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Studying the effect of solvent polarity on the value of Sun Protection Factor of Rosmarinus officinalis extracts prepared using Various Solvents

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ABSTRACT

This research aims to study the effect of different extraction solvents (water, ethanol 70%, and hexane) on the value of sun protection factor (SPF) of rosemary (Rosmarinus officinalis) extracts obtained by ultrasonic extraction technique. Water produced the highest extract yield at 17.88%, followed by 13.46% for ethanol 70%, and 2.04% for hexane.

Among the three extracts, ethanol 70% produced the strongest photoprotective effect, achieving an SPF of 35.18. This high SPF is attributed to its rich content of phenolic and flavonoids compounds, known for their strong UV-absorbing properties and antioxidant activity. The water extract demonstrated moderate protection with an SPF of 17.62, reflecting its abundance of polar compounds such as phenols and tannins. In contrast, the hexane extract exhibited minimal protection (SPF 7.52) due to its low yield and limited presence of UV-absorbing molecules.

Overall, the findings show that solvent polarity strongly influences the SPF value of rosemary extracts, with ethanol 70% emerging as the most effective solvent.

KEYWORDS: Rosmarinus Officinalis, Sun Protection Factor, Ultrasonic, Extraction Yield, Solvent Polarity.

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1. Introduction

Rosemary (Rosmarinus officinalis L.) is an aromatic evergreen shrub native to the Mediterranean region and widely cultivated across the world for its culinary, medicinal, and cosmetic applications [1]. Traditionally, rosemary has been valued for its preservative, antimicrobial, and antioxidant properties, which have been attributed to its rich composition of bioactive phytochemicals. The plant contains a diverse array of secondary metabolites, including phenolic acids, flavonoids, and terpenes (most notably carnosic acid, carnosol, and rosmarinic acid) that collectively contribute to its strong free radical–scavenging and metal-chelating activities [2]. These compounds have been shown to exert various biological effects, including anti-inflammatory, neuroprotective, and anticancer properties, making rosemary a subject of increasing pharmacological interest [3].

In recent years, natural antioxidants derived from plants have attracted considerable attention as potential alternatives to synthetic compounds in cosmetic and dermatological formulations [4] [5] [6]. Among the many applications explored, their use in photoprotection has gained particular momentum [7]. Prolonged or excessive exposure to ultraviolet (UV) radiation is known to induce a cascade of deleterious effects on the skin, including oxidative stress, inflammation, DNA damage, and degradation of collagen and elastin fibers. These changes not only accelerate cutaneous aging but also increase the risk of developing skin cancers [8]. Sunscreens containing synthetic UV filters are currently the primary means of protection [9]; however, growing environmental concerns and reports of allergic or irritant reactions have stimulated the research for safe and, naturally derived alternatives [10].

The phytochemical profile of rosemary suggests that it could play a promising role in mitigating UV-induced skin damage [11]. Compounds such as carnosic acid and carnosol are potent lipid-soluble antioxidants capable of stabilizing cell membranes and preventing lipid peroxidation under oxidative stress [12]. Rosmarinic acid, on the other hand, has been reported to absorb UV light in both the UVA and UVB ranges and to enhance cellular antioxidant defenses by upregulating endogenous enzymes such as superoxide dismutase and catalase [13]. These combined mechanisms suggest the potential of rosemary extracts to act as multifunctional sun protective agents, both absorbing harmful radiation and neutralizing reactive oxygen species generated by UV exposure [14] [15].

The initial and most critical step in exploiting the biological potential of plant material is extraction. The efficiency and selectivity of this process are predominantly governed by the principle of "like dissolves like" [16]. where solvent polarity directly determines the spectrum of compounds that will be solubilized. Polar solvents such as water (polarity index ~9.0) are effective for extracting hydrophilic compounds like polysaccharides, tannins, and certain phenolic acids. Hydroethanolic mixtures (e.g., ethanol 70%, with a polarity index of ~6.5-7.0) offer a balanced intermediate polarity, efficiently extracting a wide range of semi-polar compounds, including many flavonoids and phenolic diterpenes, which are often associated with the strongest antioxidant activities in rosemary [17]. In contrast, non-polar solvents like hexane (polarity index ~0.1) are selective for lipophilic constituents, such as essential oils, waxes, and non-polar terpenoids [18].

