



## Multivariate Optimization of Extraction Methods for Enhanced Rosmarinic acid Yield in *Salvia rosmarinus* and *Salvia sclarea*

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### ABSTRACT

Efficiency of ultrasound-assisted extraction Soxhlet and reflux extraction techniques was evaluated comparing recovery of rosmarinic acid from *Salvia* species pretty thoroughly using ethanol-water and methanol-water solvents. Compound quantification was done employing high-performance liquid chromatography pretty accurately with reasonably sophisticated equipment and some technical expertise. UAE when merged with ethanol-water mixture emerged as most effective method yielding 15.2 mg/g in *Sclarea* pretty effectively. *Sclarea* contained 14.4 mg/g and *Rosmarinus* had some similarly high concentration. *Sclarea* takes effect remarkably quickly within 15 minutes. Soxhlet and reflux extractions necessitated considerably longer durations yielding somewhat lower concentrations overall. Hierarchical Cluster Analysis and Principal Component Analysis further validated superior performance of UAE remarkably well under certain conditions. Study findings reveal an optimized rapid eco-friendly rosmarinic acid extraction protocol with promising implications for nutraceuticals and various pharmaceutical industries.

**Keywords:** Rosmarinic acid, *Salvia rosmarinus*, Ultrasound-assisted extraction, Phytochemical analysis, Green extraction, Multivariate analysis.

### INTRODUCTION

Plant-derived bioactive compounds are increasingly valued for their roles in pharmaceuticals and functional foods. Among them, rosmarinic acid, a polyphenol abundant in the Lamiaceae family, has gained attention for its potent antioxidant and therapeutic properties<sup>1</sup>. Species such as *Salvia rosmarinus* (rosemary) and *Salvia sclarea* are widely

utilized in culinary, cosmetic, and herbal applications due to their aromatic foliage and high secondary metabolite content<sup>2,3</sup>. Rosmarinic acid yield is influenced by species type, solvent polarity, and extraction method<sup>4</sup>. *S. rosmarinus*, a xeromorphic plant common to the Mediterranean basin, thrives on rocky, dry terrains across Europe, Africa, and temperate Asia<sup>5,6,7</sup>. Its strong antioxidant potential is attributed to its capacity to scavenge free radicals



and reactive oxygen species<sup>8,9</sup>. Beyond antioxidant activity, rosemary exhibits diverse pharmacological properties, including anti-inflammatory<sup>10</sup>, analgesic<sup>11</sup>, diuretic<sup>12</sup>, antidepressant<sup>13</sup>, anticancer<sup>14</sup>, antidiabetic<sup>15</sup>, antifungal<sup>16</sup>, and antibacterial<sup>17</sup> effects. Efficient extraction is crucial to maximize yield while preserving compound integrity. Conventional methods such as Soxhlet and reflux offer reliability but are time-intensive<sup>18</sup>. Ultrasound-assisted extraction (UAE) has emerged as a faster, greener alternative, enhancing extraction through cavitation<sup>19</sup>. Binary solvents like ethanol-water and methanol-water often outperform pure solvents in phenolic recovery<sup>20</sup>. However, comparative studies on rosmarinic acid extraction from *Salvia* species remain limited, especially those using optimized protocols and validated techniques like HPLC<sup>21,22</sup>.

## Methodology

### Plant Material Collection

Leaves of *Salvia rosmarinus* and *Salvia sclarea* were collected from the Medicinal and Aromatic Plants Research Farm, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India (Fig. 1). Healthy and mature leaves were harvested from approximately one-year-old plants during the active growth seasons of 2022 and 2023. Collected samples were shade-dried, powdered using a mechanical grinder, and stored in airtight containers until extraction.

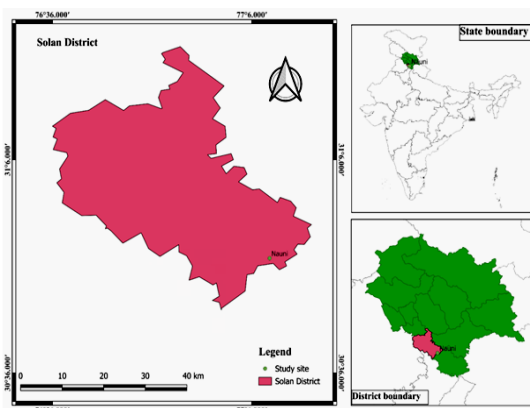


Fig. 1. Graphical representation of the Study Area (Source: QGIS)

### Extraction Techniques

An overview of the extraction protocols is illustrated in Fig. 2. Three extraction techniques viz., Soxhlet extraction, reflux extraction, and ultrasound-assisted extraction were optimized

using different solvent systems and time durations to compare efficiency.

