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Optimisation of Physico-Chemical Parameters for the Extraction of Chebulinic Acid from the Composition of 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 optimisation 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 as solvent and pH for the extraction of chebulinic acid from the composition of Medicinal herbs. The Chebulinic acid concentration for optimised conditions was 7.7 mg/ml.

Key words: Chebulinic acid, Terminalia chebula fruit, Amla fruit, Longan seed, Ethanol.

INTRODUCTION

Chebulinic acid is a phenolic compound [1] commonly found in the fruits of Terminalia chebula, leaves and fruits of Phyllanthus emblica and seeds of Dimocarpus longan species, which has many potential uses in medicine. It is also found in the leaves of Dendrophthoe falcata, Lumnttzera racemosa, Terminalia macroptera species. 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 leukemia K562 cells [4] colon adenocarcinoma HT-29 cell lines [5], anti-neisseria gonorrhoeae activity, anti-hypertensive [6], inhibiting the contractile responses of cardiovascular muscles[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]. The present paper Optimization of Physico-Chemical Parameters for the Extraction of Chebulinic acid from the composition of fruits of Terminalia chebula, Phyllanthus emblica and seeds of Dimocarpus longan species.

MATERIAL AND METHODS

2.1 Chemicals and Reagents

Folin-Denis reagent, sodium carbonate (Na_2CO_3), methanol, ethanol, ethyl acetate, hexane and distilled water.

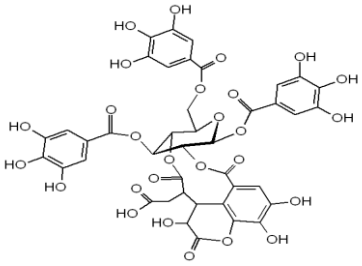

Fig. 1: Molecular structure of chebulinic acid

2.2 Collection of Plant Material

The dry fruits of Terminalia chebula, Amla and seeds of longan collected from local market in Visakhapatnam, Andhra Pradesh, India.

2.3 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 containers.

2.4 Extract Preparation

The pulverized powders of the 3g of Terminalia chebula fruit, 1g of Amla fruit and 2g of Longan seeds are mixed in the flask and add ethanol (80%) solvent and makeup this solution up to 50 ml. Soak the solution for 1 day. After soaking, filtrate the solution by using Whatman No.1 filter paper and heat the filtrate solution at 78°C. So that the solvent which is taken in the glass ware is evaporated and make up this solution up to 25 ml with distilled water to this solution add 25 ml of hexane solvent [10], and mix the solution thoroughly. Pour the entire mixture in the separating funnel by using glass funnel. Incubate the solutions of ethanolic extract for 1hr.

2.5 Determination of Chebulinic acid by Colorimeter

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By Folin-Denis Method:
1ml of ethanolic extract was withdrawn in a 10 ml volumetric flask. 0.5ml of FD reagent [11] and 1ml of Sodium carbonate were added and volume is made up to 10ml with distilled water. The mixture kept 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

3.1 Effect of Different Solvents for Extraction of Chebulinic acid:

Different organic solvents such as methanol, ethanol, ethyl acetate and water were used to extract the optimum yield of chebulinic acid from the composition of medicinal herbs. The solvent, ethanol was showed best result of chebulinic acid and its concentration was 5.4mg/ml. The results were shown in Figure.2.

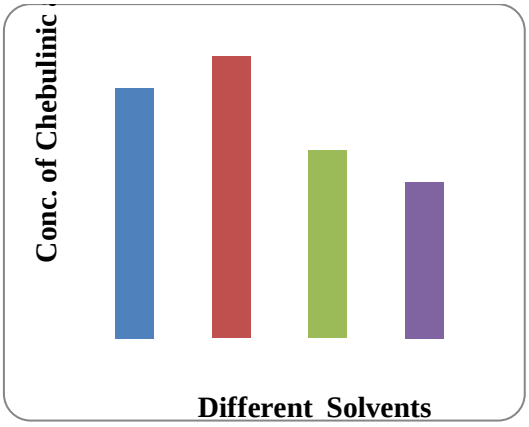

Fig 2: Effect of Different Solvents for Extraction of Chebulinic acid

3.2 Effect of Soaking Time for Extraction of Chebulinic acid:

The samples were incubated under proper conditions at different time intervals viz., 12, 24, 36 and 48 hr to investigate the influence on extraction of chebulinic acid. It was observed that first day was the best soaking time and the concentration was 6.0mg/ml. The results were shown in Figure.3.

