

# Isolation and Purification of bioactive component from *Rosmarinus officinalis*

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## Abstract

*Rosmarinus officinalis*, commonly known as Rosemary or Gulmehendi (in India), belongs to the Lamiaceae family of mints and Basil. In India, rosemary is a rare herb. *Rosmarinus officinalis*, is proved to possess various beneficial anti-properties due to the presence of various bioactive compounds. In the present research work a 5% aqueous and ethanolic extracts of leaves, roots and stems of Rosemary plant was prepared, which was used for the isolation and purification of the bioactive compounds from *Rosmarinus officinalis*. The phytochemical screening of Rosemary plant extracts revealed the presence of chemical constituents like tannins, flavonoids, terpenoids, alkaloids, and saponins. Qualitative analysis of *Rosmarinus officinalis* crude extracts was performed using thin layer chromatography which indicated the presence of alkaloids and flavonoids. Partially purified ethanolic Rosemary leaf extract was further subjected to HPLC for the purification of alkaloids in the extract. The result revealed 6 peaks indicating the presence of alkaloids in the ethanolic extract.

**Keywords:** Anti-properties, Aqueous extract, Ethanolic extract, HPLC, *Rosmarinus officinalis*, TLC.

## INTRODUCTION

*Rosmarinus officinalis*, more commonly known as rosemary, is a Mediterranean herb that belongs to the Lamiaceae family of mints. It might, however, be found all over the world. It's an aromatic annual with shrub-like branches brimming with leaves. *R. officinalis* is used in cooking as a spice, as a natural preservative in the food industry, and as an ornamental and medicinal plant. [1-4].

Flowers in a variety of colours, including white, pink, purple, and blue, bloom in the winter or spring. They bloom in clusters of five to seven from a pair of short, opposite spikes that alternate along the stalk's sides. Two seeds are produced by each flower. [5]. Bees are attracted to the tiny bluish flowers that are borne in auxiliary clusters. Rosemary is immune to most pests and plant.

*R. officinalis* consists of a volatile fraction and phenolic compounds. The compound is made up of flavonoids, Rosmarinic acid, and a couple of diterpene compounds derived from carnosic acid, carnosol, including Rosmanol [6].

Rosemary has been shown to have antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antithrombotic, antioxidant properties, etc.[7-9]. Nervous, cardiovascular, gastrointestinal, genitourinary, menstrual, hepatic and reproductive system disorders, as well as respiratory and skin conditions, are all treated with *R. officinalis*. [7]. Oral administration of rosemary leaf extract lowers blood TG levels, lowers TC, lowers LDL-c, and raises HDL-c. [10].

An extract of *Rosmarinus officinalis* was discovered to have a major inhibitory effect against hRSV (Human respiratory syncytial virus) infection, making it a potential therapeutic agent [11]. *R. officinalis* has also shown to inhibit hormone responsive lipase and pancreatic lipase in vitro. [12]. Carnosol, betulinic acid, ursolic acid, and polyphenols are antidepressant compounds contained in rosemary extract and essential oil. [13-15].

In a recent study, carnosol and carnosic acid had an antiproliferative effect that was concentration dependent. [16]. Carnosic acid and carnosol present in Rosemary

plant are the most important antioxidant constituents, accounting for 90% of the properties (Fe+2 fundamentally), reducing the formation of reactive oxygen species. [17]. It has been observed that, there has been an increasing interest and research in determining the therapeutic properties of Rosemary plant considering the various compounds present in Rosemary plant.

The present investigation aims at identification and purification of the potent bioactive molecules present in *Rosmarinus officinalis* by high performance liquid chromatography to study the drug likeness of the purified compound.

## MATERIALS AND METHODS

**Chemicals :** Ethanol, ferric chloride, sodium hydroxide, hydrochloric acid, glacial acetic acid, chloroform, sulphuric acid, Dragendroff's reagent, n-butanol, ethyl acetate, acetic acid, benzene was obtained from Fischer Scientific. TLC Silica gel plate was obtained from Merck, Quercetin was of analytical grade from Hi Media, Karnataka.

**Plant source :** The Fresh Rose Mary (*Rosmarinus officinalis*) plant was collected from Lal Bagh Botanical garden from Kaval Bysandra, Bangalore through random selection.