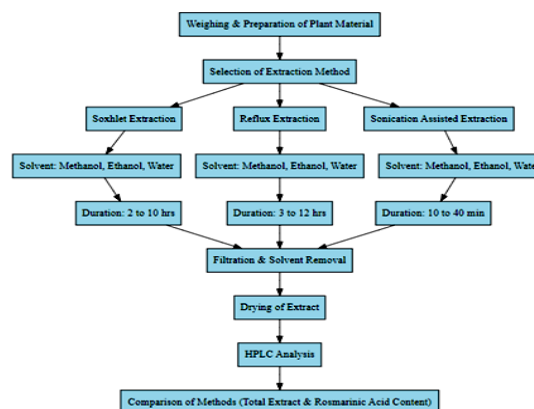


Fig. 2. Procedure of Extraction Methods (Source: MS presentation)

### Soxhlet Extraction

Two grams of powdered leaf material were subjected to Soxhlet extraction using 100 mL of four solvent systems: methanol-water (70:30, v/v), ethanol-water (70:30, v/v), pure methanol, and distilled water. Extractions were carried out for 2, 4, 6, 8, and 10 hours. The obtained extracts were concentrated under reduced pressure using a rotary evaporator and subsequently dried to constant weight. Extract yield (%) was calculated on a dry weight basis.

### Reflux Extraction

Two grams of leaf powder were refluxed with 100 mL of each solvent system (methanol-water, ethanol-water, methanol, and water) for 3, 6, 9, and 12 hours. The resulting extracts were concentrated using rotary evaporation, dried to constant weight, and weighed to determine extraction yield.

### Ultrasound-Assisted Extraction

Two grams of powdered samples were extracted with 100 mL of each solvent system (methanol-water, ethanol-water, methanol, and water) using a bath-type ultrasonic extractor (40 kHz) at  $30 \pm 1^\circ\text{C}$  for 10, 20, 30, and 40 minutes. Following ultra-sonication, the mixtures were filtered and concentrated using rotary evaporation. The dried extracts were weighed accurately to calculate yield percentages.

### HPLC Sample Preparation and Quantification

Dried extracts were diluted 500-fold with

70% methanol (v/v), filtered through a 0.2  $\mu\text{m}$  membrane, and analyzed using High-Performance Liquid Chromatography (HPLC) equipped with a Waters 515 pump and 2487 UV detector. Separation was performed on an XBridge C18 column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ ) using a binary gradient system comprising 0.2% formic acid in water (Solvent A) and acetonitrile (Solvent B), at a flow rate of 1 mL  $\text{min}^{-1}$ . Detection was conducted at 330 nm, with rosmarinic acid exhibiting a retention time of approximately 8.4 minutes.

#### Calibration Curve and Quantification

A calibration curve for standard rosmarinic acid (3.125-100  $\mu\text{g mL}^{-1}$ ) demonstrated excellent linearity ( $Y = 7.50 \times 10 X + 6.18 \times 10^0$ ,  $R^2 = 0.999$ ). The concentration of rosmarinic acid (mg  $\text{g}^{-1}$  DW) in extracts was calculated from the regression equation based on peak area values, and extraction yield (%) was derived from the final dry extract weight relative to the initial sample weight.

#### Statistical and Multivariate Analysis

All experiments were performed in triplicate under controlled laboratory conditions following a Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) to assess significant differences among treatments. Multivariate statistical analyses, including Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were performed using SPSS (Version 20.0) and R software to elucidate patterns of variation among extraction techniques, solvent systems, and durations based on standardized variables such as extract yield and rosmarinic acid content.

