



DETERMINATION OF PARTITION COEFFICIENT FOR CHEBULINIC ACID EXTRACTION FROM VARIOUS EXTRACTS OF *TERMINALIA CHEBULA* SPECIES

D. V. SURYA PRAKASH, G. SATYENDRA DINESH^a, N. SREE SATYA,
M. SUMALATHA, S. VENKATESWARA KUMAR^b and
MEENA VANGALAPATI*

Centre of Biotechnology, Department of Chemical Engineering, AUCE (A), Andhra University,
VISAKHAPATNAM (A.P.) INDIA

^aDepartment of Biotechnology, Satyabhama University, CHENNAI (T.N.) INDIA

^bDepartment of Chemical Engineering, S. V. University, TIRUPATHI (A.P.) INDIA

ABSTRACT

Chebulinic acid is the main compound in the *Terminalia chebula* species. It acts as effective anti-bacterial, anti-fungal activities. The present study was for increasing the production of chebulinic acid from different extraction process. Among the extraction process (Batch, Soxhlet and Fermentation process) the highest chebulinic acid production was obtained from fermentation process. From the batch process the chebulinic acid concentration was observed to be 3.4 mg/mL at 60 min and the concentration was increased to 6.6 mg/mL at 75 min from soxhlet extraction. Similarly the chebulinic acid concentration was increased to 8.6 mg/mL at 192 hrs from fermentation process. The partition coefficient for fermentation, soxhlet and batch extraction were found to be 3.83, 2.0 and 0.64.

Key words: *Terminalia chebula*, Chebulinic acid, Fermented extract, Soxhlet extraction, Partition coefficient.

INTRODUCTION

Terminalia chebula is species of *Terminalia* and commonly called as Black myrobalan¹ and belongs to the family *Combretaceae*. This is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. It is used for the treatment of number of diseases like

* Author for correspondence; E-mail: meena_sekhar09@yahoo.co.in

cancer, paralysis, cardio vascular diseases, ulcers, leprosy, arthritis, gout, epilepsy etc. It has been reported as antioxidant², antidiabetic, antibacterial³, antiviral, antifungal, anticancerous, antiulcer, antimutagenic⁴, wound healing⁵ activities etc. It contains the triterpenes arjun glucoside, arjungenin and the chebulosides. Other constituents contains tannins up to 30%, chebulic acid⁶ 3-5%, chebulinic acid⁷ 30%, tannic acid 20-40%, ellagic acid⁸, 2,4-chebulyl- β -D-glucopyranose, gallic acid, ethyl gallate, punicalagin terflavin A, terchebin, some purgative of the nature of anthraquinone, flavonoids like luteolin, rutins and quercetin etc.⁹

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. It helps to remove toxins and unwanted fat from the body. Act as an effective anti-bacterial, anti-fungal, improves skin glow and complexion.

The Partition (P) or Distribution (D) coefficient is the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium.

$$P = [X_E] / [X_R] \text{ Where } [X_E] : \text{Extract phase and } [X_R] : \text{Raffinate phase.}$$

Hence these coefficients are a measure of the difference in solubility of the compound in these two phases. In medical practice, partition coefficients are useful for example in estimating distribution of drugs within the body. Pharmacology, consumer products, environmental, metallurgy and agrochemicals are applications of partition coefficient.

EXPERIMENTAL

Materials and methods

The dry fruits of *Terminalia chebula* were collected from the local market at Visakhapatnam. Clean the fruits and dried under sunlight for 1 day. The dried fruits were powdered and used as a raw material and stored in the air tight container. This powder was sieved by using different particle sizes ranging from 354 to 125 microns.

Preparation of extracts

In batch process, the amount of 2 g of powder and add ethanol (25%) in the flask and makeup this solution up to 50 mL. Soak the solution for 1 day. After the soaking time filtrate the solution by using Whatman No. 1 filter paper and heat the filtrate solution at 78⁰C. 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, mix the solution thoroughly. Pour the entire mixture in the separating funnel by using glass funnel. Incubate the solutions of ethanolic extract for 1 hr.

In soxhlet extraction¹⁰, the amount of 8 g of dried powder was taken in thimble and 50% (v/v) ethanol was taken as a solvent in the round bottom flask of the soxhlet extractor. After 24 hrs. the sample was collected and used as ethanolic extract. Then the ethanolic extract was purified with hexane in 1 : 1 ratio for two times in separated funnel and the sample was treated as hexane extract. It was incubated at 75 min.

The 50% (v/v) ethanolic extract was taken as sample for fermentation process after soxhlet extraction. 1.0 mL of 50% ethanolic extract was taken in flask. Add 20 mL of distilled water and 20 mL in a 20% of sucrose solution, the contents are maintained at pH = 4.0 and then autoclaved. After cooling add 2 mL of activated yeast suspension which was activated was inoculated into the clear liquid. They were kept for 192 hrs incubation at room temperature and centrifugation at 5000 rpm for 3 min. After centrifugation, collect the fermented sample (supernatant) of extract.

Determination of chebulinic acid

After incubation periods of each extract process, 1.0 mL of ethanolic extract was withdrawn in a 10 mL volumetric flask separately. To each flask 0.5 mL of Folin Denis reagent and 1 mL of Sodium carbonate were added and volume is made up to 10 mL with distilled water. The mixture was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 700 nm using colorimeter.

