ORIGINAL ARTICLE

Microwave-assisted extraction of Lupeol from *Cuscuta reflexa* Roxb. growing on different hosts and its quantitation by high-performance thin layer chromatography

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Microwave-assisted extraction (MAE) of phytochemicals has grown as a promising technique in recent years. MAE method was developed for extraction of Lupeol from *Cuscuta reflexa* Roxb. growing on different hosts. An open vessel modified microwave system has been used for extraction of Lupeol. Various critical parameters such as use of solvent, solvent volume, power and time of irradiation were optimized. Methanol was selected as an extraction solvent based on its high dielectric constant value and maximum solubility of Lupeol. The solvent volume, microwave power and irradiation time were optimized to 10 mL, 160 Watts and 3 min respectively. The developed method is a good alternative to time consuming conventional extraction techniques.

Key words: Conventional extraction, *Cuscuta reflexa*, Lupeol, microwave-assisted extraction

INTRODUCTION

Cuscuta reflexa Roxb. (CR) is an unusual parasitic vine, growing in a prolific manner over host plants with inter-twined stems and commonly found throughout India.[1] Cuscutin, Kaempferol, Quercetin, Lupeol, ß-sitosterol, α-amyrin, ß-amyrin, Stigmasterol are the pharmacologically active markers reported from CR.[2] Lupeol [Figure 1] is a triterpenoid reported to possess biological activities such as anti-malarial,[3] hepatoprotectant,[4] anti-protozoal, anti-inflammatory, anti-tumour and anti-microbial.[5]

Lupeol has been extracted from herbal raw materials by conventional extraction techniques (such as solid-liquid extraction), which are time consuming and often leads to incomplete extraction of phytoconstituents. This is the first report for extraction of Lupeol from CR growing on two different hosts *Vitex negundo* L. and *Wedelia chinensis* L. by microwave-assisted extraction (MAE) and its comparison with conventional extraction technique like solid-liquid extraction.

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MATERIALS AND METHODS

Plant Material

CR (Whole plant) growing on host *Vitex negundo* and *Wedelia chinensis* were collected from Lonavala in the month of February. Plant specimens were authenticated by Agharkar Research Institute (Auth 11-26) and preserved in Herbal Research Lab, Ramnarain Ruia College. Plant materials were shade dried for five days and kept in a hot air oven maintained at 35±2°C for 15 days. It was then powdered, sieved (BSS sieve, 80 mesh) and stored in airtight plastic bottle at room temperature for further analysis.

Apparatus

Open vessel microwave system Model: GMC25E09-MRGX, Godrej, India was used for the extraction of phytoconstituents from plant matrix. Microwave is equipped with a magnetron of 2450 MHz with nominal maximum power of 700 watts operated at 3 power levels and the time controller. Conventional microwave was specially modified for carrying out extractions.

Chemicals and Reagents

Analytical grade solvents were procured from Qualigens Fine Chemicals, Mumbai. Lupeol (≥97% purity) was procured from Sigma Aldrich, Germany.

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Derivatizing reagent, i.e., Anisaldehyde sulphuric acid was prepared as per standard procedure.[6]

Sample Preparation Technique

Microwave-assisted extraction

To the accurately weighed one gram of plant powder, methanol was added and the mixture was irradiated in microwaves under optimized power, solvent volume and time. It was filtered through Whatmann filter paper No. 41 and used further for high-performance thin layer chromotagraphy (HPTLC) analysis.

Optimized power

Extraction was carried out at three different power levels (160, 320 and 640 watts). It was observed that at 160 watts, Lupeol got extracted efficiently, whereas at 320 watts interference of other phytochemicals was observed on TLC, while at 640 watts Lupeol degraded completely. Hence, the power was optimized to 160 watts.

Optimized time

Sample was exposed to microwaves for 1, 3, 5 and 10 minutes at 160 watts. Degradation of Lupeol was observed at 5 and 10 minutes of exposure while its extraction increased from 1–3 min. Hence, optimized time for extraction of Lupeol using MAE was 3 min.

Optimized solvent

Methanol is one of the commonly used solvent in MAE [Table 1]. One gram of plant powder was dissolved in methanol (5, 10, 15 and 20 mL). Each mixture was irradiated in microwaves for 3 min at 160 watts. Maximum Lupeol was extracted in 10 mL methanol.

Conventional extraction

One gram of plant powder was extracted with methanol (10 mL), kept on shaker for 6 hr at 80 rpm and filtered through Whatmann filter paper No. 41. Filtrate was further used for HPTLC analysis.