**Preparation of plant extract :** The plant was washed properly under tap water to remove the dust particles and other impurities from the plant. The plant parts were then oven dried overnight at 60° and it was later made into a coarse powder. A 5% aqueous and ethanolic (50% ethanol) extracts of the root, stem and leaves of the Rosemary plant was prepared by treating the coarse powder with the solvent system and placing it on a magnetic stirrer for 30 minutes. The solution was then centrifuged at 8,000 rpm for 18 mins at 25°C. The supernatant was used for further experimental analysis.

## Phytochemical analysis

The prepared plant extracts were used for the screening of phytochemicals.

**Qualitative analysis**

**Test for Tannins:** A known volume of the crude extracts were heated on the water bath and filtered. A few drops of 1% ferric chloride solution was added to the filtrate. Dark green solution indicates the presence of tannins.

**Test for Saponins:** A known volume of the crude extract were shaken and heated to boil. Formation of a stable persistent froth and creamy mass of small bubbles indicates the presence of saponins.

**Test for Flavonoids:** Diluted NaOH and HCL were added to a known volume of the crude extracts. A yellow solution that turns colourless indicated the presence of flavonoids.

**Test for Terpenoids:** A known volume of the crude extracts were filtered and mixed with 2ml of chloroform. Then 3ml of concentrated sulphuric acid was carefully added to form a layer. Formation of reddish- brown coloration indicates the presence of terpenoids.

**Test for Cardiac glycosides:** A known volume of the crude extracts were shaken with 1ml of glacial acetic acid. A drop of ferric chloride and concentrated sulphuric acid were added. Green blue colour to the upper layer and reddish-brown colour at the junction of two layers indicates the presence of cardiac glycosides.

**Test for Alkaloids:** A known volume of the crude extracts were heated with 2% sulphuric acid for two minutes .The mixture was filtered and few drops of dragendroff's reagent were added . Orange red precipitate shows the presence of alkaloids.

**Test for Phenols:** Distilled water 15ml was added to a known volume of the crude extracts. Then a few drops of neutral 5% ferric chloride solution was added. Formation of a dark green colour indicates the presence of phenolic compounds. [18-19]

**Quantification of flavonoids**

In this assay, 0.2 to 1 ml aliquots of standard Quercetin(100µg/ml) solution were added in test tubes except in the blank tube. 0.1 ml of the plant extracts were also pipetted into different test tubes. The volume in the tubes were made to 2 ml using methanol. 0.1 ml of 10% Aluminium chloride was added in each tube followed by 0.1 ml of 1M Potassium acetate. 2.8 ml of distilled water was added into all the tubes so that the total volume of contents in each tube would finally be 5ml. The tubes were incubated for 30 mins at room temperature. The absorbance was read at 450nm using colorimeter against a suitable blank.[20].

**Partial purification by thin layer chromatography.**

TLC was carried out to isolate the principle components that were present in ethanolic extracts of different parts of the Rosemary plant (stem, leaf and root). TLC was performed on a silica gel plate. 2-4µL of different plant extract was deposited on the origin of the TLC plates from 1.5 cm of the origin with the help of capillary tubes.

**•Development of chromatogram**

After the application of the sample on the plates, the plates were kept in TLC chamber (solvent saturated) then mobile phase was allowed to move through adsorbent phase upto

¾th of the plate. TLC was performed for alkaloids and flavonoids.

**•Solvent system**

The different solvent system used were:

- Alkaloids :- Benzene: Acetic acid: Water (125:72:3)
- Flavanoids:- n-Butanol: Ethyl acetate: Water(5:10:15)

TLC plates were visualized under ultraviolet light (254λ).

**Preparative thin layer chromatography**

Silica gel plate was used for the study. The mobile phase solvent system consisting of Benzene: Acetic acid: Water (125:72:3) was used for the separation of alkaloids and n-Butanol: Ethyl acetate: Water (5:10:15) was used for the separation of flavonoids. The ethanolic extract of Rosemary plant was deposited as the concentrated band 1.5cm from the edge of its respective TLC plate and allowed to dry. The TLC plate were placed in the chromatogram. Then the respective spots were scraped and was further used for characterization using HPLC.