## RESULT AND DISCUSSION

#### Soxhlet Extraction Efficiency of *Salvia rosmarinus* and *Salvia sclarea*

Soxhlet extraction of *Salvia rosmarinus* and *Salvia sclarea* leaves was performed using four solvents namely methanol-water (70:30), ethanol-water (70:30), pure methanol, and distilled water across durations of 2, 4, 6, 8, and 10 h to assess total extract yield and rosmarinic acid (RA) content. Methanol

was prioritized due to its proven efficacy in phenolic extraction<sup>23</sup>, while ethanol and water-based systems have been validated in previous studies<sup>24,25,26,27,28,29</sup>. In *Salvia rosmarinus*, the methanol-water (70:30) solvent system delivered the highest extract yield, peaking at 40.66% after 8 h with a mean yield of 33.29%. Ethanol-water also showed strong performance with 39.40% yield at the same time point. Pure methanol displayed a gradual yield increase from 20.48% (2 h) to 33.70% (10 h), while water consistently produced the lowest yields. The highest RA content (2.23%) was achieved after 6 h using methanol-water, followed by a decline likely due to thermal degradation, as noted by Atanasova *et al.*,<sup>30</sup>. Ethanol-water peaked at 1.83% after 4 h, methanol at 1.20% (8 h), and water at 0.93% (4 h), confirming methanol-water (70:30) for 6 h as the optimal condition. Similarly, in *Salvia sclarea*, methanol-water yielded the highest mean extract (28.99%) with a peak of 30.55% at 6 hours. Ethanol-water peaked slightly higher at 31.19% after 10 h, while methanol ranged from 20.59% to 28.92%, and water remained the least effective. RA content in *S. sclarea* was also maximized with methanol-water, reaching 1.55% at 6 h, with a mean of 1.33%. Ethanol-water achieved 1.42% at 4 h, while water and methanol showed moderate peaks of 0.86% and 0.67% respectively at the same time point. The decline in RA after peak extraction times again supports thermal sensitivity of phenolic compounds under prolonged exposure. Overall, Soxhlet extraction using methanol-water (70:30) for 6 h was identified as the most effective protocol for both *S. rosmarinus* and *S. sclarea*, balancing optimal extract yield and RA recovery. These results underscore the importance of solvent polarity and controlled extraction duration in enhancing phenolic compound recovery from medicinal plants.

#### Reflux Extraction Efficiency of *Salvia rosmarinus* and *Salvia sclarea*

Reflux extraction was conducted using four solvents namely methanol-water (70:30), ethanol-water (70:30), pure methanol, and distilled water over durations of 3, 6, 9, and 12 h to evaluate total extract yield and rosmarinic

acid (RA) content. In *Salvia rosmarinus*, the ethanol-water mixture produced the highest mean extract yield (34.67%), with a maximum of 37.75% after 12 hours. Methanol-water followed closely with a mean yield of 30.75%, peaking at 35.26% at 12 hours. Pure methanol and water yielded slightly lower maximums (32.64% and 33.92%, respectively). In terms of RA content, methanol-water achieved the highest mean (1.21%), peaking at 1.43% after 6 h before declining to 1.03% at 12 hours. Ethanol-water also peaked at 6 h (1.33%), showing a similar decline pattern. Methanol and water yielded lower RA levels, with water peaking at only 0.70% (3 hours). The results indicate methanol-water (70:30) for 6 h as optimal for RA extraction, aligning with findings<sup>29,31,32</sup> that prolonged exposure may degrade thermolabile phenolics. In *Salvia sclarea*, methanol-water again produced the highest mean extract yield (26.55%), reaching a maximum of 33.00% at 12 h, followed by ethanol-water (24.63%) with a peak of 29.97% at the same time. Pure methanol and distilled water were less efficient. RA content was highest in methanol-water extracts, with a mean of 1.09% and a peak of 1.25% at 6 h, before declining to 0.88% by 12 hours. Ethanol-water showed a similar pattern, peaking at 1.14% at 6 h and tapering to 1.00% at 12 hours. Water and methanol were again the least effective, with maximum RA values of 0.72% and below 0.70%, respectively. Across both species, reflux extraction using methanol-water (70:30) for 6 h was consistently optimal, achieving a balance between high extract yields and maximal RA content. Declines in RA after extended durations underscore the importance of limiting exposure to high temperatures to preserve phenolic integrity.