Thus, the polarity indices of the solvents used in the present study can be ranked as follows: Water (9.0) > Ethanol 70% (6.5–7.0) > Hexane (0.1) [19]. This descending order provides a clear framework for comparing solvent selectivity and their expected extracted compounds.

Therefore, the choice of solvent is not merely a procedural detail but a fundamental decision that defines the chemical profile and, consequently, the biological activity of the resulting extract.

Despite the known bioactivity of rosemary, a systematic investigation about extraction solvent, resultant phytochemical composition, and photoprotective efficacy is required. Most existing studies focus on either the antioxidant activity or the chemical profile in extracts [11] [20], without directly correlating them to a functional application like sun protection factor (SPF).

Therefore, this study was designed to:

Prepare extracts of Rosmarinus officinalis L. leaves using solvents of varying polarity (water, ethanol 70%, and hexane) via ultrasound-assisted extraction.

Evaluate the extraction yield and perform a qualitative phytochemical screening to determine the presence of major metabolite classes (phenols, flavonoids, alkaloids, tannins, and terpenoids).

Determine the *in vitro* SPF of each extract using the spectrophotometric method.

Establish a clear structure-activity relationship, identifying the optimal extraction solvent to obtain a rosemary extract with maximized photoprotective potential for use in cosmetic and pharmaceutical formulations.

2.Methodology

2.1. Devices and Materials

Analytical balance with a precision of 0.0001g (Precisa),Ultrasonic Bath (JENEK / PS-80), Rotary Vacuum Evaporator (STUART), Drying Oven (Memmert), UV-Visible Spectrophotometer (Rigol 3660), Pipettes, Burettes and Beakers.

Solvents: Distilled Water, 70:30 (v/v) ethanol (≥98% purity): distilled water mixture, and Hexane (≥98% purity).

Phytochemical Test Reagents: : Ferric Chloride ($FeCl_3$), Mayer's Reagent (Mercuric chloride ($HgCl_2$), Potassium iodide (KI)) ,1% Aluminum Chloride ($AlCl_3$), Chloroform, Acetic Anhydride, Concentrated Sulfuric Acid (H_2SO_4), 1% Lead Acetate Solution,10% Sodium Hydroxide (NaOH) Solution

2.2. Plant Material Collection and Preparation

Fresh rosemary (Rosmarinus officinalis L.) leaves were collected in September 2025 from the private garden of Al-Wataniya Private University in Hama, Syria. The plant was botanically identified as Rosmarinus officinalis L. (family Lamiaceae) by the Department of Pharmacognosy at the university. The leaves were washed with distilled water to remove impurities, air-dried at room temperature (25 ± 2 °C) in the shade for seven days, and ground into a fine powder using a laboratory mill. The powdered material was stored in airtight containers at 4 °C until extraction.

2.3. Preparation of Extracts

Ultrasound-Assisted Extraction (UAE):

The extracts were prepared using three different solvents: water, ethanol, and hexane, employing ultrasound-assisted extraction to maximize efficiency.

A precise 5.0 g of rosemary powder was weighed and placed into separate 250 mL conical flasks. To each flask, 100 mL of one of the solvents (water, ethanol, or hexane) was added, achieving a solid-to-solvent ratio of 1:20 (w/v).

The flasks were sealed with parafilm to prevent solvent evaporation and placed in an ultrasonic bath. The extraction was conducted under the following optimized conditions:

- Ultrasound Frequency:40 kHz
- Temperature:30 °C (maintained by water bath)
- Extraction Time: 40 minutes

After ultrasonic treatment, the mixtures were filtered through Whatman No. 1 filter paper. The filtrates were then concentrated using a rotary evaporator at reduced pressure at 40 °C [21].