**Identification of the potent molecule by high performance liquid chromatography**

The sample was further characterized for alkaloids. The analysis was made on C18 column (symmetry, 4.6mm X 250mm) in isocratic mode with the mobile phase acetonitrile (COUTO, 2011) and water in the ratio 7:3 with the RP-HPLC C-18 column at a flow rate of 1mL/min and 20µL of the sample was injected and the elution was monitored at 230nm.

**RESULTS AND DISCUSSION**

In this current study, Rosemary plant and its parts were studied. it has wide range of phytochemicals and also for its enormous pharmacological uses, ethnobotanical uses and other miscellaneous uses. The plant is easily available in the market. Rosemary plant have enormous benefits in medical field.

**PHYTOCHEMICAL SCREENING**

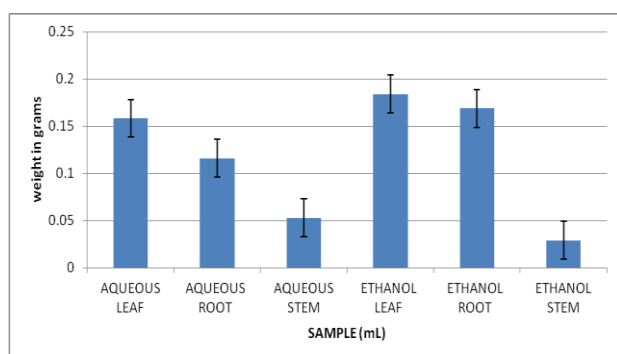
**Qualitative Analysis :** The Aqueous and ethanolic extracts of Rosemary were screened for phytochemicals. It was performed according to [21] which determined the presence of various constituents like flavanoids, tannins, saponins, terponoids, cardiac glycosides, alkaloids and phenols. The result of phytochemical screening of aqueous and ethanolic extracts has been tabulated in Table 1. Rosemary leaf and root indicated the presence of higher quantity of alkaloid in both aqueous and ethanolic extract, further more Rosemary leaf showed the presence of higher quantity of flavanoids in both aqueous and ethanolic extract, but Rosemary root proved the presence of higher quantity of flavanoids in aqueous extract. Thus, the study on phytochemical screening of Rosemary plant extract was correlated with the qualitative analysis of *Salvia officinalis*. [21], in which it revealed that the *Salvia officinalis* extract indicated the presence of tannins, flavanoids, and terpenoids whereas in the Rosemary plant extract tannins, flavonoids, terpenoids, alkaloids, and saponins were detected. Rosemary leaf had cardiac glycosides, and the root and leaf of the Rosemary plant contained phenols.

**Table-1:** Qualitative analysis of phytochemical in the aqueous extract and ethanolic extract of Rosemary Plant.

Phytochemicals	Stem		Root		Leaf	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
Tannins	-	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	++	+	++	++
Terpenoids	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	+	+
Alkaloids	+	+	++	++	+++	++
Phenols	-	-	+	+	+	+

Note: '+' indicated a positive result and '-' indicated a negative result.

**Quantification analysis :** As illustrated in figure 1, the total flavonoid content of ethanolic extract of Rosemary root (0.1689g) and leaf (0.1829g) was higher than the aqueous extract of root (0.1161g) and leaf(0.1508), but in aqueous the stem(0.0528g) content was higher than the ethanolic extract(0.0290). As compared, ethanolic extract of leaf and root was higher than the aqueous extract.



**Figure-1:** Estimation of flavonoids in Rosemary plant extract

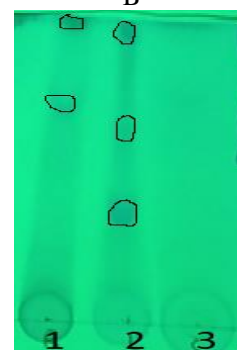
**Analysis by thin layer chromatography :** Thin layer chromatography for the Rosemary extracts were performed for qualitative analysis of Rosemary stem, root and leaf extract. Different solvent system confirmed the presence of diverse potent bioactive components in the parts of the Rosemary plant. Purple spot was observed under short UV light of 254nm confirming the presence. Partial purification was carried out by preparative thin layer chromatography with respective solvent. These potent bioactive components were further analyzed using HPLC (High Performance Liquid Chromatography).