#### **Ultrasound-Assisted Extraction of *Salvia rosmarinus* and *Salvia sclarea***

Ultrasound-assisted extraction (UAE) was performed on leaf samples of *Salvia rosmarinus* and *Salvia sclarea* using four solvents namely methanol-water (70:30), ethanol-water (70:30), methanol, and distilled water across 10, 20, 30, and 40 minutes. In *S. rosmarinus*, water and methanol-water extracts

yielded the highest mean total extracts (13.65% and 13.46%, respectively), peaking at 40 min (15.03% and 15.34%). Methanol-water yielded the highest mean RA content (0.63%), with a maximum of 0.69% at 20 min, declining to 0.54% at 40 minutes. Ethanol-water peaked similarly (0.71% at 20 min), while water and methanol produced lower RA levels. The decline in RA at longer durations is likely due to cavitation-induced degradation<sup>33</sup>. Optimal conditions were 20 min with methanol-water (70:30), consistent with findings by Rostagno *et al.*,<sup>34</sup> and Nicolai *et al.*,<sup>35</sup>. In *S. sclarea*, ethanol-water showed the highest mean extract yield (13.25%), peaking at 15.26% after 40 min, followed by methanol-water (14.34%). Methanol-water again gave the highest mean RA content (1.01%), peaking at 1.29% at 20 min before declining. Ethanol-water and methanol peaked at 0.96% and 0.87%, respectively. Water was least effective (0.67%, declining to 0.31%). Thus, 20 min with methanol-water (70:30) was optimal for RA recovery in both species. Overall, UAE using methanol-water for 20 min effectively balanced extract yield and RA content, highlighting the role of solvent polarity and controlled sonication time in maximizing phenolic recovery.

#### **Comparison of different extraction methods**

The best extraction condition from each method was compared to identify the most effective approach for extracting rosmarinic acid (RA) from *Salvia rosmarinus* and *Salvia sclarea* (Table 3). Soxhlet extraction with methanol-water (70:30) for 6 h yielded the highest RA content of 2.23% in *S. rosmarinus* and 1.55% in *S. sclarea*, while reflux extraction under the same conditions produced a lower RA yield of 1.25%. Across all methods, methanol-water consistently outperformed other solvents in RA recovery likely due to water enhancing polyphenol diffusion by swelling plant tissues<sup>36</sup>. The superior efficiency of Soxhlet is attributed to continuous solvent percolation and repeated exposure to freshly heated solvent, improving extraction kinetics. Thus, Soxhlet extraction with methanol-water for 6 h is the most effective protocol for maximizing RA content in both *Salvia* species.

**Table 1: Effect of extraction duration, solvent and extraction method on Total extract (%) of *S. rosmarinus* and *S. sclarea* leaves**

S and D	<i>Salvia rosmarinus</i>					<i>Salvia sclarea</i>				
	MeOH:H <sub>2</sub> O (70:30)	EtOH:H <sub>2</sub> O (70:30)	Methanol	Water	Mean	MeOH:H <sub>2</sub> O (70:30)	EtOH: H <sub>2</sub> O (70:30)	Methanol	Water	Mean
Soxhlet extraction										
2 H	21.47	22.16	20.48	24.84	22.24	19.09	19.72	20.59	22.72	20.53
4 H	28.36	25.91	24.23	30.40	27.30	24.33	23.53	21.98	26.50	24.09
6 H	35.07	34.45	30.61	34.24	33.59	30.55	26.17	24.28	31.48	28.12
8 H	40.66	39.40	32.50	34.81	36.84	34.98	30.71	27.98	32.52	31.55
10 H	40.91	39.69	33.70	35.55	37.46	36.04	31.19	28.92	33.69	32.46
Mean	33.29	32.32	28.31	32.03		28.99	26.27	24.75	29.38	
CD <sub>0.05</sub>										
S	0.493	0.698								
D	0.551	0.781								
SxD	1.103	1.561								
Reflux Extraction										
3 H	26.09	30.69	23.51	26.26	26.64	19.01	17.98	15.09	16.06	17.04
6 H	28.35	33.43	25.94	29.51	29.31	22.63	21.61	17.84	20.56	20.66
9 H	33.31	36.79	29.15	32.90	32.87	31.56	28.99	22.94	24.35	26.96
12 H	35.26	37.75	32.64	33.92	34.89	33.00	29.97	24.04	25.78	28.20
Mean	30.75	34.67	27.81	30.48		26.55	24.63	19.98	21.69	
CD <sub>0.05</sub>										
S	1.053	0.517								
D	1.05	0.517								
SxD	N/A	1.034								
Ultrasound-assisted										
10 m	11.92	10.87	11.29	11.94	11.51	9.61	9.92	9.97	8.83	9.58
20 m	12.95	12.08	12.98	13.14	12.79	11.95	13.29	12.14	10.31	11.92
30 m	13.63	13.64	13.39	14.46	13.78	13.13	14.53	13.32	12.06	13.26
40 m	15.34	14.92	14.78	15.06	15.03	14.34	15.26	14.15	12.90	14.16
Mean	13.46	12.88	13.11	13.65		12.26	13.25	12.39	11.03	
CD <sub>0.05</sub>										
S	0.328	0.393								
D	0.328	0.393								
SxD	N/A	0.154								