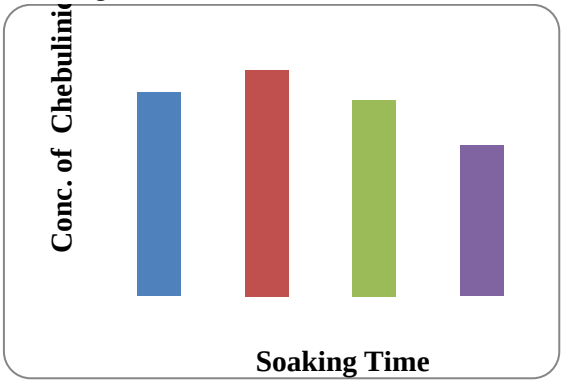

Fig 3: Effect of Soaking Time for Extraction of Chebulinic acid

3.3 Effect of Extraction Time with Hexane for Extraction of Chebulinic acid:

To investigate the influence of hexane on extraction of chebulinic acid different time intervals were taken viz., 30, 60, 90 and 120 min. Solvent-solvent extraction was done with hexane as one of the solvent. The optimum concentration was observed at the first hour of the extraction time with hexane for extraction of chebulinic acid. The concentration was 6.3mg/ml. The results were shown in Figure.4.

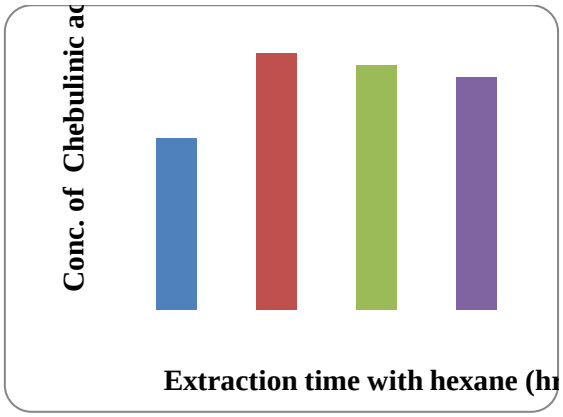

Fig 4: Effect of Extraction Time with Hexane for Extraction of Chebulinic acid

3.4 Effect of Different Particle Size for the Extraction of Chebulinic acid:

Different particle size viz., 354, 328, 250, 205, 149 and 125 microns were used to find out the optimum concentrations of chebulinic acid. The present investigation suggests that the extraction of chebulinic acid at different particle sizes indicates that the optimum particle size [12] was 125 microns. The optimum concentration was 6.8 mg/ml. The results were shown in Figure.5.

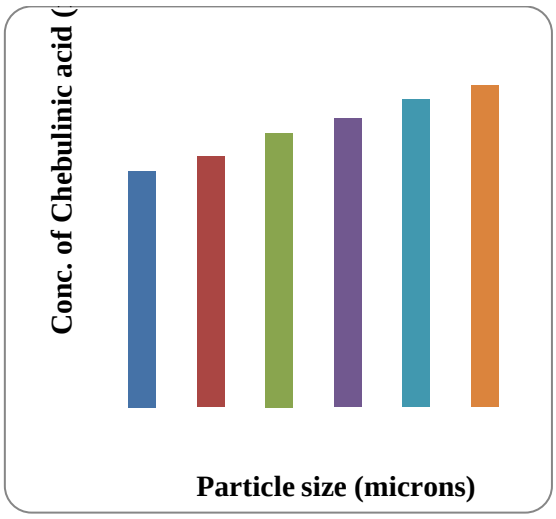

Fig 5: Effect of Different Particle Size for the Extraction of Chebulinic acid

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3.5 Effect of Different Solvent Percentages for the Extraction of Chebulinic acid:

Percentage of the solvent is also plays a vital role for the extraction of components. The study on different solvent (ethanol) percentages like 0%, 20%, 40%, 50%, 60%, 80% and 100% shows significant variations. Figure 5 shows that an optimum solvent percentage was found to be at 80% ethanol for the extraction of chebulinic acid. The optimum concentration of 80% ethanol was 7.2mg/ml. The results were shown in Figure 6.

