HPTLC Standardization and Antioxidant activity of Leaves of Rosemary (*Rosmarinus officinalis L.)*

[1]S. Farooq, [*1]Suman Lata Chhimwal and [1]Zafar Mehmood
Himalaya Wellness Company, Dehradun
[*]Email- suman15dec@yahoo.co.in

DOI 10.51129/ujpah-2025-39-2(11)

Received- 20 December, 2025
Revised- 22 December, 2025
Accepted- 24 December, 2025
Published- 27 December, 2025

Abstract- *Rosmarinus officinalis L. a fragrant perennial shrub with needle-like leaves, belongs to the mint family (Lamiaceae)* and is popularly known as rosemary. The plant is widely used for its medicinal properties, particularly its antioxidant activity, which is mainly attributed to its phenolic constituents. Proper standardization of herbal drug is essential to ensure their identity, quality and consistency. The present study focuses on the qualitative standardization and antioxidant activity of rosemary leaves. HPTLC was employed for fingerprint profiling without the use of any reference marker compound. Different mobile phase system of varying polarity were optimized to achieve effective separation of phytoconstituents and to develop a characteristic chromatographic fingerprint. Methanolic extract of rosemary leaves was subjected to HPTLC analysis using silica gel plates and suitable mobile phase. The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay. The study demonstrates the HPTLC fingerprint profiling, combined with antioxidant evaluation, provides a simple, reliable, and economical approach for the quality control and a standardization of *Rosmarins officianalis.*

Keywords: *Rosmarins officianalis, HPTLC fingerprint, Antioxidant activity, DPPH assay.*

Introduction

Rosmarinus officinalis (rosemary) is an aromatic medicinal plant widely used for its therapeutic properties. Rosemary leaves are commonly consumed as a fragrant, oxidant-rich herbal tea and are known for their benefits in enhancing memory, aiding digestion, reducing stress and alleviating inflammation. Traditionally, rosemary has been used in herbal medicine for the treatment of digestive disorders, infections and inflammatory conditions. The leaves are rich in bioactive phytochemicals, including phenolic acids, flavonoids, and diterpenes,[(1)] which are recognized for their strong antioxidant properties.

Due to the increasing demand for natural antioxidants, there has been growing interest in identifying plant based compounds that can limit or replace synthetic antioxidants. Numerous scientific studies have investigated the chemical

composition and biological activities of rosemary for this purpose. These studies highlight the plant's long standing medicinal use and its richness in secondary metabolites and phytochemicals. The therapeutic relevance of rosemary is largely attributed to its high content of bioactive compounds.

Among the various phytoconstituents present in rosemary leaves, the most significant antioxidant polyphenolic compounds include carnosic acid, rosmarinic acid[(2)]. These compounds play a major role in the antioxidant potential of rosemary extracts. Plant phenolics, the most abundant secondary metabolites, are the main source of dietary antioxidants and have many health benefits[(3)]. Rosemary and other members from the Lamiaceae family are a rich source of natural antioxidants. Rosemary were shown to have similar patterns of phenolic compounds and their antioxidant activity was attributed mainly to theier carnosic acid, carnosol and rosmarinic acid components[(4)]. This research paper standardizes the methanolic extract of rosemary with the in vitro antioxidant activity of the standardized rosemary extract.

Material and Methods

Collection of Plant Material- Fresh leaves of *Rosmarinus officinalis L.* (Rosemary) were collected from the herbal garden and the plant material was authenticated by using a standard herbarium voucher specimen.

Extraction of plant Material- Wash the rosemary leaves with distilled water to remove any dust or contaminants. Dry them by a shaded area or using an oven at low heat (around 40–45°C) to prevent degradation of the active compounds.Place the powdered rosemary leaves into a clean beaker.

