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A Process Development FOR Extraction of Marker Compound from EMBLICA OFFICINALIS FRUIT

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Abstract

The present study is done to examine and optimize the process of enzyme-assisted extraction of Gallic acid from the dried fruit of Emblica officinalis. The aqueous extraction yielded 12.09% of Gallic acid. A selective extraction process after the treatment with enzymes is proposed by using 30% (v/v) methanol which releases up to 15.68% of the Gallic acid, present in the dried fruit. The optimal conditions were as follows: pH value was 4.5, concentration of cellulase solution was 2.5 mg/mL, incubation time was 8 h, incubation temperature was 50 ºC and solid:solvent ratio was 1:8 . Enzyme-assisted extraction was proven to be highly efficient in the process development for extraction of marker compounds from herbal drugs and could be used in making enriched herbal extracts of product on a large scale.

Keywords: Emblica officinalis, Total Gallic acid, enzymatic extraction

Introduction

Emblica oficinalis is effective in the treatment of amlapitta viz.., peptic ulcer[1-2]. The fruits exhibit hypolipidaemic and antiatherosclerotic efftcts in the rabbits and rats[3-4]. The extract of amla also has antimicrobial properties[5-6]. Amla is an antioxidant with free radical scavenging properties[7]. Hepatoprotective[8], adaptogenic[9], antimutaginic[10], cytoprotective and antitumor[11] antifungal[12], were also exhibited by Amla. The Gallic acid is the basis for the quality control of *Amla* and other plant-derived drugs from the herb.

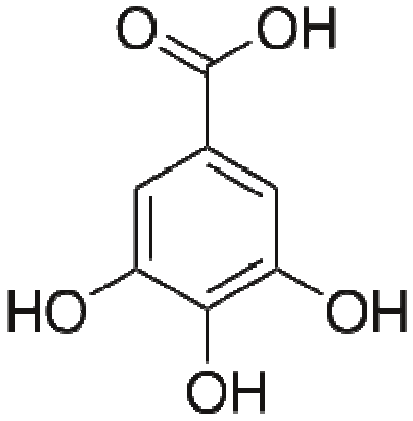

Fig. 1. Chemical structure of Gallic acid.

Although many reports about extracting Gallic acid by using different extraction methods from different plants have been published [13], some disadvantages still exist such as large organic solvent consumption, and low efficiency. Therefore, high efficient extraction method of Gallic acid represents a hot spot in *Emblica officinalis* research. Enzyme-assisted extraction is a method applied to the study of secondary metabolites releasing from biogenic materials. It possess the advantages of environmental- friendship, high efficiency and easy operation process. It has been represented as an alternative way for extracting marker compound from the herbal drug. Hydrolytic enzymes including cellulase, beta-glucosidase and pectinase, which are commonly used in extraction [14-16], can interact on cell wall, break down its structural integrity so as to increase the releasing of Gallic acid notably.

The main aim of the present study is to examine and optimize the process of enzyme-assisted extraction of Gallic acid from the Fruits of *Emblica officinalis*. For this purpose, the selection of enzyme type, pH and the concertration of enzyme solution, incubation time and temprature were studied, in order to obtain high yields of above natural products economically and environmental friendly.

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Material and Methods

Plant material

The dry Amla was collected and ground into fine powder using a high-speed blender. The dry, ground Amla was packed in a plastic bag, sealed and kept in the refrigerator (5°C) until used.

Chemicals and reagents

Gallic acid, and Cellulase were provided by Radiant Research Pvt. Ltd as gift sample. Methanol of analytical grade were purchased from Rankem Ltd. and double-distilled water was used in all experiments

Enzyme-assisted extraction and pretreatment

Cellulase was quantified accurately and dispersed in deionized water to obtain enzyme solutions of certain concentrations (0.25-4 mg/mL). 100 g dry powder was added to the enzymatic solution and adjusted to certain pH (3.5-7.0) with 0.1 M HCl solution and shaken on a flat-bed orbital shaker for a period of time (1-10 hr) at certain temperature (30-55ºC). After the treatment fulfilled, the extract was filtered through Whatmann filter paper no 1. Filtrate collected was concentrated in vacuo (55ºC) in a rotary evaporator and analyzed by spectrophotometer. All the experiments were performed in triplicate.

