Research Article

Improved Yield Of 3’, 5-Dihydroxyflavone-7-O-B-D-Galacturonide-4’-O-B-D-Glucopyranoside; A Known Rifampicin Bioavailability Enhancer From Cuminum Cuminum Using Microwave Assisted Extraction And Flash Chromatographic Separation

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ABSTRACT

3’, 5-dihydroxyflavone-7-O-β-D-galacturonide-4’-O-β-D-glucopyranoside (CC-I) is a flavonoid glycoside from Cuminum cuminum. After co-administration, CC-I improves the poor oral bioavailability of rifampicin in rats. Existing conventional hot percolation extraction followed by activity guided open column chromatographic separation method for CC-I isolation have disadvantages like high extraction period and low recovery; hence an efficient method is needed. Microwave assisted extraction (MAE) followed by flash chromatographic separation (FCS) methodology was optimized for the isolation of CC-I from C. cuminum seeds. The CC-I content of samples was determined by HPLC. Conventional extraction of C. cuminum seeds yielded 93.9 ± 4.6 mg CC-I in approximately 840 min. MAE-FCS technique resulted in 178.4 ± 16.9 mg CC-I recovery in an extraction period of 49 min. The MAE-FCS enhanced the CC-I yield by 89.98% and reduced the extraction period effectively. On the basis of yield and extraction time, MAE-FCS technique was found to be more efficient for the isolation of therapeutically important CC-I from C. cuminum.

Keywords: Flavonoid glycoside, Cuminum cuminum, Bioavailability, Microwave assisted extraction, Flash chromatographic separation

INTRODUCTION

The Cuminum cuminum (Umbelliferae) seeds, commonly known as white jeera have been used in ancient Indian medicinal system of Ayurveda since long1. The experimental studies on botanical products from C. cuminum have shown diverse pharmacological actions viz. smooth muscle relaxant2, anti-bacterial3, hypoglycemic4, anticarcinogenic5 and antioxidant6. The C. cuminum seeds are reported to contain a bewildering array of compounds belonging to variety of chemical class including glycosides and its derivatives7-10. Recently one of the flavonoid glycosides from C. cuminum seeds (Figure 1) has been found to enhance the oral bioavailability of first line anti-tubercular drug rifampicin11,12. The flavonoid glycoside, 3’, 5-dihydroxyflavone-7-O-β-D-galacturonide-4’-O-β-D-glucopyranoside [CC-I] from C. cuminum seeds has enhanced the oral bioavailability of rifampicin by 53% when co-administered.

The report demonstrated the CC-I isolation by hot percolation extraction followed by activity guided open column chromatographic separation wherein total solvent and the isolation period required was high12,13. After reviewing the wide scope for CC-I in current medical need as an oral bioavailability enhancer, the study was designed with an objective to develop and optimize advanced extraction and separation methodology demonstrating high yield in shortest possible time. The present work is the first report of CC-I isolation by microwave assisted extraction (MAE) - flash chromatographic separation (FCS) and its comparison with the hot percolation extraction-open column chromatographic separation on the basis of total yield and the required extraction time.

MATERIALS AND METHODS

Plant material, chemicals and standards: Seeds of C. cuminum were collected from the fields of Unjha area of Gujarat state, India. The sample was identified and authenticated by a senior taxonomist Dr. B.K. Kapahi. The voucher specimen (RJM 0200/P06) was preserved in herbarium of Indian Institute of Integrative Medicine, Jammu & Kashmir state, India. The CC-I standard was obtained from Natural Product Chemistry Division of Indian Institute of Integrative Medicine, Jammu & Kashmir, India. HPLC grade

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acetonitrile and acetic acid was purchased from Rankem (RFCL, New Delhi, India). HPLC grade water was obtained from Milli-Q water purification system (Millipore, USA). All other chemicals used were of analytical grade.

Instruments: CC-I extraction studies were carried out using a commercial microwave digester with extraction assembly (Model: MicroSYNTH, M/s Milestone, USA). The microwave power was linear and adjustable from 0 to 1000 W. The apparatus was equipped with stirring device (rpm range: 10–400) and a circulating water-cooling system.

Separation studies of CC-I were performed using a flash chromatography system (Model: Isolera One, M/s Biotage, Sweden) consisting of a quaternary gradient pump, SNAP cartridges, an autosampler assembly and a UV-visible detector.

Hot percolation extraction and open column chromatographic separation: CC-I was extracted from C. cyminum as described earlier with slight modifications. The extraction was performed without the activity guided fractionation.

MAE and FCS: During the optimization of MAE conditions, effect of solvents (water, ethanol and acetone), solid-solvent ratio (1:99, 5:95 and 10:90 w/v) and soaking time (6, 12 and 24 h) on CC-I recovery were studied. The un-soaked/water soaked powdered mass of C. cyminum seeds was suspended in solvent and poured in microwave extraction assembly consisting of monoblock rotor and nine cylindrical vessels (height 8.5 cm x internal diameter 3.5 cm). The stirring speed of 400 rpm was kept constant throughout the extraction period. The mass was extracted for the period of 1 to 7 min at solvent specific temperature (water: 98 °C, ethanol: 78 °C and acetone: 56 °C) and filtered. The filtrate was concentrated using rotary vacuum evaporator at 50 ± 2 °C under reduced pressure.