Maceration Process- Once dried, grind the rosemary leaves into a coarse powder using a mortar and pestle or a grinder. This increases the surface area and helps to release more active compounds into the solvent. Pour the chosen solvent over the powdered leaves to ensure that the plant material is fully submerged. Shake the beaker gently for the first few minutes to ensure that the plant material is well mixed with the solvent. You can also stir the mixture with a glass stirring rod to improve extraction. Let the mixture sit at room temperature for 48–72 hours. During this time, the solvent will slowly extract the active compounds from the rosemary leaves. Shake or stir the mixture once or twice a day to facilitate the extraction process.

TLC/HPTLC fingerprinting- TLC finger printing profile was done for methanol extract to find out the nature of compounds present. When standardizing rosemary (*Rosmarinus officinalis L.*) extracts through techniques like High-Performance Thin-Layer Chromatography (HPTLC), choosing the appropriate mobile phase is crucial for separating and identifying various bioactive compounds. The mobile phase, which consists of a mixture of solvents, plays a significant role in determining the retention factors (Rf

values) of the compounds, as well as the resolution of spots or peaks on the chromatogram.

Here are some commonly used mobile phases for the standardization of rosemary extracts,for different types of compounds (phenolic acids, flavonoids, essential oils, etc.) present in rosemary[5]:

a) Toluene: ethyl acetate: Formic acid= 5:4:1(v/v/v)- **separate non-polar to moderately polar compounds**.
b) Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26 (v/v/v/v)- **polar compound separation**,
c) Chloroform: Acetone: Formic acid= 76: 16:08 (v/v/v)- **non-polar to moderately polar compounds** such as **essential oils**, **terpenes**, and some **phenolic acids** from rosemary.

Determination of Antioxidant Activity of Rosemary Leaf Extract

DPPH radical scavenging activity

Using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich, Germany), Al Tarawneh (2022) methodology[6] was utilized to evaluate the DPPH scavenging actions of Rosemary methanol extraction 0.1 mM DPPH methanol solution was combined with 0.1 ml of different concentrations of RE. After giving the mixture a good shake and letting it at room temperature for thirty minutes, then at 517 nm, by utilizing the HITACHI U-5100 UV-VIS spectrophotometer, to get the absorbance against a blank. The following formula was utilized to compute the degree of DPPH radical scavenging activity.

Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control As is the absorbance of the sample.

Result and Discussion

a) The Rf values and color of the resolved bands were noted after development of the silica plate up to the height of 8.5cm. The bands correspond to different constituents in rosemary. Fig-1 shows the plates on the mobile phase Toluene: ethyl acetate: Formic acid= 5:4:1. This solvent system **separates non-polar to moderately polar compounds and** is useful for resolving less polar phenolic compounds or essential oils, giving moderate Rf values that allow good spot separation.

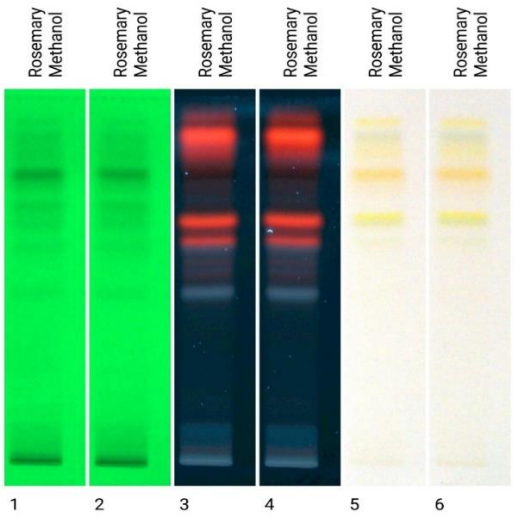

Figure-1 Separation of constituents of rosemary with mobile phase Toluene: ethyl acetate: Formic acid= 5:4:1

b) Using the mobile phase for polar compound separation is Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26. This mobile phase is used for separation of polar compound. The water content makes the mobile phase strongly polar, ideal for separating polar analytes like flavonoids and phenolic acids, yielding lower Rf values for polar substances.