RESULTS AND DISCUSSION

Among the extract process, the fermentation process was shows highest chebulinic acid concentration and partition coefficient. From the batch process the chebulinic acid concentration was observed to be 3.4 mg/mL at 60 min and the concentration was increased to 6.6 mg/mL at 75 min from soxhlet extraction. Similarly the chebulinic acid concentration was increased to 8.6 mg/mL at 192 hrs. from fermentation process. After incubation periods, the partition coefficient was found to be 0.64 for the batch process, 2.0 for the soxhlet

extraction and 3.83 for the fermented extract. The results of the batch, soxhlet and fermented extraction process were shown in Figures.

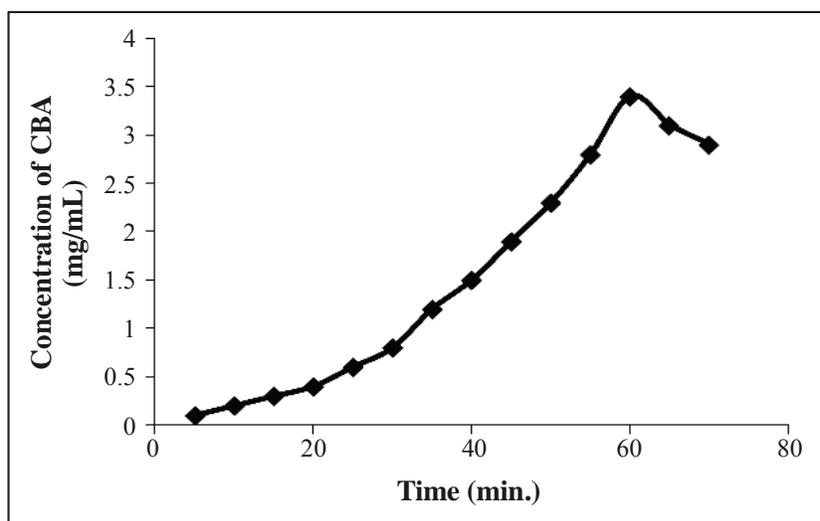


Fig. 1: Effect of incubation periods for Chebulinic acid concentrations of batch process

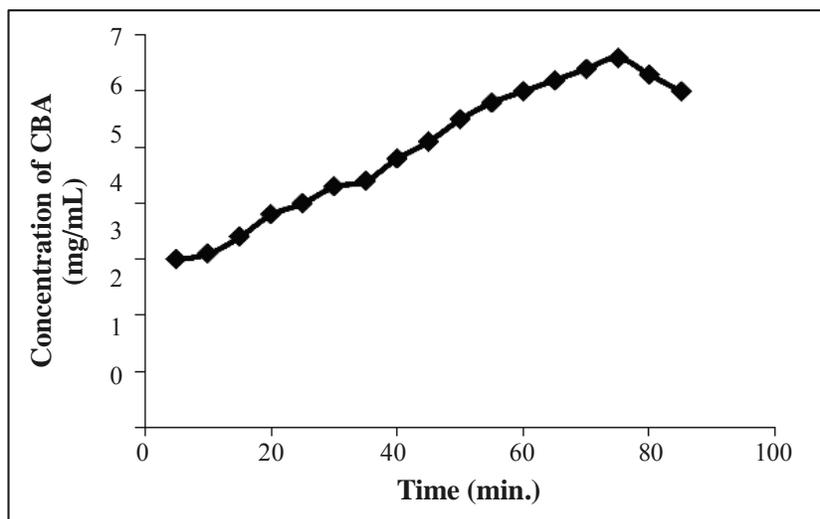


Fig. 2: Effect of incubation periods for Chebulinic acid concentrations of soxhlet extraction

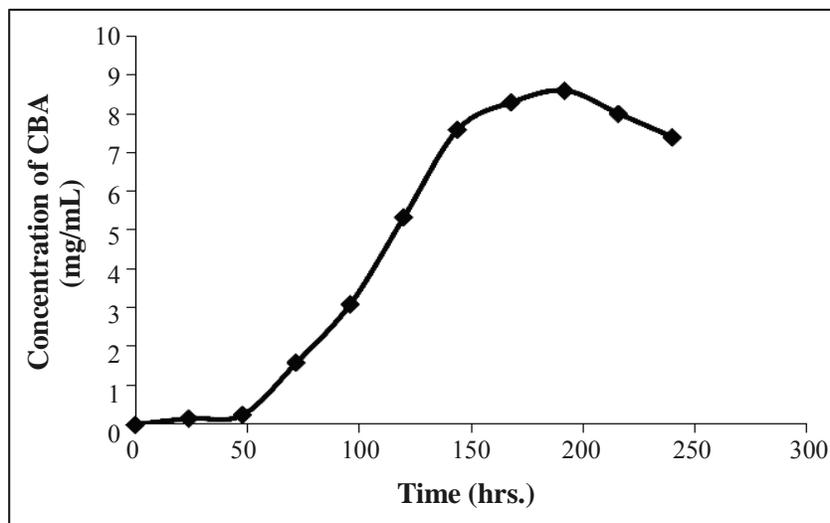


Fig. 3: Effect of incubation periods for Chebulinic acid concentrations of 50% ethanolic extract of fermentation

CONCLUSION

Chebulinic acid is main component in *Terminalia chebula* species. Act as an effective anti bacterial, anti-fungal activities. The present study was production of chebulinic acid from batch, soxhlet and fermentation process. Among the extract process, the fermentation process was shows highest chebulinic acid concentration was observed to be 8.6 mg/mL at 192 hrs, the soxhlet extraction process was 6.6 mg/mL at 75 min and batch extraction process was 3.4 mg/mL at 60 min. The partition coefficient for fermentation, soxhlet and batch extraction process were found to be 3.83, 2.0 and 0.64.

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