\*S: Solvent, D: Duration, and SxD: Solvent x Duration, H: Hours, m: minutes

**Table 2: Effect of extraction duration, solvent, and extraction method on Rosmarinic acid content (%) of *S. Rosmarinus* and *S. sclarea* leaves**

S and D	<i>Salvia rosmarinus</i>					<i>Salvia sclarea</i>				
	MeOH:H <sub>2</sub> O (70:30)	EtOH:H <sub>2</sub> O (70:30)	Methanol	Water	Mean	MeOH:H <sub>2</sub> O (70:30)	EtOH:H <sub>2</sub> O (70:30)	Methanol	Water	Mean
Soxhlet extraction										
2 H	1.71 ± 0.02	1.62 ± 0.01	0.81 ± 0.02	0.73 ± 0.05	1.21	1.13 ± 0.01	1.16 ± 0.02	0.58 ± 0.02	0.74 ± 0.02	0.90
4 H	1.81 ± 0.05	1.83 ± 0.07	0.86 ± 0.04	0.93 ± 0.06	1.35	1.18 ± 0.03	1.42 ± 0.01	0.67 ± 0.002	0.86 ± 0.01	1.03
6 H	2.23 ± 0.04	1.47 ± 0.05	1.01 ± 0.01	0.78 ± 0.07	1.37	1.55 ± 0.02	1.24 ± 0.03	0.65 ± 0.02	0.43 ± 0.01	0.97
8 H	1.92 ± 0.11	1.29 ± 0.04	1.20 ± 0.01	0.69 ± 0.04	1.28	1.42 ± 0.01	1.06 ± 0.04	0.39 ± 0.01	0.34 ± 0.004	0.80
10 H	1.13 ± 0.01	0.77 ± 0.05	0.74 ± 0.04	0.65 ± 0.02	0.82	1.35 ± 0.01	0.94 ± 0.07	0.34 ± 0.02	0.24 ± 0.01	0.72
Mean	1.76	1.40	0.93	0.76		1.33	1.16	0.53	0.52	
CD <sub>0.05</sub>										
S	0.038					0.021				
D	0.043					0.023				
SxD	0.085					0.085				
Reflux Extraction										

