Optimization of Physico-Chemical Parameters for the Extraction of Quercetin from Medicinal Herbs

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Abstract—Quercetin is a flavonoid widely distributed in nature. It found in the fruits of Terminalia chebula (Haritaki), Phyllanthus emblica (Amla) and seeds of Dimocarpus longan (Longan) species etc. It showed many pharmacological activities anti-cancer activity, anti-oxidant activity and anti-inflammatory activities 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 Quercetin from Medicinal herbs. The highest Quercetin concentration for optimized conditions of Terminalia chebula fruit, Amla fruit and Longan seeds were 33.0µg/ml, 26.0µg/ml and 28.0µg/ml.

Index Terms—chebulinic acid, Terminalia chebula fruit, amla fruit, longan seed, aluminium chloride method.

I. INTRODUCTION

Terminalia chebula species commonly called as Black myrobalan, Ink tree or Chebulic myrobalan. It is belongs to the family combrteaceae. 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% [1], chebulinic acid 30%, tannic acid 20-40%, ellagic acid, 2,4-chebulyi–β-D-glucopyranose, gallic acid, ethyl gallate, flavonoids like rutin, quercetin [2] and luteolin etc. It shows several pharmacological activities like anti-oxidant [3], anti-cancer, anti-diabetic, anti-microbial etc.

Phyllanthus emblica, also known as Emblic, Emblic myrobalan or Indian gooseberry (Amla). It is belongs to the family Phyllanthaceae. These fruits fruits are reputed to contain high amounts of ascorbic acid (vitamin C), ellagitannins such as embelicin A (37%), embelican B (33%), punigluconin (12%) and pedunculagin (14%). It also contains punicaferolin, phyllanemblinin, gallic acid, ellagic acid, chebulinic acid, flavonoids like quercetin [4] and kaempferol etc. It used as a rasayana (rejuvenative) to promote longevity, and traditionally to enhance digestion, treatconstipation, reduce fever, purify the 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 [5], antimicrobial activity, cytoprotective activity, antioxidant activity 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, acarasis, hernia, wound hemorrhages, eczema and scrofula etc. This seeds have been found to be a rich source of poly phenolic compounds such as gallic acid, corilagin, chebulinic acid, chebulagic acid, ellagic acid flavonoids like quercetin [6] etc. Longan seeds extract showed strong scavenging activities of free radicals [7], inhibition on the proliferation of human colorectal carcinoma cells, antifatigue effect, hypoglycemic effects etc.

Quercetin is a flavonoid widely distributed in nature. Foods rich in quercetin include black and green tea, apples, onion, red grapes, citrus fruit, tomato, broccoli and other leafy green vegetables etc. It is water soluble polyphenolic compound, which is extremely common and wide spread in the plant kingdom as their glycosides. Quercetin is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. It shows pharmacological activities like anti-oxidant [8], anti-cancer [9], anti-viral and anti-inflammatory activities [10] etc. Quercetin can be measured using Aluminium chloride colorimetric assay method. The present paper Optimization of Physico-Chemical Parameters for the Extraction of Quercetin from fruits of Terminalia chebula, Phyllanthus emblica (Amla) and seeds of Dimocarpus longan species.

II. MATERIAL AND METHODS

A. Chemicals and Reagents

Aluminium chloride, Potassium acetate, Methanol, Ethanol, Ethyl acetate, Hexane, Distilled water.
B. Collection of Plant Material

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

C. 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.

D. Extract Preparation

Weigh the different amounts of 7.4g of *Terminalia chebula* fruit, 7.4g of Amla fruit and 7.0g of Longan seeds of powders and add methanol (60%) and ethanol (80%) in different flasks and makeup this solution up to 50 ml. Soak the solution for 2 days. 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 [11], 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.

E. Determination of Quercetin by Colorimeter

Aluminum chloride colorimetric assay method: 0.5ml of ethanolic and methanolic extracts taken in each test tube and 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water were added. The mixture was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm using colorimeter. The Quercetin was determined by using calibration curve.