2.4. Extraction Yield Calculation

The dried extracts were weighed, and the extraction yield for each solvent was calculated using the following formula:

$$\textbf{\textit{Extraction Yield}}\ (\%) = \frac{\textit{Weight of Dried Extract}}{\textit{Weight of Dry Plant Material}} \times \mathbf{100} \qquad \ldots\ldots\ldots\ \textit{(1)}$$

The extracts were stored in dark, airtight containers at 4 °C until they were ready for analysis.

2.5. Phytochemical Screening

Preliminary qualitative phytochemical screening was performed on all extracts following standard methods. Tests for the presence of alkaloids, flavonoids, polyphenols, tannins, Coumarins, terpenoids, and steroids were conducted using conventional colorimetric and precipitation reactions.

a) Phenols: To 1 mL of extract, 2-3 drops of 1% ferric chloride ($FeCl_3$) solution were added. The formation of a blue-green or blackish coloration indicated the presence of phenols [22].

b) Alkaloid Test: A solution of 2 mL of rosemary extract was put into a beaker, and Mayer's reagent was added. If a white precipitate forms, then the sample contains alkaloids [23].

d) Flavonoid Test: Take a few drops of 1% aluminum chloride ($AlCl_3$) and add them to the extract. The appearance of a yellow color indicates the presence of a flavonoid [22].

e) Terpenoid Steroid Test: A total of 2 mL of extract solution was added to the reagent, namely 2 mL of chloroform, 10 drops of acetic anhydride, and 3 drops of concentrated H_2SO_4. A positive reaction to steroids is indicated by the formation of a green solution, and a red color is formed if it is positive for terpenoids [23].

f) Tannin TEST: Several drops of a 1% lead acetate solution were added to a test tube containing 0.5 ml of the extract. The appearance of a white, gelatinous precipitate was evidence of the presence of tannins [22].
g) Coumarin Test: 2 ml of the extract were added to 3 ml of a 10% sodium hydroxide (NaOH) solution. The appearance of a yellow color indicated the presence of coumarins [24].

2.5. Determination of Sun Protection Factor (SPF):

The *in vitro* SPF of the rosemary extracts was determined by the spectrophotometric method of Mansur *et al*. (1986) [25].
• Sample Preparation: Each extract was dissolved in its respective solvents to a final concentration of 1 mg/mL.
• UV Spectrophotometric Analysis: The absorbance of each solution was measured using a UV-Vis spectrophotometer in the wavelength range of 290 to 320 nm, at 5 nm intervals. A solution of the respective solvent was used as a blank.
• SPF Calculation: The SPF value was calculated using the following Mansur equation:

$$\boldsymbol{SPF\ spectrophotometer = CF * \sum_{290}^{320} EE * I * Abs} \ldots\ldots\ldots\ldots (\mathbf{2})$$

Where:
CF is the correction factor (=10).
EE(λ) is the erythemal effect spectrum.
I(λ) is the solar intensity spectrum.
Abs(λ) is the spectrophotometric absorbance of the solution at wavelength λ.
The values of EE × I are constants pre-determined by Sayre *et al*. (as shown in the Table (1)) [26].

Table (1): Normalized product function used in the calculation of SPF

Wavelength (nm)	EE(λ) x I(λ) (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

3. Results and Discussion

3.1. Total extraction yield

As shown in Table (2), water, the most polar solvent used in this study, produced the highest extraction yield (17.88%). This result is consistent with the fundamental principle of solubility, commonly described as "like dissolves like," where polar solvents preferentially dissolve polar phytochemicals and non-polar solvents dissolve non-polar constituents. The extraction efficiency is therefore strongly governed by

solvent polarity, which determines the types and quantities of compounds that can be solubilized from plant material [27].

The solvents employed in this study span a wide range of polarity, including water as a highly polar solvent (polarity index ≈ 9.0), ethanol of intermediate polarity (polarity index ≈ 6.5–7.0), and hexane as a non-polar solvent (polarity index ≈ 0.1), thereby reflecting their markedly different extraction capacity, as shown in Table (2).