3 H	1.33 ± 0.03	0.98 ± 0.01	0.64 ± 0.01	0.70 ± 0.01	0.91	1.05 ± 0.01	1.08 ± 0.04	0.33 ± 0.003	0.72 ± 0.01	0.80
6 H	1.43 ± 0.01	1.33 ± 0.06	0.80 ± 0.02	0.53 ± 0.04	1.02	1.25 ± 0.01	1.14 ± 0.03	0.37 ± 0.002	0.57 ± 0.003	0.83
9 H	1.06 ± 0.05	0.89 ± 0.02	1.04 ± 0.04	0.52 ± 0.01	0.88	1.17 ± 0.02	1.05 ± 0.07	0.43 ± 0.01	0.46 ± 0.01	0.78
12 H	1.03 ± 0.02	0.76 ± 0.01	0.88 ± 0.08	0.42 ± 0.02	0.77	0.88 ± 0.02	1.00 ± 0.02	0.21 ± 0.002	0.39 ± 0.02	0.62
Mean	1.21	0.99	0.84	0.54		1.09	1.07	0.33	0.53	
CD <sub>0.05</sub>										
S	0.031					0.022				
D	0.031					0.022				
SxD	0.062					0.044				
Ultrasound-assisted Extraction										
10 m	0.63 ± 0.03	0.37 ± 0.03	0.15 ± 0.01	0.11 ± 0.01	0.32	0.85 ± 0.03	0.56 ± 0.04	0.69 ± 0.01	0.48 ± 0.004	0.65
20 m	0.69 ± 0.02	0.71 ± 0.01	0.24 ± 0.002	0.19 ± 0.01	0.46	1.29 ± 0.03	0.96 ± 0.06	0.87 ± 0.02	0.67 ± 0.01	0.95
30 m	0.68 ± 0.04	0.45 ± 0.01	0.20 ± 0.01	0.27 ± 0.002	0.40	0.95 ± 0.06	0.83 ± 0.02	0.85 ± 0.02	0.39 ± 0.01	0.76
40 m	0.54 ± 0.04	0.39 ± 0.03	0.16 ± 0.003	0.25 ± 0.001	0.34	0.93 ± 0.02	0.78 ± 0.06	0.70 ± 0.03	0.31 ± 0.04	0.68
Mean	0.63	0.48	0.18	0.21		1.01	0.79	0.78	0.46	
CD <sub>0.05</sub>										
S	0.021					0.032				
D	0.021					0.032				
SxD	0.042					0.064				

(Data is expressed as ± standard deviation from 3 replications) \*S: Solvent, D: Duration, and SxD:Solvent x Duration, H: Hours, m: minutes

**Table 3: Comparison of different extraction methods**

Extraction method	Extracting solvent	Extraction duration	<i>Salvia rosmarinus</i>		<i>Salvia sclarea</i>	
			Total extract(%)	Rosmarinic acid(%)	Total extract(%)	Rosmarinic acid(%)
Soxhlet extraction	Methanol-water	6 H	35.07	2.23±0.04	30.55	1.55 ± 0.02
Reflux extraction	Methanol-water	6 H	28.35	1.43±0.01	22.63	1.25 ± 0.01
Ultrasound-assisted extraction	Ethanol-water	20 Min	12.08	0.71±0.01	11.95	1.29 ± 0.03
CD <sub>0.05</sub>			0.133	0.059	1.47	0.06

(Data is expressed as ± standard deviation from 3 replications)

### HPLC method development

Chromatographic conditions were effectively optimized using an X-Bridge C18 column (4.6 × 250 mm, 5 µm particle size). Various combinations of acetonitrile and 0.2% formic acid in water were tested under both isocratic and gradient modes at flow rates ranging from 0.8 to 1.5 mL/minute. Detection wavelengths from 270 to 380 nm were evaluated, with optimal separation of rosmarinic acid achieved at 330 nm using gradient elution with a 1 mL/min flow rate. Clear, well-resolved peaks were observed in extracts from *Salvia rosmarinus* and *Salvia sclarea* under these conditions (see Table).

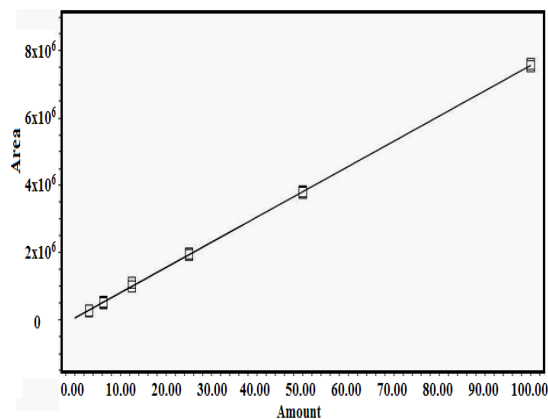
### Linearity curve of rosmarinic acid

The results obtained for linearity and range for rosmarinic acid are presented below as:

Phyto-constituent	Linear range (µg/mL)	Retention Time (minutes)	Regression equation	R <sup>2</sup>
Rosmarinic acid	3.125-100	8.429±0.030	Y=7.50e+004 X+6.18e+004	0.999

Linearity of rosmarinic acid was established for six concentrations ranging from 3.125 µg/

mL to 100.00 µg/mL. The regression equation derived was linear and the correlation coefficient (R<sup>2</sup>) value associated with it was 0.999. The regression equation derived from the linearity data was Y=7.50e+004X+6.18e+004. Rosmarinic acid showed a retention time of 8.429 minutes. The calibration curve was generated by graphing the average peak area against the concentration of each analyte (Figure 3).