Quantification of Gallic acid

Quantitative determination of total Gallic acid content in each sample of *Amla* was performed by the described method [17]. Gallic acid content was calculated using a area under standard curve. Analysis of each sample was done in triplicate. For preparation of standard solution, standard Gallic acid (2.00 mg) was accurately weighed and transferred to a 5-ml volumetric flask. Methanol was added and adjusted to a final concentration of 400 □g/ml. From this solution, concentrations of 0.8, 1.6, 2.0, 2.4 and 3.2 □g/ml were prepared and used for preparation of the calibration curve. For preparation of sample solution from Amla, the extract (300.00 mg) of each sample was separately transferred to a 10-ml volumetric flask. One milliliter of the clear supernatant liquid was transferred and diluted with methanol to 25 ml volume. This solution (1 ml) was then transferred to a 50-ml volumetric flask, and diluted to volume with methanol and % Gallic acid was calculated.

Results and Discussion

Cellulase catalyzes the breakdown of cellulose into glucose, cellobiose and higher glucose polymers; therefore, it is used to extract Gallic acid from Amla Fruit.

Effect of pH value of enzyme solution

It has been reported that the activity of cellulase can be influenced by pH very much, and it is believed that it works better with pH<7 [18]. The effect of pH was studied in this experiment in order to pick out the proper pH value which would make the cellulase work best. Fig.2 shows the effect of pH on the extraction yields of the Gallic acid. It can be observed that the yields of Gallic acid varied unregularly with different pH value. The yields of Gallic acid achieved the maximum at pH 4.5.

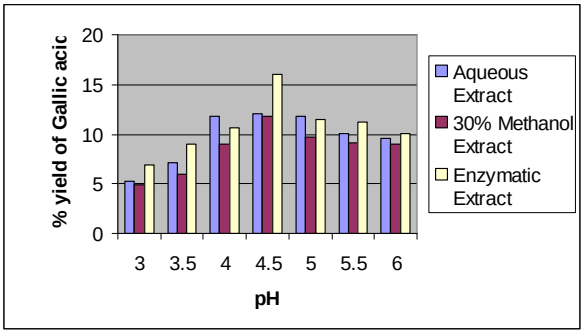

Fig. 2. Effect of pH on the yield of Gallic acid

Effect of enzyme concentration

The effect of concentration of cellulase on the extraction yields of Gallic acid was studied and the results are shown in Fig. 3. According to the results, it is obvious that with the increasing of cellulase concentration, the yields of these Gallic acid increased gradually until 2.5 mg/mL. Comparing with the yields of Gallic acid at the concenrtation of 2.5 mg/mL, 4.0 mg/mL did not show distinct advantage. Considering the economic influence, 2.5 mg/mL was selected for the pretreatment of the extraction process.

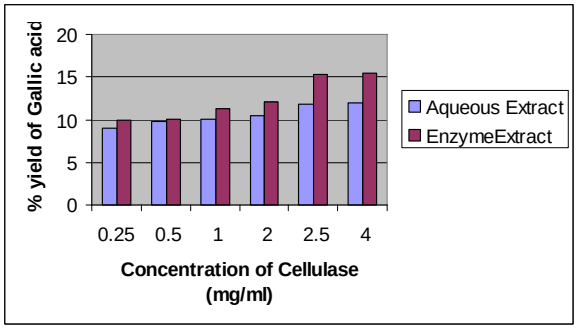

Fig. 3. Effect of conc. of Cellulase on yield of Gallic acid

Effect of incubation time

Fig. 4 showed the results of the effect of cellulase incubation time on the extraction yields of Gallic acid. The yields of these Gallic acid increased

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notablely along with the extending of incubation time. The yields of Gallic acid reached the peak (15.68 mg/g) at 8 h. And the yields began to decrease in additional time. Thus, 8 h was considered to be enough for cellulase to catalyze the hydrolysis of cell wall in Fruit of *Emblica officinalis*.