The microwave extract thus obtained (1 g per batch) was dissolved in ethanol and charged onto a SNAP samplet (6.5 x 19 mm i.d., 1 g KP-Sil silica, 40 μm particle size). Samplet was loaded on dry biotage SNAP cartridge (55 x 21 mm i.d., 10 g KP-Sil silica; 40 μm particle size). The extract was eluted in gradient mode with a mobile phase comprising of ethyl acetate (A) : ethanol (B) : water (C). Elution was performed in terms of a column volume (1 CV = 15 ml) along with a flow rate of 10 ml/min. The composition of A-B-C used in gradient mode was; 100-0-0 % (3CV), 0-100-0 % (6CV) and 0-85-15 % (20 CV). The separation was performed at 25 ± 2 °C. The detection wavelength was set at 340 nm. The fractions were collected in fraction collector tubes and analyzed for CC-I content using a pre-validated HPLC method as described below. The fractions containing CC-I were pooled, concentrated and freeze dried. HPLC analysis: HPLC analysis of CC-I was carried out using Jasco HPLC system equipped with PU 2089 quaternar...
y gradient pump, UV-2075 plus detector, LC-NetII/ADC communication module and HiQ Sil C-18W column (4.6 x 250 mm, 5-µm particle size). The mobile phase consisted of 40 % acetonitrile and 60 % water containing 1.5 % acetic acid (v/v). It was filtered under vacuum through 0.45 µm membrane filter before use. The system was isocratically run at a flow rate of 1 ml/min. Sample detection was achieved at 340 nm and injection volumes were 10 µl. The limit of quantification (LOQ) of the method was found to be 9 ng/ml. The Calibration curves over the concentration range of 10-500 ng/ml were established for the quantification of CC-I. Data analysis was carried out using ChromPass version 1.8.6.1 software.

STATISTICS
The isolation and purification process of CC-I was repeated in five separate batches of equal size. The CC-I recovery results are expressed as mean ± S.E.

RESULTS AND DISCUSSION

Figure 4: Microwave assisted extraction of CC-I using acetone.

Figure 3: Microwave assisted extraction of CC-I using ethanol.

Hot percolation extraction and open column chromatographic separation: Although percolation is conventional, high yielding extraction technique, it has several disadvantages like requirement of comparatively large amount of solvents and relatively high extraction period with a possible thermal degradation of desired phytochemicals. The classical open column chromatography is practiced worldwide because of its simplicity in operation but it has limitations like slow separation, requires more optimization trials, runs under isocratic condition and suitable for purification of comparatively small quantities. The CC-I was isolated using previously reported hot percolation extraction and open column chromatographic separation wherein 93.9 ± 4.6 mg CC-I /g seed sample was obtained.

MAE and FCS: It is well reported that MAE technique has high and fast extraction performance ability with less solvent consumption capacity. Moreover, MAE offers protection to the thermally sensitive compounds. There are several examples of isolation of therapeutically
important phyto-constituents like piperine, curcuminoids, ginsenosides, anthraquinones and capsaicinoids wherein the MAE demonstrated high extraction yield in less extraction period. FCS technique is basically an air pressure driven hybrid of medium pressure and short column chromatography which has been optimized for particularly rapid separations. In order to overcome the disadvantages of classical percolation and open column chromatography technique employed in CC-I extraction, advanced MAE technique followed by FCS was developed and optimized so as to enhance the CC-I yield. The various parameters such as suitable extraction period, solvent, solid: solvent ratio and effect of soaking were investigated for optimization of MAE. The extraction period was optimized using some preliminary experiments (Data not shown).

Effect of solvent and solid: solvent ratio: The effect of various solvents and solid-solvent ratio on the MAE of CC-I is shown in Figure 2-4. Water as an extraction solvent along with a solid: solvent ratio of 1:99 w/v demonstrated high yield of CC-I.

Effect of soaking: The effect of water soaking on the MAE of CC-I is shown in Figure 5. Before extraction, soaking of powdered mass in water for a period of 24 h resulted in enhanced CC-I yield.

Comparison among isolation methods: The extraction time of the CC-I (total yield; 93.9 ± 1.9 mg/g seeds) by conventional percolation-column chromatographic technique was around 840 min. The MAE-FCS technique yielded the CC-I within 49 min (total yield; 178.4 ± 16.9 mg/g seeds). As compare to conventional extraction technique, the MAE-FCS technique enhanced the CC-I yield by 89.98 % and reduced the overall extraction period. The results showed that CC-I can be isolated efficiently in terms of high yield and low extraction time using MAE-FCS technique.

HPLC analysis: The typical HPLC chromatograms of standard CC-I (A), CC-I after conventional hot percolation-open column chromatographic separation (B) and CC-I after Microwave assisted extraction-Flash chromatographic separation (C).

CONCLUSION
The results of this study show that the method of extraction and purification enables the extraction of CC-I with reliable performance and a significant reduction in
the extraction and isolation time and volume of solvent used in process.

REFERENCES