**Fig. 3. Calibration curve of rosmarinic acid (Reference compound)(Source: MS excel)**

The HPLC chromatogram shows a major peak at 8.402 min, indicating rosmarinic acid as the dominant compound. Minor peaks at 2.953, 3.448, and 3.867 min suggest other constituents. A stable baseline ensures accurate detection. This pattern is typical in *S. rosmarinus* and *S. sclarea* analyses for compound purity and quantification (Figure 4).

This HPLC chromatogram shows clear separation of compounds, with retention time

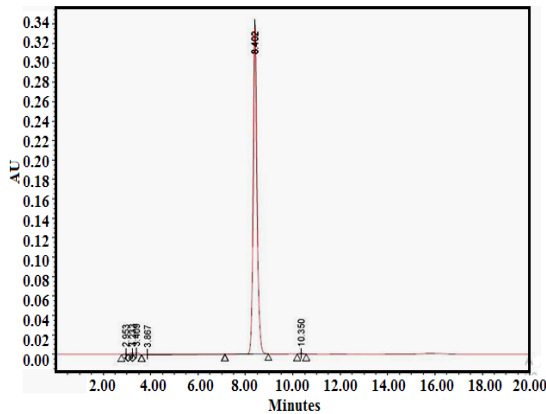


Fig. 4. Chromatogram of rosmarinic acid (Standard)(Source: MS excel)

on the x-axis and absorbance on the y-axis. A dominant peak at 8.966 min indicates the main compound, likely rosmarinic acid, while smaller peaks between 3.002 and 17.995 min suggest minor components or impurities (Fig. 5). A stable baseline reflects effective separation and instrument performance. Such profiles are common in botanical studies for identifying and quantifying key bio-actives, requiring comparison with standards for confirmation.

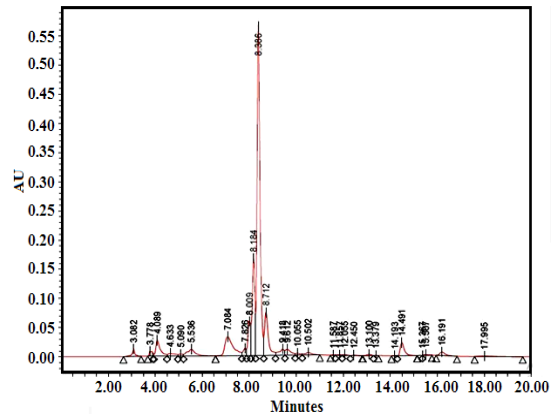


Fig. 5. HPLC chromatogram showing 70% methanolic extract of *S. rosmarinus* and *S. sclarea* (Source: MS excel)

Table 4: Mock extraction dataset for PCA and Clustering

Sample ID	Species	Method	Solvent	RA Content (mg/g)	Yield (%)	Time (min)
SR_UAE_EW	<i>S. rosmarinus</i>	UAE	Ethanol-Water	15.2	2.5	15
SR_UAE_MW	<i>S. rosmarinus</i>	UAE	Methanol-Water	14.8	2.4	15
SR_SOX_EW	<i>S. rosmarinus</i>	Soxhlet	Ethanol-Water	12	2	180
SR_SOX_MW	<i>S. rosmarinus</i>	Soxhlet	Methanol-Water	11.4	1.9	180
SR_RFX_EW	<i>S. rosmarinus</i>	Reflux	Ethanol-Water	13.2	2.2	60
SR_RFX_MW	<i>S. rosmarinus</i>	Reflux	Methanol-Water	12.8	2.1	60
SS_UAE_EW	<i>S. sclarea</i>	UAE	Ethanol-Water	14.4	2.3	15
SS_UAE_MW	<i>S. sclarea</i>	UAE	Methanol-Water	13.6	2.2	15
SS_SOX_EW	<i>S. sclarea</i>	Soxhlet	Ethanol-Water	10.5	1.8	180
SS_SOX_MW	<i>S. sclarea</i>	Soxhlet	Methanol-Water	9.8	1.7	180
SS_RFX_EW	<i>S. sclarea</i>	Reflux	Ethanol-Water	11.9	2	60
SS_RFX_MW	<i>S. sclarea</i>	Reflux	Methanol-Water	11.2	1.9	60