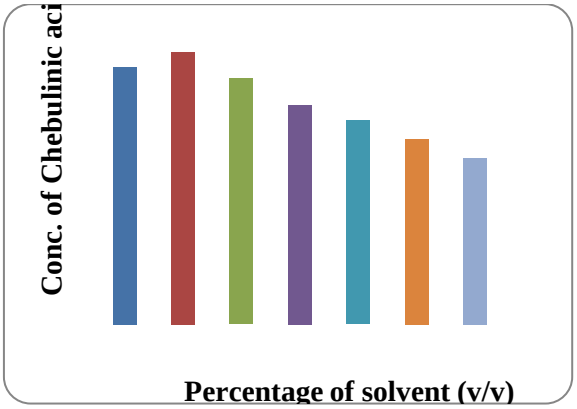

Fig 6: Effect of Different Solvent Percentages for the Extraction of Chebulinic acid

3.6 Effect of Different Volumes of Hexane for the Extraction of Chebulinic acid:

To determine the volume of hexane for the extraction of chebulinic acid, different volumes of hexane with solvent (ethanol) 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 and the optimum concentration was 7.5mg/ml. The observed results were shown Figure 7.

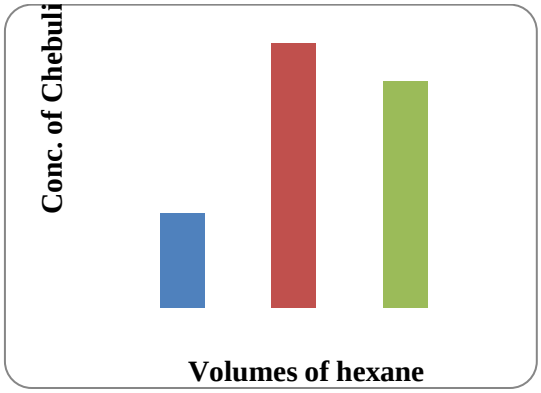

Fig 7: Effect of Different Volumes of Hexane for the Extraction of Chebulinic acid

3.7 Effect of pH for the Extraction of Chebulinic acid:

pH places a major role for the extraction of chebulinic acid. To optimize the pH for this process different pH extract samples viz., 5, 6, 7, 8 and 9 were considered. It was observed that the extraction of chebulinic acid was found to be optimum pH at 6 [13]. and optimum concentration was 7.7mg/ml. The results were shown in Figure.8.

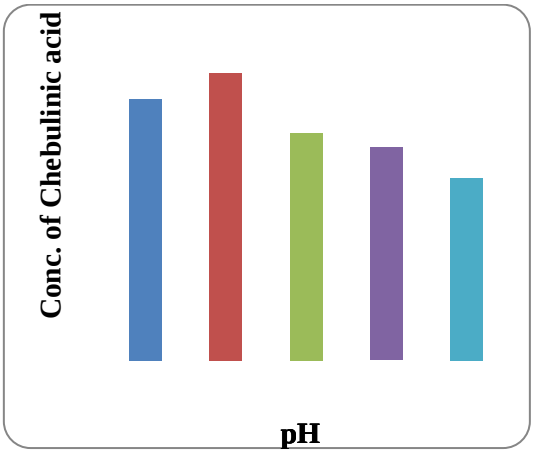

Fig 8: Effect of pH for the Extraction of Chebulinic acid

CONCLUSION

Experiments were performed for the optimisation of extraction of chebulinic acid from composition of medicinal herbs like dry fruits of Terminalia chebula, Amla and seeds of Longan fruit. Chebulinic acid estimation and optimise the physico-chemical parameters by using FD reagent method. The parameters like effects of different solvents, soaking time, extraction time with hexane, particle size, different solvent percentages, different volumes of hexane with ethanol as solvent and pH for the extraction of chebulinic acid concentrations were observed from the experimental work. The highest chebulinic acid concentration for optimized conditionwas 7.7 mg/ml.

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