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# OPTIMIZATION OF EXTRACTION METHOD AND PROFILING OF PLANT PHENOLIC COMPOUNDS OF MERREMIA UMBELLATA (HALLIER. F.)THROUGH RP-HPLC

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**ABSTRACT**

The Merremia umbellata (Hallier. f.) phenolics extraction efficiency of CAE, MAE and UAE methods were assessed by comparing their RP-HPLC chromatogram the highest recovery of phenolic compounds in terms of number and yield was obtained in CAE when compared with MAE and UAE. The CAE is traditionally successful extraction method because in this method, maceration or percolation mechanism accounts for better extraction efficiency Phenolic compounds were monitored in the range between 280, 300 to 320 nm . Among these monitoring wavelengths, at 280 nm, most of the abundant phenolic compounds were detected. It is because most of the phenolic compounds absorbance maximum is near to 280 nm.

**KEYWORDS :**

## 1. INTRODUCTION

Phenolics compounds are naturally present antioxidants, found in a variety of plant based food and vegetables. These compounds are attracting a great deal of attention due to increasing evidence suggesting that they may prevent oxidative diseases such as cancer and other neurological diseases. The reversed-phase (RP) columns have considerably enhanced HPLC separation of different classes of phenolic compounds and RP C18 columns are almost exclusively employed. It was found that column temperature may affect the separation of phenolics such as individual anthocyanin.

## 2. MATERIALS AND METHODS

### 2.1. Optimization of phenolic Extraction

### 2.2 Microwave-Assisted Extraction (MAE)

500 mg of powdered plant material was extracted with 40 mL of 70 % methanol (with 10 ml 5 M HCl) in a microwave for 10 – 40 min. On mass yield basis, an extraction time of 20 min at 650 W microwave powers was found to be optimum. The extracts were filtered and concentrated to dryness under vacuum (temperature, 40 ± 3 °C)

### 2.3. Ultrasound-Assisted Extraction (UAE)

500 mg of powdered plant leaves was sonicated with 40 mL of 70 % methanol (10 ml 5 M HCl) in an ultrasonic bath at a frequency of 40 KHz and at a controlled temperature of 30 ± 5 °C for 40 – 80 min. An extraction time of 60 min was taken as optimum on mass yield basis. The extracts were filtered and concentrated to dryness under vacuum (temperature, 40 – 45 °C) and the yield obtained used for further analysis

### 2.4. Conventional-Assistant Extraction (CAE)

500 mg of powdered plant leaves with 40 mL of 70 % aqueous methanol was taken. Then 10 mL of 5 M HCl was added. The mixture was stirred carefully. The extraction mixture was then refluxed in a water bath at 100° C for 145 min. After cooling, it was filtered with Whatman No.1 filter paper). Then filtrate was centrifuged at 5000 rpm. The supernatant was filtered with 0.22 $\mu$m membrane syringe filter prior to injection in RP-HPLC

### 2.5. Ultrasound/Conventional-Assistant Extraction (UCAE)

500 mg of powdered plant leaves with 40 mL of 70 % aqueous methanol was taken. Then 10 mL of 5 M HCl was added. The mixture was stirred carefully. The extraction mixture was then refluxed in a water bath at 100 °C for 145 min. After cooling, it was filtered with Whatman No.1 filter paper) and filtrate was sonicated at a frequency of 40 KHz in ultra sonic bath for 10 min. Then the filtrate was centrifuged at5000 rpm and the supernatant was filtered with 0.22 $\mu$m membrane syringe filter prior to injection.

### 2.6. Optimization of RP-HPLC Method

The analytical HPLC system employed consists of High Performance Liquid Chromatography (Waters, USA) coupled with a photodiode array detector (PDA-2998), USA. C18 reverse phase column of 4.6×250 mm, 5$\mu$m particle size (SYMMETRY) was used. The mobile phase used was water with 0.1% formic acid as solvent A and 100% methanol as Solvent B. The different isocratic and gradient programmes were followed for phenolic compound separation. The optimized gradient program of 0–10% B (5 min), 10–15% B (5 min), 15–20% B (5 min), 20–30% B (5 min), 30-40% B (10 min) was followed, flow rate was 1.0 ml/min, and the injection volume was 20 $\mu$l. The wavelength was monitored between 210 to 400 nm.

## 3. RESULTS AND DISCUSSION

### 3.1. Optimization of UCAE for phenolic compounds extraction

The phenolics extraction efficiency of CAE, MAE and UAE methods were assessed by comparing their RP-HPLC chromatogram . Among these extraction methods, the highest recovery of phenolic compounds in terms of number and yield was obtained in CAE when compared with MAE and UAE. The CAE is traditionally successful extraction method because in this method, maceration or percolation mechanism accounts for better extraction efficiency and little amount of water in the extracting solvent can penetrate easily into the cells of the plant matrix and facilitate better heating of the plant matrix. This in twist increases the mass transfer of the active constituents into the extracting solvent. Further, the chromatographic profile of the combination of both UAE and CAE method (UCAE) was better in terms of number of phenolics eluted and resolution of their individual peak when compared with chromatographic profile of other extraction methods Since, the conventional extraction gives better percolation of sample to solvents under boiling temperature and the ultrasound extraction technology, the sonication gives individual phenolic compounds fractions and also offers a mechanical effect to give high penetration of solvent into the sample surfaces, thereby increasing the contact surface area between the solid and liquid phase and result in quick diffusion of sample solute from the solid phase to the extraction solvent .