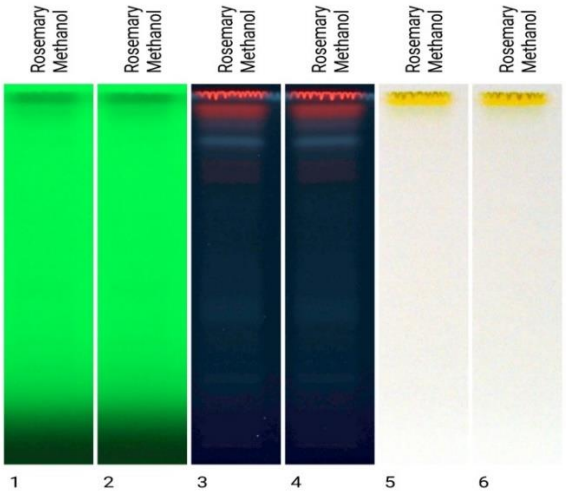

Figure-2 Separation of constituents of rosemary with mobile phase Ethyl acetate: Acetic acid: Formic acid: water= 100:11:11:26

c) Using another mobile phase to separate nonpolar to moderately polar compound, Chloroform: Acetone: Formic acid= (76: 16:08) is employed. This solvent system effectively resolves non-polar to moderately polar compounds, including essential oils and terpenes, thereby providing superior separation of medium polarity components in the rosemary extract.

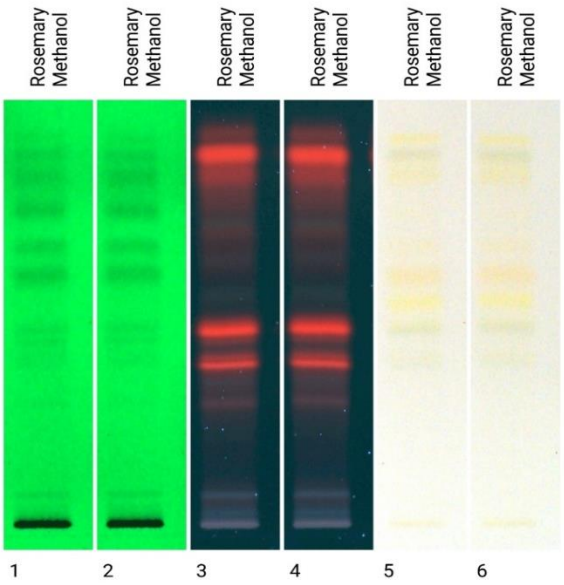

Figure-3 Separation of constituents of rosemary with mobile phase Chloroform: Acetone: Formic acid= 76:16:08

Table-1 Antioxidant activity of Methanolic extract of Rosemary leaves.

Concentration (µg/ml)	Absorbance	Plant extract (%) inhibition
Blank	0.159	-
20	0.109	31.4%
30	0.106	33.3%
40	0.086	45.9%
50	0.081	49.0%
60	0.048	69.8%
70	0.035	77.9%
80	0.010	93.7%
IC_{50}	-	51.6 µg/mL

The radical DPPH is frequently utilized to assess antioxidant activity quickly. Of the natural antioxidants, rosemary extract has been widely recognized as one of the spices/seasoning that exhibits high antioxidant activity in numerous food applications. According to Moreno et al.[8], both antioxidant and antimicrobial activities of rosemary extract are linked to its polyphenol composition. The capacity of the phytochemicals to give hydrogen, that scavenges the DPPH radical, provides the basis for DPPH scavenging action. When a DPPH solution is combined with an element that may supply an electron or a hydrogen atom to DPPH, neutralizing its free radical properties, the resulting reduced form of DPPH (non-radical) loses its violet hue. As the fraction of free radical inhibition rises, so does the radical scavenging activity [7]. The stable radical DPPH's absorbance decreased and its hue changed from purple to yellow at varying doses.