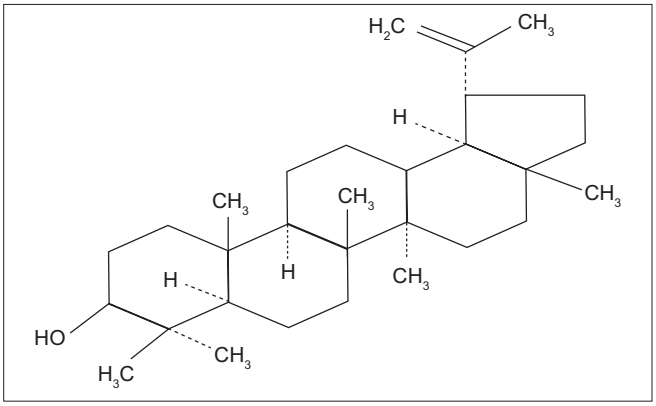

Figure 1: Structure of Lupeol

Optimized high-performance thin layer chromotagraphy condition

Chromatographic separation was achieved on HPTLC plates pre-coated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness with aluminium sheet support. Samples were spotted using Camag Linomat IV Automatic Sample Spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton, 100 µL). Plates were developed in a glass twin trough chamber (Camag) pre-saturated with mobile phase for fifteen minutes. Scanning device used was Camag TLC Scanner II equipped with CATS 3 software. The experimental condition was maintained at 22±2°C. Photodocumentation of the HPTLC plates were carried out using Camag Reprostar 3 system.

Solvent system

Toluene: Chloroform: Ethyl acetate: Glacial acetic acid (10:2:1:0.03 v/v/v/v) was used in this method to resolve and quantitate Lupeol from plant matrix of CR.

Preparation of standard solution

Stock solution of Lupeol (1000 µg mL^{-1}) was prepared in methanol. Aliquots of 5-50 µg mL^{-1} were prepared from this stock solution for calibration curve. Three quality control samples ((low quality control (LQC), mid quality control (MQC) and high quality control (HQC)) of Lupeol (7.5, 17.5, 40 µg mL^{-1}) were prepared for precision, accuracy and ruggedness studies.

Method Validation

International Conference on Harmonization (ICH) guidelines were followed for the validation of the developed analytical method.[7]

Specificity

Specificity was ascertained by analysing standard compound and sample. The bands for Lupeol from sample solutions were confirmed by comparing the R_f and spectra of the bands to those of the standard. The peak purity of the compound [Figure 2] was analysed by comparing the spectra at three different levels, i.e. start, middle, and end positions of the bands.

Instrumental precision

Instrumental precision was affirmed by repeated scanning (n=7) of the same spot of Lupeol (5 µg mL^{-1}) and further

Table 1: Physical and dielectric constants of methanol used in microwave-assisted extraction

Solvent	Dielectric constant (ε')	Dielectric loss factor (ε'')	Dissipation factor tan δ × 10^{-4}	Boiling point (°C)	Lupeol solubility
Methanol	32.7	20.9	6400	64.7	Soluble

expressed as relative standard deviation (% relative standard deviation (RSD)).

Repeatability

Repeatability of the method was affirmed by analysing 5 µg mL^{-1} of Lupeol on HPTLC plate (n=5) and expressed as % RSD.

Inter- and intra-day precision

Variability of the method was studied by analyzing Quality control samples of Lupeol on the same day (Intra-day precision) and on different days (Inter-day precision). Results were expressed as % RSD.

Limit of detection and limit of quantification

For the evaluation of limit of detection (LOD) and limit of quantification (LOQ) different concentrations of the standard solutions of Lupeol were applied along with methanol as blank and determined on the basis of signal-to-noise (S/N) ratio. LOD was determined at S/N of 3: 1 and LOQ at S/N of 10:1.

Recovery

Accuracy of the method was assessed by performing recovery study at three different levels (25, 50 and 100% spiking of Lupeol in plant matrix). Percent recovery and the average percent recovery for each level were calculated.

Ruggedness

Ruggedness of the method was assessed by deliberately incorporating the small variations in the optimized chromatographic condition. Effect of change in analyst, change in mobile phase composition [Toluene: Chloroform: Ethyl acetate: Glacial acetic acid (10:2:1:0.02 v/v/v/v) and Toluene: Chloroform: Ethyl acetate: Glacial acetic acid (10:2:1:0.04 v/v/v/v)] and change in spotting volume (9 µL and 11 µL) on the response and R_f of quality control samples was observed.

