different weight dosages were used to find out the optimum concentrations of Chebulinic acid of these plants.

The present investigation suggests that the extraction of Chebulinic acid at different weight dosages indicates that the optimum weight dosages were 7.2 gm of *Terminalia chebula* fruit, 8.0 gm of Amla fruit and 7.4 gm of Longan seeds.

The optimum concentrations were 6.2 mg/mL, 4.8 mg/mL and 5.7 mg/mL.

Conclusion

Experiments were performed for the optimization of extraction of Chebulinic acid from medicinal herbs like dry fruits of *Terminalia chebula*, Amla and seeds of Longan fruit. Chebulinic acid estimation and optimize the physico-chemical parameters by using FD reagent method.

The parameters like effects of different solvents, soaking time, and extraction time with hexane, particle size, different solvent percentages, and different volumes of hexane with ethanol and methanol as solvents, pH and different weight dosages for the extraction of Chebulinic acid concentrations were observed from the experimental work.

The highest chebulinic acid concentration for optimized conditions of *Terminalia chebula* fruit, Amla fruit and Longan seeds were 6.2 mg/mL, 4.8 mg/mL and 5.7 mg/mL respectively.

References

1. Anil, D. Mahajan; Nandini, R. Pai; Development and validation of HPLC method for quantification of phytoconstituents in *Haritaki Churna*. *International journal of ChemTech research*, **2011**, 3(1): 329–336.
2. Asmawi, M.Z.; Kankaanranta, H.; Moilanen E.; Vapaatalo, H.; Anti-inflammatory activities of *Emblia officinalis* Gaertn leaf extracts. *Journal of Pharmacy and Pharmacology*, **1993**, 45(6): 581–4.
3. Atita, Panyathepa; Teera, Chewonarina; Khanittha, Taneyhillb; Usanee, Vinitketkumnuen; Antioxidant and anti-matrix metalloproteinases activities of dried longan (*Euphoria longana*) seed extract, *Journal of Science Asia*, **2013**, 39: 12–18.
4. Avani, Patel; Amit, Patel; Patel, Dr. N.M.; Estimation of Flavonoid,



- Polyphenolic Content and In-vitro Antioxidant Capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). *International Journal of Pharma Sciences and Research (IJPSR)*, **2010**, 1(1): 66–77.
5. Bostan, C.; Butnariu, M.; Butu, M.; Ortan, A.; Butu, A. Allelopathic effect of *Festuca rubra* on perennial grasses. *Romanian Biotechnological Letters*. **2013**, 18 (2), 8190–8196.
 6. Butnariu, M. Armon, E. Tonea R. Quantity determination of molybdenum from *Pisum sativum* plants and the influence of heavy metal to chemical elements accumulation. *Jurnal Lucrări Științifice-Zootehnie și Biotehnologii, Universitatea de Științe Agricole și Medicină Veterinară a Banatului Timișoara*, **2008**, 41(1), p. 735–743.
 7. Butnariu, M.; Bostan C. Antimicrobial and anti-inflammatory activities of the volatile oil compounds from *Tropaeolum majus* L. (Nasturtium). *African Journal of Biotechnology*, **2011**, 10 (31), 5900–5909.
 8. Ekta, Singh; Sheel, Sharma; Ashutosh, Pareek; Jaya, Dwivedi; Sachdev, Yadav; Swapnil Sharma. Phytochemistry, traditional uses and cancer chemopreventive activity of Amla (*Phyllanthus emblica*): The Sustainer. *Journal of Applied Pharmaceutical Science*, **2011**, 2(1); 176–183.
 9. Harpreet, Walia; Subodh, Kumar; Saroj, Arora; Comparative antioxidant analysis of hexane extracts of *Terminalia chebula* Retz. Prepared by maceration and sequential extraction method. *Journal of Medicinal Plants Research*. **2011**, 5(13): 2608–2616.
 10. Juree, Charoenteeraboon; Chatri, Ngamkitidechakul; Noppamas, Soonthornchareonnon; Kanjana, Jaijoy; Seewaboon, Sireeratawong; Songklanakarin. Antioxidant activities of the standardized water extract from fruit of *Phyllanthus emblica* Linn. *J. Sci. Technol*, **2010**, 32(6): 599–604.
 11. Karel D. Klika.; Ammar, Saleem; Jari, Sinkkonen; Marja, Kähkönen; Jyrki, Loponen; Petri Tähtinen; Kalevi, Pihlaja; The structural and conformational analyses and antioxidant activities of chebulinic acid and its thrice-hydrolyzed derivative, 2,4-chebuloyl- β -D-glucopyranoside, isolated from the fruit of *Terminalia chebula*. *Journal of ARKIVOC*, **2004**, 7: 83–105.
 12. Lokeswari, N.; Jayaraju, K.; Optimization of gallic acid production from *Terminalia chebula* by *Aspergillusniger*. *E-Journal of Chemistry*, **2006**, 4(2): 287–293.
 13. Manosroi, A; Jantrawut, P.; Akazawa, H.; Akihisa, T.; Manosroi, J.; Biological activities of phenolic compounds isolated from galls of *Terminalia chebula* Retz. (Combretaceae). *Natural products research Journal*, **2010**; 24(20): 1915–26.
 14. Saleem, A.; Husheem, M.; Harkonen, P.; Pihlaja, K.; Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz fruit. *Journal of Ethnopharmacology*, **2002**, 81(3): 327–336.
 15. Surya Prakash, D.V.; Meena, Vangalapati; A. Review on Chebulinic acid from medicinal herbs. *World Journal of Pharmaceutical Research*, **2014**, 3(6): 2127–2139.
 16. Yean Yean, Soong; Philip John, Barlow; Isolation and structure elucidation of phenolic compounds from longan (*Dimocarpus longan* Lour.) seed by high-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography A*, **2005**, 1085: 270–277.
 17. Yi, Z.C.; Wang, Z.; Li, H.X.; Liu, M.J.; Wu, R.C.; Wang, X.H.; Effects of chebulinic acid on differentiation of humanleukemia K562 cells. *Journal of Acta Pharmacological Sinica*, **2004**, 25(2): 231–238.

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