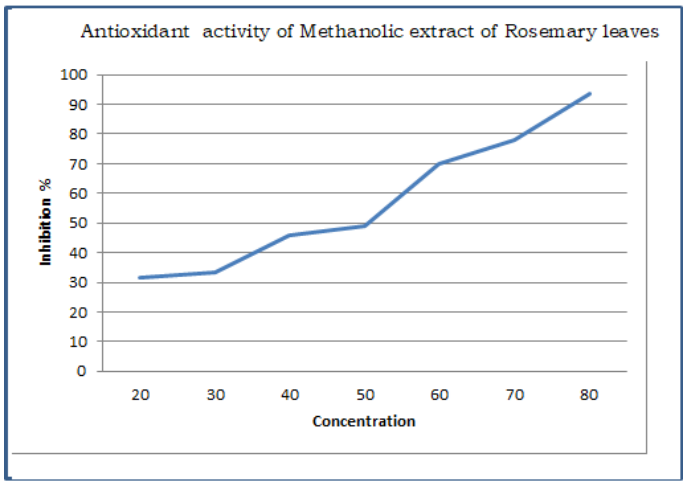

Figure-4 Antioxidant activity of Methanolic Extract of rosemary leaves

According to the research findings, the extraction of methanol exhibited the greatest DPPH scavenging activity (93.7% for rosemary extract at 80µg/ml), while the lowest scavenging value was (31.4 % for rosemary at 20µg/ml). The methanolic extract of rosemary leaves demonstrated a concentration –dependent antioxidant activity, with an IC_{50} of 51.6µg/ml. this indicates that the extract exhibits moderate free radical scavenging potency, effectively inhibiting 50% of oxidative stress at this concentration.

Conclusion

The HPTLC standardization of rosemary extract, conducted using three mobile phases, generated reproducible fingerprint profile. The selected mobile phase system ensured effective separation and quantification of bioactive compounds in the extract and can be used for quality control outline of rosemary. Rosemary also exhibited potent antioxidant activity according to the DPPH assay. The result showed notable antioxidant activity (IC_{50}= 51.6ug/ml). Thus rosemary extract emerges as a validated, moderate-strength natural radical scavenger, rich in polyphenol and capable of protecting against oxidative stress.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Saini, A.; Pandey, A. and Sharma, S. et al. Assessment of antioxidant activity of rosemary (*Rosmarinus officinalis*) leaves extract. *J Pharmacogn. Phyto-chem.*; 2020; 9(3):14-17.

2. Nieto, G.; Ros, G. and Castillo J. Antioxidant and anti-microbial properties of rosemary (*Rosmarinus office-nalis*, L.): A review. *Medicines*. 2018; 5(3):98.

3. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F. and Bonaduce, D.; et al. Oxidative stress, aging, and diseases. *Clin. Interv. Aging*; 2018; 13:757–772.

4. Frankel, E.N.; Huang, S.W.; Aescbbach, R. and Prior, E. Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid, in Bulk Oil and Oil-in-Water Emulsion. *J. of Agricultural and Food Chemistry*; 1996; 44: 131-135.

5. Altarawneh, R.M.; Al-Jaafreh, A.M.; Qaralleh, H. and Al-Qaralleh, OS. Chemical profiling of *Punica granatum* peels from Jordan using LC–MS/MS and study on their biological activities. *Int. J. Food Sci. Technol*.; 2022; 57(8): 5256-5267.

6. H.Wagner and S.Bladt. A thin layer Chromatography Atlas. Plant Drug Analysis; 1996.

7. Schreiner, M.; Beyene, B.; Krumbein, A. and Stutzel, H. Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in Brassica carinata leaves as affected by water supply. *J. Agric Food Chem*.; 2009; 57(16):7259-7263.

8. Moreno, S.; Scheyer, T.; Roman, C.S. and Vojnov, A.A. Antioxid-ant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic. Res.;* 2006; 40(2): 223-231.