Multivariate Analysis of Extraction Methods

PCA and HCA simplified the dataset by highlighting key patterns in extraction performance. PC1 and PC2 explained 92.0% and 7.8% of the variance, respectively (Fig. 7,8). Ultrasound-assisted extraction with ethanol-water clustered in the negative PC1 region, indicating high RA content and short duration. Soxhlet and Reflux formed distinct HCA clusters due to longer times and moderate yields (Fig. 8), supporting method comparison and optimization.

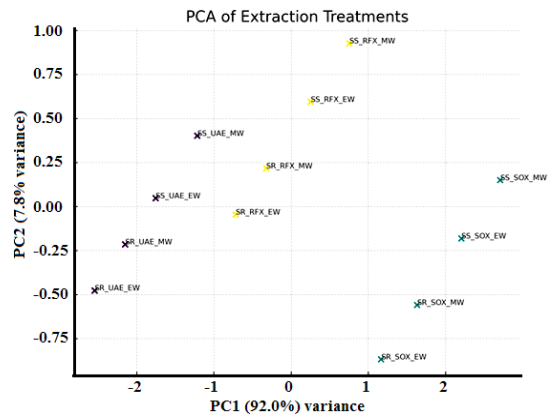
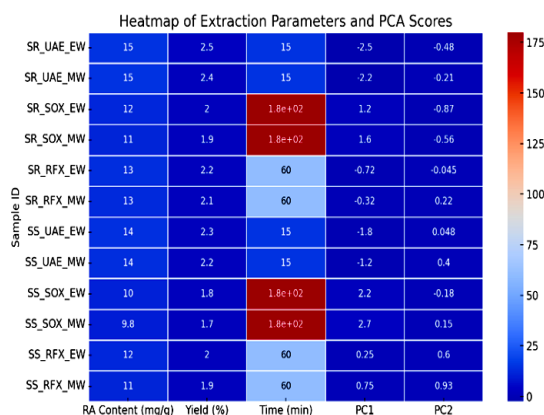
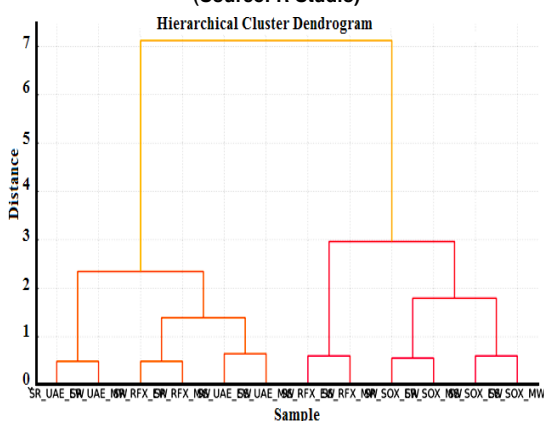


Fig. 6. Graph showing Principal Component Analysis (Source: R Studio)



**Fig. 7. Principal Component Analysis (PCA) plot of extraction parameters and variation in variables (Source: R Studio)**



**Fig. 8. Hierarchical cluster dendrogram (Ward's method) showing similarity among extraction treatments (Source: R Studio)**

## CONCLUSION

Ultrasound-assisted extraction particularly with ethanol-water mixture proves superior for rapid extraction of rosmarinic acid from *Salvia* species efficiently. UAE significantly shortened extraction time to just 15 min while racking up highest concentrations of rosmarinic acid 15.2 mg/g. UAE method's distinct efficiency across various solvents and species was confirmed largely through multivariate statistical analysis including PCA and HCA. These findings highlight potential of UAE as scalable eco-friendly alternative for industrial herbal medicine applications and food supplement formulation effectively nowadays. Validated HPLC quantification paired with comparative extraction performance analysis offers a highly reproducible framework for recovering various bioactive compounds effectively. Future work might delve into utilizing response surface methodology for optimizing processes further and examining bioactivity in various functional applications deeply.

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## Conflict of interest

No potential conflict of interest was reported by the author(s).

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