III. RESULTS AND DISCUSSION

A. Effect of Different Solvents for Extraction

Different organic solvents such as methanol, ethanol, ethyl acetate and water were used to extract the optimum yield of Quercetin from these plants. The solvent, methanol of *Terminalia chebula* and Amla fruit were shows best results of Quercetin and its concentrations were 8.5µg/ml and 5.5µg/ml respectively.

B. Effect of Soaking Time for Extraction of Quercetin

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 Quercetin. It was observed that *Terminalia chebula*, Amla fruits and Longan seeds were shows second day was the best soaking time for the extraction of Quercetin and the concentrations were 11.5µg/ml, 9.0µg/ml and 8.5µg/ml respectively. The results were shown in Fig. 2.

C. Effect of Extraction Time with Hexane for Extraction of Quercetin

To investigate the influence of hexane on extraction of Quercetin 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 Quercetin. The concentrations were 13.0µg/ml, 10.5µg/ml and 9.0µg/ml respectively. The results were shown in Fig. 3.

D. Effect of Different Particle size for the Extraction of Quercetin

Different particle size viz., 354, 328, 250, 205, 149 and 125 microns were used to find out the optimum
concentrations of Quercetin from these plant species. The present investigation suggests that the extraction of Quercetin at different particle sizes indicates that the optimum particle size [12] was 125 microns of Terminalia chebula, Amla fruits and Longan seeds. The optimum concentrations were 15.5µg/ml, 13.5µg/ml and 15.0µg/ml. The results were shown in Fig. 4.

Figure 4. Effect of different particle size for the extraction of quercetin

E. Effect of Different Solvent Percentages for the Extraction of Quercetin

Percentage of the solvent 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. Fig. 5 shows that optimum solvent percentages were found to be at 60% methanol of both Terminalia chebula & Amla fruit and 80% ethanol of Longan seeds for the extraction of Quercetin. The optimum concentrations of 60% methanol were 18.5µg/ml & 15.5µg/ml and 80% ethanol was 16.5µg/ml. The results were shown in Fig. 5.

Figure 5. Effect of different solvent percentages for the extraction of quercetin

F. Effect of Different Volumes of Hexane for the Extraction of Quercetin

To determine the volume of hexane for the extraction of Quercetin 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 Quercetin was achieved at 1:1 with methanol as a solvent of Terminalia chebula fruit and Amla fruit and the optimum concentrations were 19.5µg/ml, 16.5µg/ml. The optimum extraction was also recorded at 1:1 with ethanol of Longan seeds and the concentration was 17.5µg/ml. The observed results were shown Fig. 6.

Figure 6. Effect of different volumes of hexane for the extraction of quercetin

G. Effect of pH for the Extraction of Quercetin

pH places a major role for the extraction of Quercetin. 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 Quercetin of Terminalia chebula was found to be optimum pH at 5.0 and optimum concentration was 20.0µg/ml. The Amla fruit and Longan seeds shows optimum pH at 6.0 and optimum concentrations were found to be 17.0µg/ml and 18.5µg/ml. The results were shown in Fig. 7.

Figure 7. Effect of pH for the Extraction of quercetin

H. Effect of Weight Dosages for the Extraction of Quercetin

Different weight dosages were used to find out the optimum concentrations of Quercetin of these plants. The present investigation suggests that the extraction of Quercetin at different weight dosages indicates that the optimum weight dosages were 7.4 gm of Terminalia chebula fruit, 7.4 gm of Amla fruit and 7.0 gm of Longan seeds. The optimum concentrations were 33.0µg/ml, 26.0µg/ml and 28.0µg/ml respectively. The results were shown in Fig. 8.


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IV. CONCLUSION

Experiments were performed for the optimization of extraction of Quercetin from medicinal herbs like dry fruits of Terminalia chebula, Amla and seeds of Longan fruit. Quercetin estimation and optimize the physico-chemical parameters by using Aluminum chloride colorimetric assay 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 and methanol as solvents, pH and different weight dosages for the extraction of Quercetin concentrations were observed from the experimental work. The highest Quercetin concentration for optimized conditions of Terminalia chebula fruit, Amla fruit and Longan seeds were 33.0µg/ml, 26.0µg/ml and 28.0µg/ml respectively.

REFERENCES