Table (2): Extraction yield of rosemary plant

Solvent	**polarity index**	***Dried simplicia weight* (g)**	**dry extract mass (g)**	***Yield*(%)**
Ethanol 70%	6.5–7.0	5	0.673	13.46
Water	9.0	5	0.894	17.88
Hexane	0.1	5	0.102	2.04

The high yield confirms that a significant portion of the rosemary biomass is composed of water-soluble materials. This makes aqueous extraction a cost-effective method for obtaining a high mass of total soluble solids, though it may include compounds with varying bioactivity [28] [29].

The intermediate yield of 13.46% obtained using ethanol 70% highlights its effectiveness as a balanced and versatile solvent for extracting specific valuable secondary metabolites.

Ethanol-water mixtures are particularly effective at extracting semi-polar compounds such as phenolics and terpenes, while minimizing the simultaneous extraction of highly polar substances like sugars and proteins. This reinforces the notion that ethanol 70% provides an optimal polarity for accessing a diverse range of bioactive molecules [30].

The yield of 13.46% represents a concentrated extract rich in the compounds commonly associated with rosemary's antioxidant and antimicrobial properties. This extraction method strikes a balance between the high-yield but less specific water extraction and the low-yield but highly specific hexane extraction.

Hexane, the least polar solvent examined, produced the lowest extraction yield (2.04%). This result is consistent with reports indicating that nonpolar solvents primarily extract lipophilic components, including essential oils and terpenoids, which typically represent only a small proportion of the total biomass of rosemary [31] [32].

The extraction yields observed in this study demonstrate the expected relationship between solvent polarity and the solubility of the phytochemical constituents found in rosemary. The trend of solvent effectiveness—ranging from water, to ethanol, and then to hexane—highlights the importance of selecting the appropriate solvent to optimize extraction efficiency for different types of bioactive compounds.

3.2. Phytochemical screening

The qualitative phytochemical screening of extracts from Rosmarinus officinalis (As shown in Table (3)) revealed significant variation in secondary metabolite content depending on the solvent used. Water, ethanol 70%, and hexane all produced distinct profiles that aligned with their polarity and the known chemical characteristics of the compounds found in rosemary.

Abundance was estimated qualitatively based on the intensity of color formation or the amount of precipitate in each test: (+++) = high intensity/abundance, (++) = moderate, (+) = low/weak, (-) = absent [33].

Phenolic compounds were most abunda in the water extract, moderately present in the ethanol extract, and absent in the hexane extract. This pattern reflects the strong polarity of phenols, which are preferentially soluble in aqueous solutions. Flavonoids were strongly detected in the ethanol extract and weakly present in the water extract, but were absent in hexane, which aligns with the semi-polar nature of flavonoid aglycones and glycosides. Alkaloids were detected at moderate levels in the ethanol extract and at low levels in the water extract, with no detection in hexane, indicating their partial solubility in polar and semi-polar solvents.

Tannins were highly abundant in the water extract and present at low levels in ethanol, confirming their strong hydrophilic nature. In contrast, terpenoids were predominantly found in the hexane extract, with minimal presence in ethanol and none in water, consistent with their nonpolar and lipophilic characteristics. Coumarins were not detected in any of the extracts, likely due to their low concentration or limitations of the qualitative assay used.

Overall, the results illustrate the expected relationships between solvents and phytochemicals: water preferentially extracted polar compounds (such as phenols and tannins); ethanol extracted a broader range of semi-polar constituents (including flavonoids and alkaloids); and hexane selectively extracted nonpolar terpenoids. These findings support previously reported phytochemical profiles of rosemary and confirm the specificity of solvents in extracting its secondary metabolites.

Table (3): Phytochemical screening

secondary metabolite	water	Ethanol	Hexan
Phenols	+++	++	-
Flavonoids	+	+++	-
Alkaloids	-	++	-
Tannins	++	+	-
Terpenes	_	+	+++
Coumarins	_	_	_

Low+, Moderate ++, High +++, Absent-

Although qualitative screening provides preliminary insight into phytochemical composition, advanced analytical techniques such as gas chromatography–mass spectrometry (GC–MS) are required for definitive compound identification and structural confirmation.