RESULTS AND DISCUSSION

In recent years Lupeol, a triterpenoid, has attracted interest from researchers for activities such as its extraction, isolation and synthesis due to its broad spectrum of biological activities.[8] Conventional techniques such as heating, boiling or refluxing can be used to extract terpenoids; however, the disadvantages are their loss due to ionization, hydrolysis and oxidation during extraction as well as the long extraction time. Other techniques which include supercritical carbon dioxide extraction, subcritical water extraction, ultrasonic-assisted extraction (UAE) and MAE have also become of interest as alternatives for the conventional methods. Among these, MAE is the simplest and the most economical technique for extraction of many plant derived compounds.[9,10] Enhancement of product recovery by microwave is generally attributed to its heating effect, which occurs due to the dipole rotation of the solvent in the microwave field. This causes the solvent temperature to rise, which then increases the solubility of the compound of interest. Specifically, solvent heating by microwave occurs when molecules of the polar solvent could not align themselves quickly enough to the high frequency electric field of microwave. This discrepancy causes the solvent molecules to dissipate the absorbed energy in the form of heat.[10]

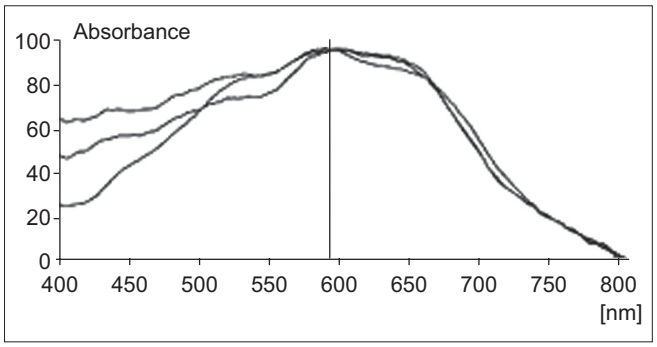

Figure 2: Specificity of method showing Lupeol standard with Lupeol from *Cuscuta reflexa* growing on two different hosts

Table 2: Validation parameters

Parameters	Results
Specificity (selectivity)	Specific
Accuracy (% recovery)	97-99
Precision (% RSD)	
Intra-day	1.11
Inter-day	1.28
Repeatability (% RSD, n=5)	0.89
Limit of detection (µg mL^{-1})	2.00
Limit of quantitation (µg mL^{-1})	5.00
Ruggedness	Rugged

Table 3: Calibration parameters

Phytoconstituent	Linear working range (µg mL^{-1})	Regression equation	Correlation coefficient (r^2)
Lupeol	5-50	y = 15.935x + 14.412	0.992

Table 4: Assay of Lupeol

Extraction method	Amount (mg/g) of Lupeol from *Cuscuta reflexa* Roxb	
	Host *Vitex negundo* Linn.	Host *Wedelia chinensis* Linn.
Conventional extraction method (solid-liquid extraction)	0.0423±0.0028	0.0462±0.0034
Microwave-assisted extraction	0.1268±0.0169	0.1195±0.0101

There are HPTLC methods reported for quantification of Lupeol.[11,12] These methods make use of conventional extraction technique but there are no reports on extraction of Lupeol by MAE from any plant matrix.

In the current method Lupeol was extracted using MAE and the method was compared with conventional extraction technique (solid-liquid extraction). MAE was optimized for selection of solvent, solvent volume, microwave power and irradiation time.

Optimized microwave conditions were as follows:
Solvent: Methanol
Solvent volume: 10 mL
Microwave power: 160 watts
Time: 3 min

Extracts obtained from MAE and solid-liquid extraction were further applied to estimate respective Lupeol content using HPTLC. Of the various solvent systems tried, mixture containing Toluene: Chloroform: Ethyl acetate: Glacial acetic acid (10:2:1:0.03 v/v/v/v) gave the best resolution of Lupeol (R_f=0.44) from the other components of CR. Identity of Lupeol in plant matrix was confirmed by overlay in visible spectra with standard Lupeol using Camag TLC scanner II. Purity of Lupeol in the plant extract was confirmed by overlaying the absorption spectra at the start, middle and end position of the bands [Figure 2]. Developed method was validated in terms of precision, repeatability and accuracy [Table 2]. Response of Lupeol was found to be linear over the range of 5-50 μg mL^{-1} with correlation coefficient (r^2 value) 0.992 [Table 3]. The TLC densitometric method was found to be precise with % RSD for intra-day precision in the range of 0.80-1.11 and for inter-day precision in the range of 1.10–1.28 (for Quality control samples) [Table 2]. This indicated that the method was precise. LOD and LOQ value for Lupeol was 2 and 5 μg mL^{-1}, respectively [Table 2]**.** Average recovery at three different concentrations of Lupeol was 97-99% [Table 2]. Ruggedness of the method in terms of change in analyst and change in mobile phase composition showed variations within acceptable limits. Change in spotting volume (9 and 11 μL) did not affect the R_f of Lupeol but response was altered within acceptable limits. Lupeol content of CR growing on *Vitex negundo* and *Wedelia chinensis* by solid-liquid extraction was 0.0423±0.0028 and 0.0462±0.0034 mg/g, respectively, while by MAE it was 0.1268±0.0169 mg/g and 0.1195±0.0101 mg/g, respectively [Table 4].

CONCLUSION

This is the first report for MAE of Lupeol from CR growing on two different hosts. Comparative evaluation of the results concluded that, MAE was determined to be time effective and efficient method for extracting Lupeol from CR. This method has a great potential for industrial application in the near future.

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