### 3.2. Optimization of RP-HPLC condition

A separation of mobile phase composition with a constant flow throughout the procedure is termed isocratic (meaning

constant composition). In gradient programme, which the mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. Both isocratic and gradient program was used for optimization of RP-HPLC condition for ME phenolic compound identification. The Figure 1. shows phenolic compound elution by isocratic program. Figure 2, 3 and 4 shows the different gradient programs such as 1, 2 and 3 respectively. Among this gradient elution, the gradient program no.3 was chosen since it gave more phenolic compounds interms of yield and number compared to isocratic and other gradient programs.

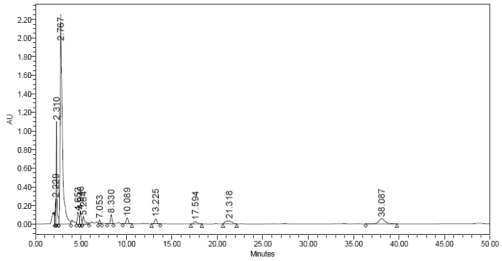

**Figure 1. Isocratic program RP-HPLC chromatogram at 280 nm**

**Mobile phase**

Solvent A: Water with 0.1% formic acid, Solvent B: 100% Methanol Isocratic program: Solvent A - 50 % Solvent B - 50 %

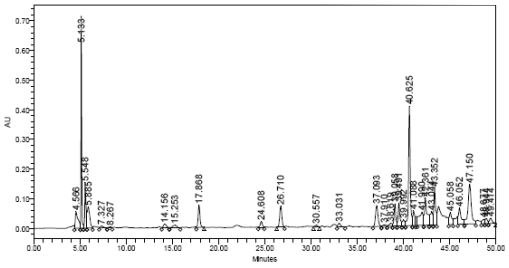

**Figure2. Gradient program 1 RP-HPLC chromatogram at 280 nm**

Mobile phase =Solvent A : Water with 0.1% formic acid, Solvent B : 100% Methanol (Program 1)

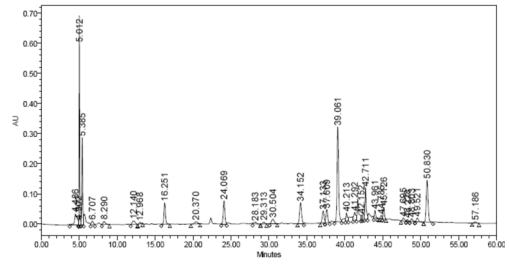

**Figure3. Gradient program 2 RP-HPLC chromatogram at 280 nm**

Mobile phase=Solvent A : Water with 0.1% formic acid, Solvent B : 100% Methanol (Program 2)

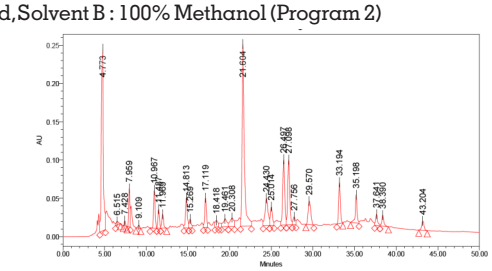

**Figure 4. Gradient program 3 RP-HPLC chromatogram at 280 nm**

Mobile phase=Solvent A : Water with 0.1% formic acid, Solvent B : 100% Methanol(Program 3)

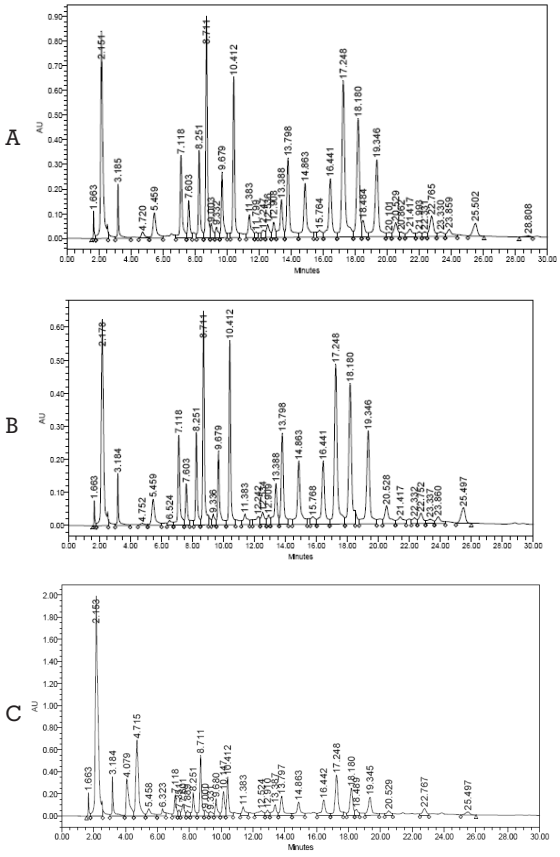


**Figure 5. Different nm absorbance of RP-HPLC chromatogram detection**

a.Ultrasound Conventional-Assistant extraction (UCAE) RP-HPLC chromatogram at 280 nm

b.Ultrasound Conventional-Assistant Extraction (UCAE) RP-HPLC chromatogram at 320 nm

c.Ultrasound Conventional-Assistant Extraction (UCAE) RP-HPLC chromatogram at 340 nm

## 4. CONCLUSION

In this chapter, we successfully investigated the optimization of phenolic compounds extraction using CAE, MAE, UAE and UCAE methods. The optimized phenolic compound extraction method was named as ultrasound-conventional assistant extraction (UCAE). Further, the optimized RP-HPLC condition for phenolic compound detection was chosen as gradient programme No.3 which gave more number of phenolic compounds yield at 280 nm wavelength.

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