3.3. Determination of Sun Protection Factor (SPF):

The sun protection factor (SPF) of the different Rosmarinus officinalis extracts was evaluated spectrophotometrically in the UV-B (290–320 nm). The absorbance values of the hexane, ethanol (70%), and aqueous extracts at a concentration of 1 mg/mL are presented in Table (4), followed by the calculated SPF values illustrated in Figure (1).

Table (4): Absorbances of samples at a concentration of 1 mg/ml

UV (nm)	**water**	**Ethanol**	**Hexan**
290	1.098	2.975	0.958
295	1.167	3.094	0.813
300	1.675	3.574	0.812

305	1.769	3.579	0.709
310	2.074	3.602	0.729
315	2.095	3.601	0.705
320	2.105	3.607	0.704

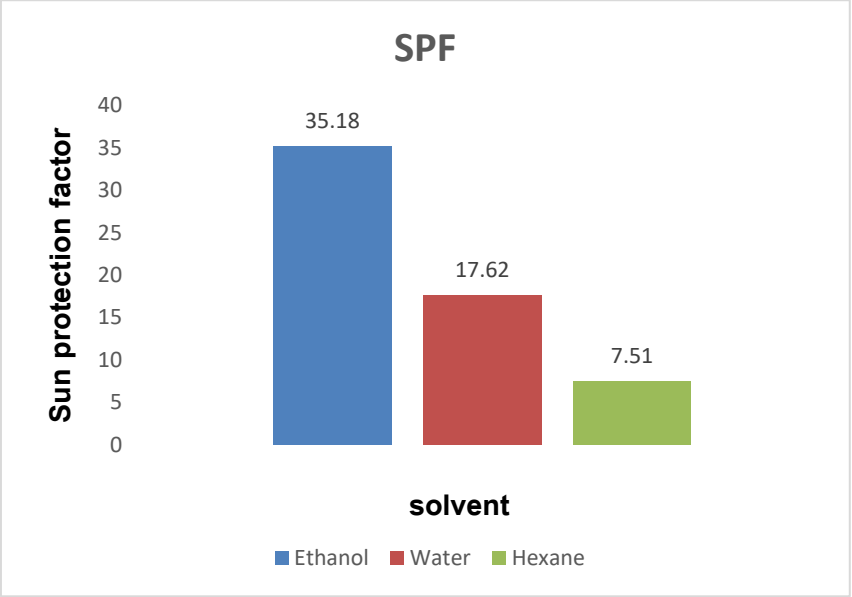

FIGURE (1): SUN PROTECTION FACTOR FOR ROSEMARY EXTRACTS

The SPF evaluation of Rosmarinus officinalis extracts (As shown in Figure (1)) revealed substantial variation in UV-protective capacity depending on the extraction solvent. Ethanol extract exhibited the highest SPF value (35.18), followed by the water extract (17.62), while the hexane extract showed the lowest level of photoprotection (7.52). These differences reflect both the chemical composition of the extracts and the UV-absorbing characteristics of the phytochemicals present.

The ethanol extract demonstrated a remarkably high SPF of 35.18, which categorizes it as an "Ultra High" protection product according to international standards (SPF 30-50). This outstanding performance can be directly attributed to its unique phytochemical composition, as revealed in the phytochemical screening.

The superior efficacy of the 70% ethanol extract is primarily associated with its optimal solvent polarity, which enables efficient extraction of both polar phenolic acids and semi-polar flavonoids. This hydroalcoholic solvent system therefore provides broader phytochemical coverage [34]. Although alternative ethanol–water ratios were not evaluated in the present study, extensive phytochemical literature consistently reports that 70% ethanol yields maximal recovery of polyphenols and flavonoids from plant matrices, thereby explaining its enhanced SPF performance [35] [36] [37].