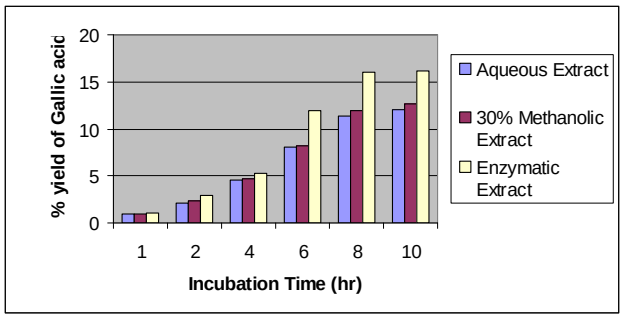

Fig. 4. Effect of Incubation time on the yield of Gallic acid

Solid Solvent Ratio

Different solid: solvent ratios ranging from 1:2 to 1: 12 were studied and the optimum ratio for the extraction of Gallic acid was found to be 1:8 g/ml (Fig 5).

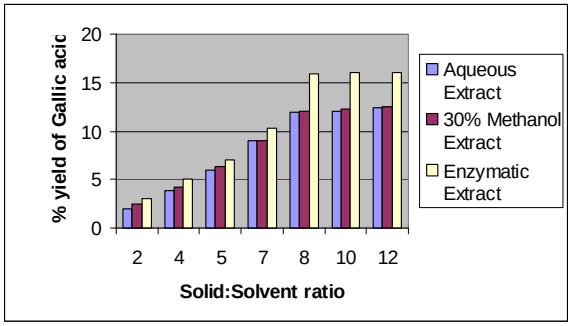

Fig. 5. Effect of Solid:Solvent ratio on the yield of Gallic acid

Effect of temperature on enzyme activity

The study of the thermal effect on the extraction yields was also carried out in this work. The results are presented in Fig.6. The yields of Gallic acid varied with the change in temperature. With the increase in temperature, the yields of Gallic acid increased gradually until 50 ºC. The yields of Gallic acid increased upto 15.68 mg/ml. Therefore, 50 ºC was chosen for cellulase incubation temperature in this assay.

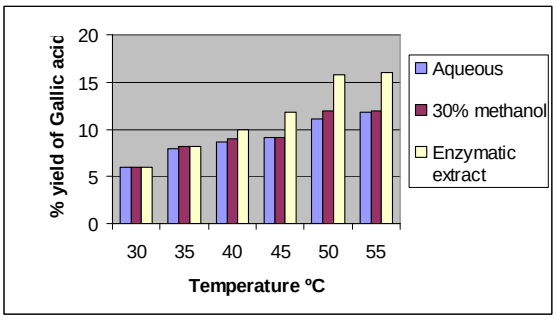

Fig. 6. Effect of Temperature on the yield of Gallic acid

Statistical analysis

All results were subjected to statistical analyses. Mean values of all data were obtained from triplicate experiment.

Conclusion

Enzyme–assisted extraction Gallic acid, from Fruits of *Emblica officinalis* was carried out in present study. The effect of hydrolytic enzyme was studied and it was proved that cellulose at a concentration of 2.5mg/ml to be most effective for extracting Gallic acid from Fruit of *Emblica officinalis*. As per the economic effect, cellulase was chosen for the treatment of the Fruit. The extraction conditions including pH and the concentration of cellulase solution, solid:solvent ratio, incubation time and incubation temperature were optimized. Results showed that all these factors were important for the extraction of Gallic acid. The optimal conditions were as following: pH value was 4.5, concentration of cellulase solution was 2.5 mg/mL, incubation time was 8 h, incubation temperature was 50 ºC and solid:solvent ratio was 1:8 . Pass through the treatment by cellulase, the contents of Gallic acid were 1.23–fold, of those in the control which showed that cellulase destroy the structures of plant cells and results in higher extraction yields of Gallic acid. Enzyme-assisted extraction may provide a feasible way for the extraction of Gallic acid from *Emblica officinalis* and other species of Amla, it has the advantages of environment friendship, lower cost, easy operation and higher efficiency, and it is promising for industry application broadly.

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