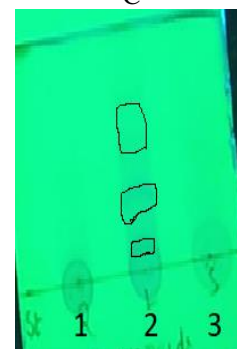
A



B



C



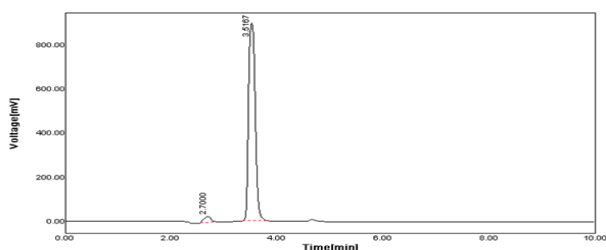
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Lane 1: Root : Lane 2: Leaf :Lane 3: Stem

**Figure 2:** (A) TLC profile for Alkaloid (aqueous); (B) TLC profile for alkaloid (ethanol); (C) TLC profile for Flavonoids (ethanol); (D) TLC profile for Flavonoids(aqueous)

**Purification by HPLC :** High performance liquid chromatography was performed to identify the alkaloids present in Rosemary leaf. Partially purified samples were subjected to HPLC for further purification and

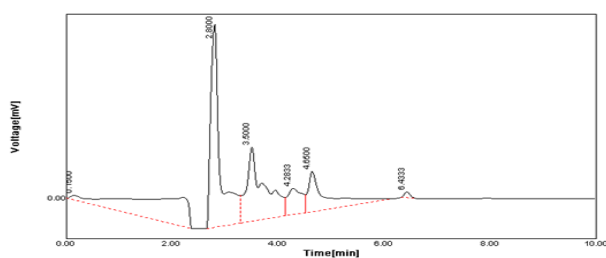
identification. The sample showed 6 peaks, in which one major peak was 2.8000 and 5 minor peaks at 0.1500, 3.5000, 4.2833, 4.6500, 6.4333. With previous studies it was found that codeine was found to be present at 2.8 minutes, Caffeine showed a peak at a retention time of 3.5 minutes in Jasmine plant, oxymatrine was found to be present in *Corydalis yanhusuo* showing a peak at a retention time of 2.8 minutes and harmaline was found to be in Passion fruit showed a peak at a retention time of 6.6 minutes.



**Figure 3:** Chromatogram of the standard used- Caffeic Acid by HPLC.

**Table2:** HPLC profile of standard caffeic acid

No.	RT[ <i>min</i> ]	Area[mV*s]	Height[mV]
1	2.7000	231.4191	24.9182
2	3.5167	7887.3906	895.4562
Sum		8118.8096	920.3744



**Figure 4:** Chromatogram of the purification of ethanolic extract of Rosemary leaf by HPLC.

**Table3:** HPLC profile of purified ethanolic leaf extract of *Rosmarinus officinalis*

No.	RT[ <i>min</i> ]	Area[mV*s]	Height[mV]
1	0.1500	682.9336	9.8608
2	2.8000	846.5366	67.6208
3	3.5000	580.7429	24.7682
4	4.2833	166.3126	8.7567
5	4.6500	316.5809	13.3570
6	6.4333	13.7282	1.8331
Sum		2606.8352	126.1966

### CONCLUSION

The present study indicated that the *Rosmarinus officinalis* plant contained significant amount of various secondary metabolites. The presence of tannins, flavonoids, terpenoids, alkaloids, and saponins in the *Rosmarinus officinalis* plant was analysed via phytochemical screening in this research.

Rosemary root had a higher concentration of flavonoids in its aqueous extract, which was confirmed using thin layer chromatography profiling. The ethanolic extract of Rosemary leaf (0.1829g) was found to have the highest amount of flavonoid content when compared to the others extracts used in this study. The test for alkaloid in both the ethanolic and aqueous extracts of rosemary leaf and root, showed an evident colour change.

Later, flavonoids of the ethanolic extract of Rosemary leaf were purified using HPLC. In samples chromatogram, 6 peaks were observed at different retention times. The flavonoids found to be present was Codeine that showed a peak at 2.8 minutes, Caffeine showed a peak at a retention time of 3.5 minutes, Oxymatrine showed a peak at a retention time of 2.8 minutes and Harmaline showed a peak at a retention time of 6.6 minutes.

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