Phytochemical analysis revealed that the ethanol extract is particularly rich in flavonoids and phenolic compounds, which are recognized as key contributors to photoprotection. These molecules possess conjugated aromatic systems that efficiently absorb ultraviolet radiation, especially within the UV-B region (290–320

nm) [38] [39]. The major photoprotective constituents expected in rosemary extracts include rosmarinic acid, carnosic acid, and carnosol [40]. (Figure (2))

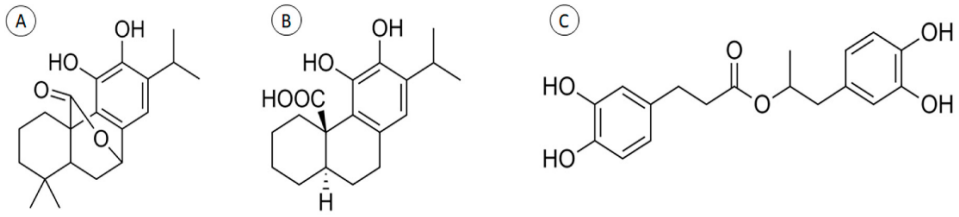

FIGURE (2): CHEMICAL STRUCTURE OF SOME ROSMARINUS OFFICINALIS SECONDARY METABOLITES: CARNOSOL (A), CARNOSIC ACID (B), ROSMARINIC ACID (C

The high and consistent absorbance values of the ethanol extract across the entire tested wavelength spectrum (as seen in Table (4)) suggest a broad-spectrum UV absorption profile. This is likely due to the synergistic action of various flavonoids and phenolic compounds, each absorbing light at slightly different wavelengths, thereby creating a more effective cumulative shield than any single compound alone [39].

The water extract provided a "High" level of protection with an SPF of 17.62. This is a significant value for a natural extract and confirms that water-soluble components of rosemary contribute substantially to its photoprotective effect.This activity is primarily driven by the very high concentration of phenols detected in the aqueous extract. While it contained fewer flavonoids, the abundance of polar phenolic acids (such as rosmarinic acid, a known potent antioxidant and UV absorber [41]) was sufficient to confer considerable UV-filtering capacity.

Its lower SPF compared to the ethanol extract underscores the critical role of flavonoids, which were less abundant in the water fraction. This indicates that while phenolic compounds are crucial, the full photoprotective potential of rosemary is best unlocked with a solvent that can also extract semi-polar flavonoids.

The hexane extract showed a low SPF of 7.52, offering only minimal protection. This result was anticipated based on its phytochemical profile.

Although the hexane extract was rich in terpenes, these non-polar compounds are generally less effective at absorbing UV radiation in the critical erythemal range compared to phenolics and flavonoids [42]. The complete absence of these key UV-absorbing molecules in the hexane fraction explains its poor performance in the SPF assay.

This finding clearly demonstrates that the lipophilic fraction of rosemary, while valuable for other properties like aroma or antimicrobial activity, is not the primary source of its sun-protective compounds.

The SPF results powerfully demonstrate that the choice of extraction solvent is paramount in obtaining a rosemary extract with high sun protection activity. The ethanol 70% extract emerges as the unequivocally superior candidate, yielding an SPF value that competes with many synthetic sunscreen agents.

The strong correlation between the high SPF value and the high concentration of flavonoids and phenols in the ethanol extract provides a compelling scientific rationale for its use. This aligns perfectly with the existing literature on natural UV filters, which consistently identifies polyphenols and flavonoids as key photoprotective agents [42] [41] [43].

4.Conclusion

This research confirms that the extraction solvent plays a crucial role in determining both the phytochemical richness and photoprotective capacity of rosemary extracts. Among the tested solvents, ethanol 70% proved the most effective, yielding an extract with high concentrations of phenols and flavonoids and demonstrating the strongest UV-protective activity (SPF 35.18). Water, while producing the highest extraction yield, delivered only moderate SPF due to its lower flavonoid content. Hexane, a nonpolar solvent, extracted mainly terpenoids and showed minimal photoprotective potential.

These findings highlight the superior suitability of hydroethanolic extraction for producing multifunctional rosemary extracts capable of acting as natural sunscreen agents. The strong correlation between phenolic/flavonoid content and SPF underscores the importance of targeting semi-polar bioactive compounds in the development of effective, plant-based photoprotective formulations. Consequently, ethanol 70% extract of Rosmarinus officinalis represents a promising candidate for incorporation into cosmetic and pharmaceutical sunscreen products, offering a safe and natural alternative to synthetic UV filters.

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